EFFECTS OF MEGALAC®-R SUPPLEMENTATION ON MEASURES OF INFLAMMATION AND PERFORMANCE IN TRANSPORT-STRESSED BEEF CALVES

By

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To my mother Arlete, my father Dudu and my sister Mazinha
“Saudade existe para quem sabe ter...”

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ADG	average daily gain
APP	acute-phase proteins
ARA	arachidonic acid
BH	biohydrogenation
BW	body weight
CCK	cholecystokinin
CLA	conjugated linoleic acids
CNS	central nervous system
CO	control
COX	cyclo-oxygenases
Cp	ceruloplasmin
CP	crude protein
CSFA	calcium salts of fatty acids
CV	coefficient of variation
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DMI	dry matter intake
DPA	docosapentaenoic acid
EN	Energy Booster 100®
EPA	eicosapentaenoic acid
ETA	eicosatetraenoic acid
FA	fatty acids
Fb	fibrinogen
FL	Florida

G:F	gain to feed ratio
GLNA	γ-linolenic acid
HETE	hydroxyl-eicosatetraenoic acid
HPETE	hydroperoxy-eicosatetraenoic acid
Hp	haptoglobin
ID	identification
IGF-1	insulin-like growth factor 1
IL-1	interleukin 1
IL-6	interleukin 6
LA	linoleic acid
LNA	α-linolenic acid
LPS	lypopolysaccharide
LSD	least significant difference
LT	leukotrienes
MG	Megalac®-R
MUFA	monounsaturated fatty acids
NDF	neutral detergent fiber
OM	organic matter
PG	prostaglandins
PUFA	polyunsaturated fatty acids
SEM	standard error of the mean
SFA	saturated fatty acids
SD	standard deviation
TDN	total digestible nutrients
TNF-α	tumoral necrosis factor α

TMR	total mix ratio
TX	thromboxanes
UFA	unsaturated fatty acids
VFA	volatile fatty acids

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EFFECTS OF MEGALAC®-R SUPPLEMENTATION ON MEASURES OF INFLAMMATION AND PERFORMANCE IN TRANSPORT-STRESSED BEEF CALVES

By

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Two studies were conducted to evaluate measures of performance and inflammation in transport-stressed beef calves supplemented with saturated or unsaturated fatty acids. In the first study, prior to transport (d -40 to 0), 64 weaned, Braford steers were stratified by initial BW and age, and randomly allocated to 2 pastures. Each pasture was randomly assigned to receive 1 of 2 treatments, which consisted of grain-based supplements with (EN) or without (CO) the inclusion of a prilled saturated fat source (Energy Booster 100®). On d 0, steers were loaded onto a commercial trailer and transported for approximately 1,600 km over a 24 h period and delivered to a feedlot. Upon arrival (d 1), steers were stratified by pre-shipping treatment and randomly re-assigned in to receive EN, CO, or MG (grain-based supplement containing Megalac®-R). Shrunk BW was recorded on d -40, 0 and 30 to determine ADG. Individual DMI was recorded daily during the post-shipping period using the GrowSafe® system (Model 4000E). Blood samples were collected on d 0, 1, 4, 8, 15, 22 and 29 for determination of fibrinogen and ceruloplasmin concentrations. No pre-treatment effects or pre- x post-shipping treatment interactions were observed. During the post-shipping phase, steers fed MG had decreased ($P < 0.05$) ADG and lower mean DMI ($P < 0.01$) compared to CO-fed steers (0.80 and 1.04 kg/d, and 2.37 and 2.80% of BW, respectively). Steers fed MG had poorer G:F ($P < 0.05$) compared to EN steers, and

tended (P = 0.10) to have decreased G:F compared to CO-fed steers (0.37, 0.35 and 0.29 mean G:F for EN, CO and MG steers, respectively).

In the second study, prior to shipping (d -30 to 0), 48 Brahman-crossbred heifers were stratified by initial BW and randomly allocated to 6 pastures. Each pasture was randomly assigned to receive 1 of 2 daily supplement treatments, consisting of a grain-based supplement, with (MG) or without (CO) the inclusion of Megalac®-R. On d 0, heifers were transported for approximately 1,600 km over a 24 hour period. Upon arrival (d 1), 24 of the 48 heifers were stratified by BW and assigned to individual feedlot pens. Pre-shipping treatment allocation continued in the post-shipping phase. Shrunk BW was recorded on d -30, 1 and 28 to determine ADG. Individual voluntary hay intake was recorded daily from d 1 to 28. Blood samples were collected on d 0, 1, 4, 8, 15, 22 and 28 were used to determine plasma concentrations of ceruloplasmin, haptoglobin, and cortisol. A treatment x time interaction was detected for haptoglobin ($P < 0.01$) because MG-fed heifers had decreased ($P < 0.05$) haptoglobin concentrations on d 1, 3 and 5, relative to transport, compared with CO-fed heifers.

These data imply that that Megalac®-R supplementation appears to negatively affect performance of transport-stressed beef calves; decreasing ADG, DMI and G:F. In addition, the acute-phase reaction following transport appears to be modulated when Megalac®-R is supplemented to beef calves at least 30 d prior shipping.

CHAPTER 1
INTRODUCTION

In 2007, the beef cow herd of Florida (FL) was composed of 936,000 head and 68% of this herd was located in the Southern half of the state. The calves marketed from FL in 2007 totaled 721,000 head, over 80% of the calf crop (USDA-NASS, 2008). Nationally, FL ranked 12th in beef cows and 18th in total cattle, and its beef industry consists basically of cow-calf enterprises with high British-Brahman crossbred genetic influence and grazed pasture as the major source of nutrition.

Florida is the leading state in the United States for the number of large cow/calf operations (> 2,500 cows; USDA, 2002), with no commercial feedlot industry in FL, nearly all market steers are weaned and shipped outside of the state for further growing and finishing (Arthington et al., 2008). For example, Texas is the state which receives the majority of FL-market calves, and it is located approximately 2,400 km to the west. This isolation results in important impacts on subsequent animal health and performance, caused mainly by stressors associated with weaning, weather changes and transportation. In the U.S., the calf morbidity and mortality associated with respiratory disease and shipping fever complex is estimated to cost approximately $500 million to the beef industry (NASS, 1996). Many factors contribute to the cost of these diseases, such as pharmaceutical purchase, feed resources lost, increased labor, death and poor performance of animals (Loerch & Fluharty, 1999).

Marketing processes at feedlot arrival are crucial events causing a considerable amount of stress for cattle. Most of the health problems with newly arrived calves occur within the first 2 weeks due to the impact of stress on feed intake and immunocompetency (Fluharty & Loerch, 1997). Previous studies have evaluated the effects of weaning management and transportation on the acute-phase reaction and performance of beef calves (Arthington et al., 2003 and 2005), and

indicate that plasma concentrations of acute-phase proteins (APP) are significantly affected by these procedures, and may be used as an indicator of stress and performance of these animals.

The inclusion of polyunsaturated fatty acids (PUFA) into diets has been shown to modulate immune responses (Calder et al., 2002). The majority of PUFA originating from common feedstuffs are extensively modified in the rumen (Palmquist & Jenkins, 1980), and the addition of calcium soaps of fatty acids into diets may provide protection of these PUFA from the rumen microorganisms (Ngidi et al., 1990). Being a rumen-inert technology, the supplementation of Megalac®-R (Church & Dwight Co., Inc. Princeton) might be a management option for increasing the delivery of PUFA to the small intestine, providing essential precursors for modulating the immune system.

For these reasons, two experiments were conducted to evaluate the effect of supplemental Megalac®-R on measures of performance and physiological responses of growing cattle following transportation.

CHAPTER 2
LITERATURE REVIEW

Lipids

Definition of Dietary Lipids

Lipids are a chemically diverse group of compounds defined commonly by their insolubility in water, but are generally soluble in organic solvents. Dietary lipids of significant importance include fatty acids (FA), triglycerides, cholesterol and esters of cholesterol, and fat-soluble vitamins (Spallholz et al., 1999). Lipids have multiple functions including supplying dietary energy, serving as a source of heat, insulation and protection for the animal body, providing essential FA, and serving as a carrier for absorption of fat-soluble vitamins (Jurgens, 2002).

The fats and oils used almost universally as stored forms of energy in living organism are derivates of fatty acids (Nelson & Cox, 2005). Although fat usually comprises less than 5% of the ruminant diet, ruminants depend more on nonglucose metabolites for energy metabolism than nonruminants (Palmquist & Jenkins, 1980). Traditionally, in beef cattle grazing systems, pasture is the primary feed source and the range of FA content in forages varies widely from 1.6 to 10% (Clapham, 2005). In green plants, FA are predominantly in the form of glycolipids and phospholipids. Glycolipids account for 70 to 80% of ether extract in plant leaves whereas the concentration of triacylglycerol is negligible in the leaf (Harfoot, 1978). In contrast, triacylglycerols are the primary lipid in grains and whole seeds, and triacylglycerol are the major lipid class in the diet of cows fed in confinement management programs, unless fat sources containing free FA are included as dietary components of the ration (Doreau & Ferlay, 2004).

Linoleic acid (LA) is the predominant FA in most oilseeds, whereas in fresh forages α-linolenic acid (LNA) is usually greatest. Linoleic acid and LNA are considered essential FA

because they cannot be synthesized by mammals and ruminal microorganisms (Sprecher, 1981), and both LA and LNA are considered PUFA, because they contain more than one point of unsaturation, or double-bond. Fatty acids are classified and abbreviated according to the length of the acyl chain, number of unsaturations, and location and configuration of the unsaturation. By convention, essential FA are indentified according to simplified nomenclature of the ω-numbering system, which begins numbering carbons starting at the methyl end of the FA. For example, LA has 18 carbons and 2 double bonds (C18:2), and it belongs to the ω-6 family, also called n-6, because the first of its double bonds is localized at the sixth carbon from the methyl end. Processing of FA in one family can only generate fatty acids of the same family. For example, FA of the n-3 family cannot be converted into a member of n-6 family and vice versa (Mattos et al., 2000).

Supplemental Fat Intake and Digestion

Beef cattle production systems are traditionally classified into two broad categories: grazing and feedlot. In addition to the lipids provided by the forages, grazing cattle can obtain triacylglycerol and other FA through supplements. Most feedlot diets are grain-based to increase their energy concentration, which typically improves the efficiency and cost of gain (Gibb, 2004). Although containing less energy than grain, sources of fiber are often included in diets to help maintain rumen function (Bull et al., 1965) and animal health (Cheng et al., 1998). In confinement, lipids are often fed to increase energy density without decreasing fiber of the diet (Gibb, 2004).

The inclusion of fat to cattle diets can affect dry matter intake (DMI; Jerred et al., 1990). The type of fat fed, and the type and amount of forage offered, also have an effect on the extent to which DMI is affected (Allen, 2000). The mechanisms involved with the FA-induced depression of DMI are unclear, but may include alterations in palatability, gut motility, brain

signaling satiety centers and fiber digestion. The palatability of a fat can vary according to type (saturated or unsaturated), source (oil or whole seeds), and concentration in the diet (Miller et al., 1958). Grummer et al. (1990) stated that adaptation to supplements improved fat acceptability, and the differences in acceptability among fats can be minimized by mixing fats with other dietary ingredients. Chemical nature and other factors, such as odor, physical form, and appearance also influence fat acceptability (Grummer et al., 1990)

Fat is a potent stimulator of cholecystokinin (CCK) release and evidence exists that CCK contributes to satiety control (Reidelberger, 1994). It is probable that the CCK suppresses feed intake by inhibiting gastric emptying (Moran, 1992). Choi and Palmquist (1996) observed decreased DMI following decreased postprandial plasma concentrations of insulin and increased postprandial plasma concentrations of CCK by feeding fat to lactating dairy cows. Also, an intravenous injection of exogenous CCK depressed feed intake of sheep (Grovum, 1981). Reidelberger et al. (1994) suggested that peripheral action of gut CCK may activate vagal and splanchnic afferent neurons that inhibit the brain satiety centers, and an increased rate of oxidation of FA in the liver can alter signals generated by hepatic vagal afferent nerves to brain satiety centers (Allen, 2000). In addition, gut motility may be decreased by the presence of PUFA in the small intestine, which could decrease DMI (Drackley et al.,1992).

Devendra and Lewis (1974) summarized four theories to explain the negative effect of supplemental fat on fiber digestibility; 1) Physical coating of the fiber with fat, thus preventing microbial interaction; 2) Modification of the ruminal microbial population from possible toxic effects of fat on certain microorganisms; 3) Inhibition of microbial activity from surface active effects of fatty acids on the cell membrane; and 4) Reduction of cation availability to key ruminal microbes resulting from formation of insoluble complexes with long chain fatty acids.

The substitution of non-fiber carbohydrate with fat sources can reduce microbial protein production since carbohydrates are the primary energy source for ruminal microbes. Further, excessive unsaturated FA (UFA) supplementation may lead to depressed fiber digestion due to the toxic effects of UFA on ruminal microorganisms (Jenkins & Jenny, 1992). To avoid the inhibitory effects of free FA on ruminal bacteria, the calcium soaps technology was developed (Jenkins & Palmquist, 1982; Sukhija & Palmquist, 1990). Calcium salts of FA (CSFA) should be inert in the rumen, dissociating in the abomasum at low pH (Wu & Palmquist, 1991, Wu et al., 1991), thus becoming available in the small intestine for further absorption (Jenkins, 1993).

Palmquist and Jenkins (1980) reported beneficial effects of fat added to diets of high producing dairy cows. Cows fed fat from 3 to 5% of the total diet increased energy intake without negative effects on fiber digestion. Feedlot finishing diets containing 2 to 5% of total fat may stimulate weight gain of beef cattle (Moore et al., 1986), although apparent digestibility increased, whereas true digestibility decreased when fat was added up to 8% of dietary DM (Haaland et al., 1981). Grummer (1988) reported no effect on nutrient digestion and ruminal fermentation in dry Holstein cows fed CSFA or prilled fat when supplemented at 3.5% or less of the total ration DM, but cows fed prilled fat consumed a greater amount of fat than those fed CSFA due to the greater fat content in the prilled fat. Differently, Chalupa et al. (1986) reported reduced feed intake and lower milk yields from low producing dairy cows fed prilled fat at 6 or 9% of the total ration DM compared to prilled fat-fed cows at 0 and 3%.

The effects of fat supplementation on digestibility and performance were reported by several authors. Garcia et al. (2003) fed whole sunflower seeds (containing approximately 70% LA) at 5% of dietary DM and reported no differences in the live weight, average daily gain (ADG), or carcass weight of beef heifers compared to those fed no dietary lipid supplement.

Atkinson et al. (2006) supplemented sheep with increasing amounts of high-linoleate safflower oil (0, 3, 6, or 9% of dietary DM) and sugested no effects on apparent ruminal digestibility of organic matter, neutral detergent fiber (NDF), and nitrogen. Elliot et al. (1997) demonstrated a decrease in ruminal organic matter (OM) digestibility but no difference in postruminal OM digestibility when tallow was supplemented to beef steers consuming a 60% forage diet. Brokaw et al. (2002) sugested that total tract OM digestibility decreased when soybean oil (2.25% of dietary DM) was supplemented to ruminants consuming high-forage diets, and Brokaw et al. (2000) noted that heifers supplemented with high-oil corn at 0.5 % of body weight (BW) selected forage that was less digestible than forage selected by heifers fed conventional corn. However OM intake was not affected by feeding supplemental high-oil corn at 1.5% to 1.74% of dietary DM (Brokaw et al, 2001). Additionally, forage intake and diet digestibility were not affected in steers offered switchgrass hay and canola seeds to provide approximately 4% of dietary DM as crude fat (Leupp et al., 2006).

Hess et al. (2008), in a review of summarized results, indicated that an optimal inclusion rate for supplemental fat is less than 3% of dietary DM if the goal is to maximize the use of forage-based diets, and that supplemental fat should be limited to 2% of DM or less if the goal is to prevent substitution of forage with intake of supplemental fat. However, ruminants fed high-concentrate diets may receive up to 6% supplemental fat in the diet without ill effects on utilization of other components (Kucuk et al., 2004; Atkinson et al., 2006; and Hess et al., 2008).

Fatty Acid Metabolism in the Rumen

The first step in lipid metabolism in the rumen is hydrolysis of ester bonds via microbial lipases (Dawson et al., 1977). The end-products produced by ruminal hydrolysis are free FA, glycerol and galactose, which are converted to volatile fatty acids (VFA), mainly propionate and butyrate (Hazlewood & Dawson, 1975). The extent of hydrolysis ranges from 85 to 95% for

most unprotected lipids, and this percentage is greater for diets rich in fats than for conventional diets, in which most of the lipids are in the cellular structure (Bauchart et al., 1990). Lipolysis is a prerequisite to biohyrogenation (BH), which provides the free carboxyl group from UFA (Chalupa & Kutches, 1968). The microbial BH only occurs on free FA, released from triacylglycerols, adsorbed on feed particles or microbial cells (Doreau & Ferlay, 1994).

The BH process involves a series of microbial enzymes called isomerases and reductases that initially isomerizes UFA and sequentially hydrogenate the double bonds (Kepler et al., 1966). Because the BH of PUFA is very extensive, 92% for LNA and 80% for LA (Doreau & Ferlay, 1994), most of the dietary PUFA is modified and therefore not absorbed as such in the small intestine. Consequently, the composition of absorbed FA does not reflect the same composition of FA intake from the diet. Gulati et al. (2005) evaluated BH of prilled fat, CSFA, extruded oilseeds, formalin-treated oilseeds and untreated oilseeds. Formalin treatment provided the most protection from BH at around 90%, followed by CSFA at about 60% and then prilled fat or extruded fat at 30%.

Therefore, CSFA can be used as one of the alternatives to increase delivery of PUFA postruminally. This method of protection of FA promotes a chemical linkage between the free carboxyl group and a molecule of calcium, making the FA carboxyl group unavailable for microbial enzymes (Wu et al., 1991), avoiding reducing ruminal BH (Chalupa & Kutches, 1968). Megalac®-R is a commercial example of CSFA, composed of a mixture of palm and soybean oils, which contains about 39% LA and 3% LNA (Church & Dwight Co, Princeton, NJ).

Fatty Acids Metabolism in Tissues

The amount of each FA incorporated into organs and tissues depends on the amount of precursors present in the diet (Mattos et al., 2000). Lessard et al. (2003) showed an increased concentration of blood FA in Holstein cows fed whole flaxseed, micronized soybean and CS of

soybean oil prior to and during the breeding period. Cows fed CS of soybean oil presented a lesser blood concentration of LNA on d 5 and 21 after calving; and on d 0 and 20 after first artificial insemination when compared to cows fed flaxseed. Cows fed flaxseed had a blood n-6 to n-3 ratio three times less than cows fed CS of soybean oil. Increased milk fat content and oleic acid concentration were observed when lactating dairy cows were supplemented with CSFA (Megalac®) at 3 and 6% of the total diet DM compared to cows not fed CSFA (Schauff & Clark, 1992). In contrast, Harrison et al. (1995) reported a decreased concentration of oleic acid and an increased concentration of LA in milk when lactating dairy cows were fed CSFA (Megalac®; 2.7% of dietary DM) during the 3 months after calving compared to cows fed saturated fat source. Biohydrogenation of dietary LA and oleic acid in lactating dairy cows decreased slightly when soybean oil was fed to cows as CSFA (Lundy, III, et al., 2004).

The term conjugated linoleic acids (CLA) refers generically to a class of positional and geometric conjugated dienoic isomers of LA, two of which (cis-9,trans-11 and trans-10,cis-12 CLA) are known to posses biological activity (Pariza et al., 2001). These two types of CLA are produced in the rumen of cattle and other ruminant animals during an incomplete microbial BH of LA and LNA (Pariza et al., 2000), thus their principal dietary sources for humans are dairy products, beef and other foods derived from ruminants (Dhiman et al., 1999). Changes in substrate supply and extent of BH will affect the supply of intermediate and end products of BH resulting in an altered LA, LNA and CLA content of milk and meat (Kelly et al., 1998). The CLA are primarily recognized for their anti-carcinogenic and lipolytic effects (Park et al., 1997), but CLA also influence immunity (Pariza, 2001) and reproduction (Garcia, 2003) affecting the synthesis of eicosanoids, cytokines, and steroid hormones.

Gassman et al. (2000) fed CS of CLA to finishing steers, with diets containing 0, 1 and 2.5% of CLA (DM basis) and reported decreased feed intake and BW gain as the intake of CLA increased. Additionally, increased CLA concentrations in adipose and lean tissue were suggested. In contrast, Gillis et al. (2004) reported an increased ADG when finishing steers were supplemented with CS of CLA (2% of dietary DM) when compared to steers fed corn oil (4% of dietary DM) or no oil supplement. Haddad & Younis (2004) also observed decreased DMI when Awassi lambs were supplemented with CS of CLA added at 2.5 and 5% of total dietary DM. Park et al. (1997) fed CLA to mice at 0 or 0.5% of total dietary DM in 2 experiments and no differences in DMI and ADG were reported between CLA-fed group and the control group, although the concentration of CLA was 2.5 times greater in the body of mice fed CLA, indicating that greater dietary CLA increases CLA content in body tissues.

Cholesterol is a precursor of several reproductive hormones such as steroids and prostaglandins (Mattos et al., 2000). Dietary fat supplementation in cows consistently increases plasma cholesterol concentration (Grummer & Carroll, 1991) and the greater availability of cholesterol can result in increased secretion of progesterone (Staples et al., 1998). Oldick el al. (1997) reported an increased plasma concentration of non-esterified FA, triglyceride and cholesterol when ruminally cannulated dairy cows received abomasal infusion of tallow or yellow grease (0.45 kg/d) compared to cows which received an infusion of glucose (1 kg/d). Lloyd et al. (2002) suggested increased serum cholesterol and triglyceride concentrations when CSFA (Megalac®; 0.113g/heifer daily) were supplemented to pubertal Angus heifers prior to breeding. These data agree with Lucy et al. (1991), who suggested increased plasma concentrations of cholesterol and basal LH in early postpartum dairy cows receiving CSFA (Megalac®).

Dietary supplementation of LNA reduced the in vitro synthesis of prostaglandin (**PG**) $F_{2\alpha}$, one important eicosanoid responsible for luteolysis. Feeding fish meal (Mattos et al., 2004) or abomasally infusing a fat source rich in LA (Oldick et al., 1997) resulted in an attenuation of the induced PGFM (a measurable metabolite of $PGF_{2\alpha}$) response in peripheral plasma compared with control animals. These results indicate that high concentrations of PUFA can decrease the endometrial secretion of prostaglandins resulting in greater chances of pregnancy establishment (Thatcher et al., 1997). Conclusively, feeding fats and targeting of FA to reproductive tissues may be a potential strategy to integrate nutritional management to improve animal productivity (Santos et al., 2008).

Among these multiple biological functions, dietary PUFA also appear to impact multiple immunological functions. Lessard et al. (2003) indentified that blood concentration of PGE_2 was reduced in cows fed flaxseed compared with those fed CS of soybean oil or micronized soybean, while progesterone concentrations were increased in cows fed flaxseed compared with those fed CSFA during the breed period. Essential FA, such as LA and LNA, may modulate immune reactions and inflammatory responses, by influencing biological membrane fluidity (Schmitz & Ecker, 2008), and activating cellular communication by stimulating eicosanoids biosynthesis (Calder & Grimble, 2002; Yaqoob, 2004).

Immune Function

The specific immune response can be classified into two types, humoral and cell-mediated. Humoral immunity is mediated by antibodies that are released by B-lymphocytes into the bloodstream and are responsible for specific recognition and elimination of antigens. Cell-mediated immunity involves specific antigen recognition by T-lymphocytes Miles & Calder, 1998). According to Calder et al. (1996) and Miles & Calder (1998) the leucocytes are the principal group of cells of the immune system, consisting of: B-lymphocytes, T-lymphocytes,

dendritic cells, natural killer cells, mononuclear phagocytes (including monocytes and macrophages), and granulocytes (including neutrophils, eosinophils and basophils)

The Acute-Phase Reaction

The acute-phase reaction involves an organism's response to disturbances of its homeostasis due to infection, tissue injury, neoplastic growth, or immunological disorders (Heinrich et al., 1990). It consist of an early and local reaction at the site of injury characterized by an accumulation and activation of leukocytes, mainly granulocytes and mononuclear cells, fibroblasts and endothelial cells, which in turn release acute-phase glycoprotein mediators called cytokines. The acute-phase reaction may be beneficial to the injured organism with the objective of restoring the disturbed homeostasis (Pepys & Balyz, 1983).

Cytokines act on specific receptors in several organs and tissues, leading to a systemic reaction characterized mainly by fever, leukocytosis, increase of adrenocorticotropic hormone and glucocorticoids secretion, activation of the clotting cascade, and dramatic changes in the plasma concentration of some liver-derived proteins called APP (Heinrich, 1990). Macrophages and monocytes secrete three cytokines that have a profound metabolic effect on the organism: interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Because macrophages and monocytes represent the first line of defense in the immune system, collectively these three immune mediators are recognized as pro-inflammatory cytokines (Johnson, 1997). Tumor necrosis factor-α is the first cytokine to be released in response to bacterial endotoxin, followed by IL-1 and IL-6, and TNF-α also stimulates the production of IL-1 and IL-6 (Peck, 1994).

The analysis of plasma concentrations for APP is commonly used as a sensitive indicator of inflammation in mammals (Breazile, 1996). In addition to their secondary response to an activated immune system, the APP also have other vital functions in the organism, specific of

each protein. Ceruloplasmin (Cp), also known as ferroxidase, is an enzyme containing 6 atoms of carbon in its structure. It is the major copper-carrying protein in the blood and participates in iron homeostasis. Concentrations of Cp may increase due to inflammation. Haptoglobin (Hp) is responsible to bind free hemoglobin in the blood, forming the Hp-hemoglobin complex. Also, it prevents the loss of body iron and its concentrations are normally undetectable in bovine blood unless there is tissue damage. Fibrinogen (Fb) is involved in the clotting cascade and in the formation of the fibrin matrix for tissue repair. Increased Fb concentrations are detected during internal hemorrhage or tissue damage.

According to Johnson (1997), the proinflammatory cytokines are large, hydrophilic molecules (17 to 26 kDa) and therefore incapable of crossing the blood-brain-barrier. The mechanism by which cytokines communicate with the brain is through accessing circumventricular organs devoid of a blood-brain-barrier, following production of secondary signals, such as prostaglandins. Receptors for IL-1 have been observed in the hippocampus and choroid plexus (Farrar et al., 1991) and binding sites for TNF-α have been identified also in the brainstem, cortex, cerebellum, thalamus, and basal ganglia of rats' brain (Kinouchi et al., 1991).

In cattle, the APP reaction following transport has been characterized by Arthington et al. (2003). The magnitude of this response may be a key indicator of subsequent productivity in the feedlot, especially during the initial receiving period (Qiu et al., 2007; Arthington et al., 2005). Arthington et al. (2005) observed increased plasma concentrations of Cp and Hp in steers following 24 hours of transportation, and the concentration of these APP (Cp and Hp) was greater in calves weaned normally were compared with calves which were weaned 211 d prior to transport. In another experiment, Arthington et al. (2008) evaluated performance and the acute-phase reaction following transportation of steers submitted to 4 weaning management strategies.

During the 29-d receiving period, plasma Cp concentrations were decreased for the pre-weaned group when compared to control or creep-fed steers. Also, ADG, DMI, and feed efficiency were greater for pre-weaned steers compared with control steers. These data are supported by Dhuyvetter et al. (2005), who indicated that preconditioning of calves before marketing helped to avoid depression of performance and health upon feedlot arrival. Weaning and diet management prior to shipment can become an important practice to decrease morbidity and mortality rates of transported beef cattle.

Physiological Stress and Growth

In terms of the marketing process, weaning is likely the greatest stress imposed to cattle, and according to Loerch & Fluharty (1999) many other factors are able to cause stress in calves. For example, weaning breaks the bond between dam and calf and causes prolonged vocalization. Marketing and transportation causes depravation of water and feed, which is a common occurance in cattle shipments from Florida to other states. Weather changes, over-crowding, unexpected and loud noises, poor air quality, and poor sanitization are extra stressors often experienced by the calves during the weaning and transport process. Upon arrival at the feedlot, calves are exposed to new diets and unfamiliar feeders and waterers. In the new feedlot environment, calves also may have to acclimate to a new social dominance and new pathogens. Processing procedures, such as vaccination, castration, commingling and dehorning are stressors commonly found in feedlot facilities.

The effects of weaning and transportation on blood serum components have been studied by several groups. Large increases in blood concentrations of cortisol, epinephrine, and norepinephrine have been observed in steers after weaning and transportation (Cole et al., 1988; Lefcourt & Elsasser, 1995), although corticosteroids were most responsive to transport stress.

Agnes (1990) compared isolated metabolic effects of stress caused by transport, loading and noise; all three caused rapid increases in cortisol.

Growth is a genetically programmed sequence of events in the young animal (Klasing & Korver, 1997), and when this sequence is disrupted by stress, several physiological changes contribute to favor a process of reallocation of animal resources important in survival. Conceptually, the status of the immune system (immunosuppression versus immunopotentiation) will depend upon the net effect of these changes (Khansari et al. 1990). Domestic food animals with clinical and subclinical infections eat less, grow slower, and convert feed to body tissues and products in an inefficient manner (Johnson, 1998), suggesting that a suppressed immune status can interact with the central nervous system and modulate feed intake. Summarily, the immune system is able to use cytokines to delivery information to other systems, including the central nervous system, regarding the level of immunologic activity.

Selye (1976) stated that biological stress is the non-specific response of the body to any demand, and when animals are exposed to stress, they react in a three-step process named "General Adaptative Syndrome." The first answer to this syndrome is called "Alarm Reaction" and it is characterized by vocalization, hypothalamic-pituitary-adrenal axis response, and catabolism. The second stage is called "Resistance" which is characterized by anabolism and increased feed intake. When resistance is not successful, the animals start the third and last step of the syndrome called "Exhaustion." In this stage the adaptative capability of the animal is limited, resulting in exhaustion before it adapts to the stressor. Survival and/or recovery of stressed animals are dependent on the level of stress they were exposed to prior, during, and after the marketing process.

Changes in the ruminal environment are normally observed in cattle after a long period of water and feed depravation. Cole & Hutcheson (1985) reported that fermentative capacity of ruminal micorbes was reduced by 75% following a 48-h period without feed as well as total reduction in bacterial numbers by 10 to 25% of baseline (Baldwin, 1967). Animals returned to control levels by 7 d after re-alimentation. According to Loerch & Fluharty (1999), DMI, ruminal volume, and weight of ruminal contents decreased as duration of feed and water absence increased; however, 4 d after arrival, there were no longer differences in DMI or weight of ruminal contents. These results may indicate that fermentative and digestive capacities play a minor role in low feed intakes during the first two weeks after arrival into the feedlot. Probably, the most important impact of stress on feed intake is due to its negative effects on animal immunocompetency.

The inflammatory response is marked by accelerated muscle degradation and increased hepatic APP synthesis. At least 60% of the amino acids liberated by body protein degradation are utilized by the liver as fuel for production of APP (Johnson, 1997). The cytokines IL-1 and TNF-α induce production of liver APP in vivo, and IL-6 induces in vitro (Richards et al., 1991). Muscle degradation is mediated by IL-1, IL-6, and TNF-α (Spurlock, 1997), while IL-1 has been shown to inhibit the anabolic effects of insulin on skeletal muscle (Klasing & Johnstone, 1991). Increased plasma concentration of nonesterified FA and hyperglyceridemia is associated with a variety of infections (Grunfeld et al., 1989). Tumor necrosis factor-α modulates lipid metabolism by increasing hepatic FA synthesis, inhibiting lipase activity, and stimulating lipolysis (Memon et al., 1994). High cortisol and plasma urea nitrogen concentrations have been observed in pigs exposed to environments that provide high immunological challenges (Williams, 1993).

Ban et al. (1992) proposed a neural link between the periphery and CNS. In this association, peripheral cytokines are able to stimulate vagal afferent nerves in the viscera, avoiding the necessity of an elevated concentration of peripheral cytokines to activate afferent nerves which lead to cytokine synthesis within direct CNS involvement. It is concluded, therefore, that inflammatory stimuli in the periphery induces synthesis of pro-inflammatory cytokines in the brain. Peripheral and central injection of recombinant IL-1 induced anorexia as well as a number of profound behavioral alterations that are reminiscent of sickness in rats (Dantzer & Kelley, 1989). This reduction in food intake is attributed to a reduction in both meal size and meal duration. Peripheral intra-venous and intra-parenteral administration of recombinant human TNF-α in rodents suppressed food intake and appetite (Johnson, 1998). Sonti et al. (1996) reported synergistic effects of IL-1 and TNF-α in inducing anorexia in rats when these cytokines were administered intravenous or intra-cerebroventricular.

It is known that pro-inflammatory cytokines suppress appetite and feed intake by acting directly on the CNS, although the same cytokines can regulate appetite in immune-challenged animals indirectly. Tumor necrosis factor-α stimulated leptin secretion by acting directly on adipocytes (Finck et al., 1998). Both peripheral and central injection of leptin reduced food intake, increased energy expenditure, and deplete adipose tissue in lean mice (Hallas et al., 1995). Interleukin-1 induces a decrease in the plasma concentrations of thyroxin and an even larger decrease in triidothyronine concentrations (Klasing & Korver, 1997), and in combination with TNF-α, both cytokines reduced concentrations of growth hormone (Elsasser et al., 1988) and insulin-like growth factor 1(IGF-1) in the circulation, liver, skeletal muscle, and pituitary (Klasing & Kover, 1997), which presumably contributes to impaired growth. In addition, the release of growth hormone-releasing hormone from medial basal hypothalamic explants was

decreased by IL-1, whereas somatostatin release wass increased (Spurlock, 1997). In conclusion, pro-inflammatory cytokines can act on a number of targets that are likely to contribute to poor intake and growth of immunologically challenged animals (Klasing, 1988). The immunological stress is characterized by the direct and indirect impacts of cytokines on CNS and other organ systems (Johnson, 1997).

Supplemental Fat, Stress and Growth

Eicosanoids are a group of chemical messengers synthesized from 3 types of 20-carbon chain PUFA: dihomo-γ-linolenic acid (DGLA; 20:3n-6), arachidonic acid (ARA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3). Eicosanoids include mainly PG, thromboxanes (TX), leukotrienes (LT), and other inflammatory mediators. The PUFA precursors for eicosanoid synthesis are stored in an esterified form in cell and organelle membrane phospholipids or in cytoplasmatic lipid bodies bound to glycerides and phospholipids at the cytosolic surface (Schmitz & Elker, 2008). They are mobilized and re-hydrolized usually by the action of phospholipase A_2 activation in response to a cellular stimulus (Calder et al., 2002). Because the majority of cell membranes contain predominately ARA compared to EPA and DGLA, ARA is the key precursor for eicosanoid biosynthesis.

Linoleic acid is converted to γ-linolenic acid (GLNA; 18:3n-6) by action of delta-6-desaturase. A specific enzyme called elongase 5 converts GLNA into DGLA which is converted to the key intermediate ARA by action of delta-5-desaturase. Arachidonic acid is further metabolized to docosapentaenoic acid (DPA; 20:5n-6) and eicosanoids. The n-3 LNA is converted to stearidonic acid (18:4n-3) and to eicosatetraenoic acid (ETA; 20:4n-3) to form EPA using the same series of enzymes as those used to synthesize ARA. The EPA is further metabolized to docosahexaenoic acid (DHA, 22:6n-3) or eicosanoids.

The n-6 ARA can be converted to eicosanoids of the PG_2, TX_2, $LT_,$ derivates of HPETE and HETE, lipoxin A_4 by action of COX and lipo-oxygenases. In contrast, EPA is converted to PG_3, TX_3, and LT_5. Resolvins, docosatrienes, and neuroprotectins are synthesized from DHA. For example, PGE_2 and PGI_2 are pro-arrhythmic while PGE_3 and PGI_3 are anti-arrhythmic; TXA_2 is a platelet activator and TXA_3 is platelet inhibitor; TXB_2 causes vasoconstriction and the TXB_3 causes vasodilatation; LTB_4 is pro-inflammatory and HPETE and HETE are involved in inflammation processes, in contrast to LTB_5, resolvin E_1, resolvin D, and neuroprotectin D_1 which promote anti-inflammatory responses. According to Schmitz & Ecker (2008), the main difference between n-6 and n-3 FA-derived eicosanoids is that most of the mediators formed from ARA are pro-inflammatory whereas those formed from EPA and DHA are anti-inflammatory.

Several studies have investigated the effects of the amount and type of fat in the diet on immune reactions (Yaqoob & Calder, 1993; Jolly et al, 1997; Cullens, 2005), and the inclusion of PUFA into diets have been shown to modulate immune cell function (Calder et al., 2002). The mechanism by which FA might modulate immune functions is not yet understood, but apparently PUFA alter the production of mediators involved in communication between cells of the immune system through the synthesis of eicosanoids, cytokines and nitric oxide (Miles & Calder, 1998), and to alter the expression of adhesion molecules which are involved in direct cell-to-cell contact (Calder, 1999).

Farran et al. (2008) fed flaxseed, rolled full-fat soybeans and tallow at 13, 20 and 4% of dietary DM to beef heifers, respectively. Heifers supplemented with flaxseed and full-fat soybeans showed greater ADG than heifers fed tallow. Also, the flaxseed-fed heifers had increased plasma concentrations of total n-3 PUFA, whereas, full fat-soybean-fed heifers had

increased plasma concentrations of total n-6 PUFA. In another experiment, the same authors fed flaxseed (12.9% of dietary DM), rolled full-fat soybeans, and tallow (both at 20% of dietary DM) to lipopolysaccharide-challenged (LPS) heifers. After LPS challenge, rectal temperatures were less for flaxseed- and soybean-fed heifers than those fed for tallow; and concentrations of plasma TNF was greater in heifers supplemented with soybean than tallow. In agreement, Chang et al. (1992) observed increased serum TNF for LPS-challenged mice fed n-3-enriched fish oil compared with those fed corn, coconut oil, or a low-fat diet. Pomposelli et al. (1989) reported that diets containing fish oil reduced fever response in guinea pigs.

Flaxseed is rich in LNA while fish oil is abundant in EPA and DHA, all members of the n-3 FA family. Because many studies have demonstrated the anti-inflammatory effects of n-3 FA, these results may explain the decreased fever and serum concentrations of TNF. Calder et al. (2002) stated that DHA and LNA can be converted to EPA in animal cells. Additionally, EPA, by virtue of its ability to compete with ARA receptors, can competitively inhibit production of eicosanoids such as the 2-series PG and 4-series LT from ARA, thereby reducing inflammation (Calder, 1999).

Silvestre (2009) observed a lesser n-6 to n-3 FA ratio in neutrophils of dairy cows fed CS of fat-enriched fish oil compared with those fed CS of palm oil. In addition, mean concentration of TNF-α in supernatants of isolated neutrophils were lower for cows fed CS of fish oil. Further, the neutrophil concentration of EPA, DPA and DHA was greater in cows fed CS of fish oil than cows fed CS of palm oil. Do Amaral (2008) reported greater plasma concentrations of acid soluble proteins after calving when Holstein cows were fed CS of *trans* C18:1 (55% C18:1 trans) starting five wk prepartum than cows fed sunflower oil (80% C18:1 *cis*). In another experiment using PUFA supplementation prior to calving in heifers and mature cows, the same author

observed an effect of fat source and parity on plasma concentration of APP. Plasma concentration of Cp were greater in heifers fed n-6 FA (CS of sunflower oil) when compared with heifers fed n-3 FA (CS of palm and fish oils), although heifers fed n-3 FA had greater plasma concentrations of Fb compared to heifers fed n-6 FA. Cows and heifers fed n-3 FA from linseed-oil had lower concentrations of blood neutrophils than these fed n-6 FA from Megalac®-R (Do Amaral, 2008).

Lower postpartum Fb concentrations were reported by Cullens (2005) when primiparous lactating cows were fed CSFA (Megalac®-R; 2% of dietary DM) during the prepartum period compared to those not fed fat. In the same experiment, multiparous cows fed CSFA had greater plasma PGFM concentrations at approximately d 5, 6, and 7 postpartum compared with those not fed fat prepartum. Juchem et al. (2008) sugestedd greater plasma concentrations of PGFM at d 1 postpartum when primiparous cows where fed prepartum with CS of fat enriched in LA and trans-octadecenoic acids. This response was associated with a lesser incidence of uterine infection compared to cows fed CS of palm oil. Polyunsaturated FA, such as EPA and DHA, inhibited production of IL-1β and TNF-α by human monocytes (Purasiri et al., 1994). These results agree with Farran et al. (2008) and Caughey et al. (1996), who demonstrated that diets enriched with flaxseed and fish oil inhibited IL-1 and TNF-α production by monocytes in cattle.

Palm and coconut oils provide mainly saturated FA (SFA). Megalac®-R, sunflower oil, and soybean oil are sources of n-6 FA. Opposite to effects of the n-3 FA, n-6 FA are associated with pro-inflammatory and inflammatory responses, observed by the stimulation of cytokines and other physiological mediators, such as PGE_2 and LTB_4. Greater concentrations of blood neutrophil in n-6 FA-fed cows suggested by Do Amaral (2008) may indicate that n-6 FA stimulate neutrophil activity during the peripartum period, which may explain the lesser post-

partum uterine infection and greater serum concentrations of PGFM observed by Juchem (2008) in primiparous cows fed CS of fats enriched in *trans* C18:1 and LA.

Conclusively, dietary FA participate in immunomodulatory effects in mammals, and the role of lipids in immunity is focused on PUFA, especially the n-3 and n-6 families. The nature of the difference between these two families on immunomodulation is not certain, although speculations about changes in lipid sources and their interactions on physiological responses of immune-challenged animals have been made.

CHAPTER 3
EFFECTS OF MEGALAC®-R INCLUSION IN RECEIVING DIETS OF WEANED FEEDER STEERS

Materials and Methods

This experiment was conducted from July to September 2006, and was divided into a pre-shipping (d -40 to 0) and a post-shipping phase (d 1 to 30). The pre-shipping phase was conducted at the University of Florida – IFAS, Range Cattle Research and Education Center, Ona, and the post-shipping phase at the University of Florida – IFAS, North Florida Research and Education Center, Marianna.

The animals utilized in these experiments were cared for in accordance with acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals and Facilities

Sixty-four weaned Braford steers (BW ± SD = 218 ± 23 kg; age ± SD = 226 ± 27 d) were utilized in this experiment. For the pre-shipping phase (d -40), steers were stratified by initial BW and age, and randomly allocated to two bahiagrass (*Paspalum notatum*) pastures (32 steers/pasture) of 2.04 ha. Each pasture was randomly assigned to one of the two following supplementation treatments: 1) No fat control (CO) or 2) Saturated Fat (EN).

On d 0, all steers were loaded into a commercial livestock trailer and transported 1,600 km from Ona, FL to a research feedlot facility in Marianna, FL. Steers remained in the truck for 24 h, before being received into the feedlot. Upon arrival, steers were stratified by pre-shipping treatment and current BW, received electronic ear ID tags (Allflex USA, Inc., Dallas-Ft. Worth, TX) for the measurement of individual feed intake with the GrowSafe® System (Model 4000 E, GrowSafe® Systems Ltd., Airdrie, AB, Canada), and re-allocated into three feedlot pens (104.6 m^2) during the first 7 d. Each pen was concrete floor, covered, and provided of two feed bunks

and one waterer. Animals were then randomly assigned, in a 2 x 3 factorial arrangement, to one of three supplemental treatments: 1) CO, 2) EN, or 3) Megalac®-R supplementation (MG). On d 8, three more feedlot pens were added (two pens/treatment; eleven animals/pen).

Diets

Pasture quality during the pre-shipping phase was estimated to be 54.0% TDN and 9.6% CP (DM basis) from hand-plucked samples collected at the beginning of the trial and analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Samples were taken at 30 locations per pasture according to procedures determinated by Vendramini et al. (2006). The pastures utilized in this experiment were not fertilized prior to or during the experimental period.

Treatments consisted of two grain-based supplements (Tables 3-1 and 3-2) with (EN) or without (CO) the inclusion of a prilled saturated fat source (Energy Booster 100®; MSC Co, Carpentersville, IL). Supplement intake was limited (4.1 kg/d) with the EN providing 5.91% of dietary fat/steer daily (DM basis).

For the post-shipping phase, diets were prepared and fed as a TMR in *ad libitum* amounts daily, except the first four days when steers were offered daily with 5 kg of Tifton 85 bermudagrass (*Cynodon dactylon*) hay (as-fed) and a 70:30 mixture of concentrate:cottonseed hulls, separately.

Following this initial period, steers were offered in *ad libitum* amounts a 60:25:15 mixture of concentrate:cottonseed hulls:bermudagrass hay from d 5 to 12, and followed by a 65:28:7 mixture of the same ingredients in *ad libitum* amounts from d 13 to the end of the experiment (d 30). The EN and CO concentrate ingredients were similar to the pre-shipping phase, whereas the MG treatment consisted of a grain-based supplement with the inclusion of a source rumen-inert PUFA (Megalac®-R; Tables 3-1, 3-2) providing approximately 5.0% of

dietary fat/steer daily (DM basis; Table 3-3). Water was offered *ad libitum* throughout all phases of the experiments.

Sampling

During the pre-shipping phase, steer shrunk BW (16 h of feed restriction) was recorded on d -40, whereas full BW was recorded on d -25, -11, and immediately prior to shipping on d 0. During the post-shipping phase, steer shrunk BW was recorded on d 1 (immediately following arrival at feedlot facility) and at the end of the experiment (d 30). Full BW was recorded on d 4, 8, 15, 22 and 29. Only shrunk BW values were utilized to determine ADG during the pre-shipping and post-shipping phases.

Individual feed intake was recorded daily during the post-shipping phase using the GrowSafe® feed intake system. The GrowSafe® system is a technology which continuously measures individual feed consumption.

Representative samples of all feedstuffs were obtained weekly during each phase. Samples were dried after collection for 96 h at 60^{o}C in a forced air oven to calculate concentration of DM. The weekly samples were ground through a 1-mm Wiley mill screen (Model 4, A. H. Thomas, Philadelphia, PA) to be analyzed for nutrient composition according to analytical procedures of a commercial feed laboratory (Dairy One Forage Laboratory, Ithaca, NY).

Following transport, blood samples were collected on d 0, 1, 4, 8, 15, 22, and 29 for determination of fibrinogen and ceruloplasmin concentrations. Plasma samples used for determination of fatty acid composition were collected on d 0 and 29.

Blood analysis

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin. Samples were placed immediately on ice and centrifuged at 2,000 × *g* at 5^{o}C for 30 min (GPR Centrifuge, Model 349702; Beckman Instruments Inc., Fullerton, CA) for plasma separation and collection. Plasma was frozen at -20°C on the same day of collection.

A Coagulation Analyzer (Fibrometer, Rankin Biomedical Corp., Holly, MI) was used to determine plasma fibrinogen concentration from a standard curve using a human reference (Sigma procedure No. 880; Sigma Diagnostics, St. Louis, MO). 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 described by Demetriou et al. (1974). Ceruloplasmin concentrations were expressed as mg/dL, as described by King (1965). The intra and interassay CV were 4.8 and 6.4% for fibrinogen and 3.2 and 6.7 for ceruloplasmin, respectively.

Plasma fatty acid extraction and methylation were determined using procedures described by Kramer et al. (1997). The fatty acid methyl esters were determined using a gas-liquid chromatography (GLC; CR-3800 Gas Chromatograph, Varian, Inc. Corparate Headquaters, Palo Alto, CA) equipped with auto-sampler (Varian CP-8400), flame ionization detector, and Varian capillary column (CP-Sil 88, 100 m x 0.25 mm x 0.2 µm). The peak was indentified and calculated based on the retention time and peak area of known standards.

Statistical analysis

Performance data from the pre-shipping phase were not statistically analyzed and are reported as mean ± SD because steers were allocated to a single pasture per treatment and supplements were group-fed, therefore the experimental unit (pasture) was not replicated.

For the post-shipping phase, performance and physiological data were analyzed using the PROC MIXED procedure of SAS (SAS, 2001).

The statistical model used for plasma measurements and DMI was:

$$Y_{ijkl} = \mu + TRTA_i + TRTB_j + CALF_{k(ij)} + D_l + TRTAD_{ik} + TRTBD_{jk} + TRTABD_{ijk} + + E_{ijkl}$$

where

Y = response variable

μ = mean

TRTA = fixed effect of pre-shipping treatment

TRTB = fixed effect of post-shipping treatment

CALF = random effect of steer within pre- x post-shipping treatment

D = fixed effect of day

TRTAD = effect due to interaction of pre-shipping treatment and day

TRTBD = effect due to interaction of post-shipping treatment and day

TRTABD = effect due to interaction between pre- and post-shipping treatment and d

E = residual error

The statistical model used for ADG analysis was:

$$Y_{ij} = \mu + TRTA_i + TRTB_j + CALF_{k(i)} + E_{ij}$$

where

Y = response variable

μ = mean

TRTA = fixed effect of pre-shipping treatment

TRTB = fixed effect of post-shipping treatment

CALF = random effect of steer within pre- x post-shipping treatment

E = residual error

Plasma measurements were analyzed using values from d 0 as a covariate. Results are reported as least square means. Means were separated using LSD. Significance was determined at $P \leq 0.05$ and tendencies include $P > 0.05$ and ≤ 0.10. Only significant interactions were reported. Pearson correlation coefficients among plasma measurements, ADG and DMI were generated using the CORR procedure of SAS (SAS, 2001).

Results

Measurements of Performance

The ADG observed during the pre-shipping phase of the study was 0.35 ± 0.18 kg/d for EN-fed steers and 0.34 ± 0.20 kg/d for CO-fed steers. No pre-shipping or pre-shipping x post-shipping treatment effects were detected, therefore, all results reported herein are derived from the post-shipping treatments in the post-shipping phase of the study.

Steers fed MG had decreased ($P < 0.05$) ADG when compared with CO-, but not EN-, fed steers (1.04, 0.98, and 0.80 kg/d for CO, EN, and MG treatments, respectively; SEM = 0.08). Treatment x day interactions was observed ($P < 0.01$) for DMI (Figure 3-1). Steers fed MG had less overall DMI compared to CO-fed steers ($P < 0.01$), but not compared to EN-fed steers (2.37, 2.80, and 2.55 % of BW for MG, CO, and EN, respectively; SEM = 0.10). Steers fed MG had decreased ($P < 0.05$) G:F when compared to EN-fed steers, and tended ($P < 0.10$) to have less G:F compared to CO-fed steers (0.29, 0.37, and 0.35 kg/kg of G:F for MG, EN, and CO, respectively; SEM = 0.026).

Plasma Measurements and Coefficients of Correlation

No differences were observed among treatments for covariately adjusted mean plasma fibrinogen concentrations (331.0, 351.9, and 378.9 mg/dL for CO, EN, and MG, respectively; SEM = 27.0) and mean plasma ceruloplasmin concentrations (18.0, 17.7, and 18.9 mg/dL for CO, EN, and MG, respectively, SEM = 0.69). A day effect ($P < 0.01$) for fibrinogen and ceruloplasmin was observed (Figure 3-2 and 3-3).

Pearson correlation coefficients among ceruloplasmin, fibrinogen, DMI, and ADG are presented in Table 3-7. Significant positive correlations were observed between ceruloplasmin and fibrinogen concentrations ($P < 0.05$), and between ADG and DMI ($P < 0.01$). Fibrinogen and ceruloplasmin concentrations were both negatively correlated with ADG and DMI ($P < 0.05$).

Plasma FA concentrations from d 0 and 29 are presented in Table 3-6. Concentrations of C16:0, C17:0, C18:0, C18:1c9, SFA, MUFA, total n-6 and SFA/UFA were less ($P < 0.03$) for CO- compared with EN-fed steers on d 0 (feedlot entry). Also on d 0, steers supplemented with CO tended to have decreasedd ($P < 0.09$) concentrations of LNA, ARA and total FA when compared to EN-fed steers. On d 29, CO-fed steers had decreased ($P < 0.05$) plasma concentrations of C14:0, C16:0, C17:0, LA, SFA, PUFA, total n-6 and total FA than EN-fed steers.

When compared to MG-fed calves, concentrations on d 29 of C16:0, LA, LNA, PUFA, total n-3, total n-6 and total FA were less ($P < 0.04$) and tended to be less ($P < 0.08$) for DPA and SFA in CO-fed steers. In contrast CO-fed steers tended to have a greater ($P = 0.08$) SFA to UFA ratio than MG-fed steers. On d 29, MG-fed steers had less C14:0, C17:0 ($P = 0.05$) than EN-fed steers, and tended to have greater ($P < 0.09$) concentrations of EPA, total n-3 and n-3 to n-6 ratio.

Discussion

Performance

Growth can be disrupted by many factors including the pattern of DMI and immunological status (Baumann & Gauldie, 1994). Decreased DMI and BW gain as a result of supplemental fat has been reported previously in cattle (Pavan et al., 2007; Gibb et al., 2004; Harrison et al., 1995; Sklan et al., 1991; Beam & Buttler, 1998). Among the several types of fat sources used as supplements, the CSFA appears to have the greatest impact for inhibiting intake (Bateman et al., 1996; Simas et al., 1995; Ngidi et al., 1990), which might help describe the differences in ADG observed between the fat supplements evaluated in the current experiment. In the feedlot, MG-fed steers had decreased less overall ADG compared with CO- and EN-fed, and decreased less overall DMI when compared with CO-fed steers. In addition, MG-fed steers had reduced ($P < 0.08$) feed efficiency (G:F) compared with EN- and CO-fed steers. These results are in agreement with Allen (2000) who proposed that feed sources of PUFA, mainly CS (i.e. Megalac®-R), are more detrimental to intake than prilled fats or other sources of SFA.

Hess et al. (2008) stated that an optimal inclusion rate for supplemental fat should be less than 3% of dietary DM if the goal is to maximize the use of forage-based diets, and supplemental fat should be limited to less than 2% of dietary DM if the goal is to prevent substitution of forage with intake of supplemental fat. The maximum fat content observed in the CO diet was 3.6% (DM basis). In this experiment a TMR was utilized to feed the treatment supplement with hay and cottonseed hulls. Megalac®-R and EN contributed 1.4 to 1.7% fat to the final diets which contained a total fat content of 4.9 and 4.8%, respectively. According to Hess et al. (2008), these values indicate that the diets offered during this experiment were formulated to avoid potential negative effects on DMI and fiber digestibility. Further, there were no differences for DMI and ADG between EN- and CO-fed calves, suggesting that the dietary fat concentration in the diets

likely did not inhibit intake, but the type of fat utilized may have contributed to decreased DMI due to lower acceptability of CSFA provided by MG, than the prilled fat provided by EN.

According to Grummer et al. (1990), Energy Booster 100® is a source of prilled long-chain SFA, which is more acceptable to dairy cows than CSFA (i.e. Megalac®-R). The author's results showed that the CSFA acceptability was less than other commercial fat sources even if CSFA had been offered alone, in a top-dressing, or mixed (TMR) into other dietary components. Zinn (1988) and Zinn (1989) recommended that fat should be introduced into diets gradually, facilitating the animal's adaption for the new feed ingredient.

This experiment was developed as a 2 x 3 factorial design. During the pre-shipping phase, steers received EN or no-fat supplement (CO) for a period of 40 d prior to transport. In the feedlot, steers allocated to the MG treatment received this fat source for the first time. The absence of gradual adaptation in addition to the lower acceptability of MG, may contribute to the reduced performance measures observed in MG-fed steers compared to EN- and CO-fed steers.

Unsaturated long chain FAs are potentially more detrimental on ruminal fermentation (Schauff & Clark, 1989) because UFA are more soluble, and therefore are more likely to adsorb onto bacteria (Chalupa et al., 1984). In other words, impaired ruminal fermentation is more likely with diets containing UFA compared SFA. Calcium salts of FA are classified as rumen-inert and may decrease BH rate of PUFA and may increase absorption of PUFA in the small intestine (Wu et al., 1991). Juchem (2007) demonstrated that more than 70% of the LA and more than 85% of the LNA fed to lactating cows were BH in the rumen when fed as unprotected oils or as CS of long chain FA.

According to the manufacturer, the SFA to PUFA ratio of MG is 0.4 and of EN is 1.2, which means that MG has a greater PUFA proportion content compared to EN. It is suggested,

therefore, that MG may more negatively affect ruminal fermentation. This negative effect of MG on ruminal fermentation might decrease microbial synthesis and then decrease the availability of amino acids for protein synthesis in other tissues (Palmquist and Moser, 1981), which might have affected the growth of MG-fed steers in the current experiment. This observation is in agreement with Palmquist (1994) who suggested that feeding more PUFA may negatively affect microbial production in the rumen. Additionally, Schauff & Clark (1989) have reported that prilled saturated fat appears to depress the acetate to propionate ratio compared to CSFA, and therefore, potentially improve animal performance.

Inflammatory Reaction

In response to immunological stress, the liver will produce the acute phase proteins Cp and Fb (Baumann & Gauldie, 1994). It is unclear whether increased concentrations of APP are due to greater stress (indicating an adverse state) or due to a greater immune response (indicating a healthier state). In this study, the plasma concentration of Fb and Cp ranged from 63.5 to 922.9 mg/dL and 4.8 to 27.1 mg/dL, respectively. According to The Merck Veterinary Manual (1997), normal values of APP in cattle range from 100 to 600 mg/dL for Fb and 16.8 to 34.2 mg/dL for Cp. In this study no differences among treatments were observed for mean plasma Cp and covariately adjusted Fb concentrations during the first 29 d post-shipping.

Immune reactions have been reported to be modulated by the diet; including the PUFA composition of the diet (Yacoob & Calder, 1993; Miles & Calder, 1998; Pamposelli et al., 1989). The mechanisms involved in this regulation are not yet understood, but evidences exist that n-3 and n-6 FA composition in the diet may influence cellular activation through the synthesis of eicosanoids, steroid hormones, and cytokines (Calder et al., 2002). Several authors have reported immunological and physiological changes in animals provided diets containing PUFA during immune challenge (Calder et al., 2002; Farran et al., 2008; Cullens, 2005; Silvestre, 2009, Do

Amaral, 2008), but these studies mainly examined inflammatory processes caused by parturition or LPS-challenge, and not by transport. Lessard et al. (2004) evaluated the cellular immune function of dairy cows fed supplemental CS of palm oil, flaxseed and micronized soybeans from 6 wk pre-partum to 6 wk postpartum. The authors concluded that cellular immune function was modulated around parturition; however, feeding diets rich in n-3 or n-6 FA did not have a major impact on immune function. Cullens (2005) reported that mean plasma concentrations of Fb during the first 27 d postpartum tended to be greater for control cows than for cows fed CS of long-chain FA (Megalac®-R). In addition, the author suggested that the initiation of PUFA supplementation before parturition can affect the immune status and physiological response of mature cows after parturition. In these studies, PUFA supplementation commonly started at least 3 wk prior to immune challenge and continues at least 3 wk after. The authors suggest that a preliminary period of supplementation is necessary to observe the effects of PUFA on immunomodulation, and the duration of this supplementation period must be long enough to potentiate these effects. Continual feeding of CS of a mixture enriched with fish oil increased concentrations of EPA and DHA in endometrium, liver, mammary, muscle, subcutaneous and internal adipose tissues of dairy cows (Bilby et al., 2006), which may indicate that daily feeding CS of FA is a practical approach to manipulate tissue FA composition (Silvestre, 2009).

In the current experiment, the steers began MG consumption immediately after shipping. Because MG supplementation did not occur during the pre-shipping phase of the experiment, changes on immune responses by MG could not be expected during the first d after shipping. Further, steers provided EN in the pre-shipping phase experienced an APP reaction to shipping, which did not differ from CO-fed steers. Energy Booster 100® is a supplemental fat source that is rich in SFA and low in PUFA. In a review of research investigating effects of SFA compared

to PUFA on measures of immune function, it was concluded that SFA impacts immune competence to a lesser degree compared to PUFA (Miles & Calder, 1998). Similarly, Farran et al. (2008) observed no difference in concentrations of plasma TNF-α of LPS-challenged steers provided tallow (rich in SFA and MUFA) or no supplemental fat (control).

Ruminal BH can influence the amount of PUFA reaching the small intestine, although according to Juchem (2007), the continual feeding of PUFA, regardless the source, can increase the concentration of PUFA in cells and tissues despite BH. It is in agreement with Mattos et al. (2000) who stated that the amount of each FA incorporated into organs and tissues depend on the amount of precursors present in the diet. According to the manufacturer, EN contains 60% SFA and MUFA and 36% PUFA. Megalac®-R contains 55% PUFA, and LA and LNA represents 87% and 9% of this PUFA. It is suggested that feeding steers with EN or MG may affect concentration and composition of FA in the blood differently.

Total plasma FA concentration was increased after fat supplementation during pre- and post-shipping phases of the current experiment. On d 29, MG-fed steers tended to have increased EPA and DPA and total n-3 FA compared to EN-fed steers. Also, MG-fed steers had greater LNA and lesser SFA to UFA ratio compared to CO-fed steers. It is likely that MG increased plasma concentrations of n-3 FA such as LNA, EPA and DPA due to the greater amount of n-3 FA in this treatment. These results agree with Lessard et al. (2003) who observed no differences in plasma LA concentrations in dairy cows supplemented with Megalac® or flaxseed during the first 21 d of the feeding period, likely due to the similar amounts of LA provided by the Megalac® and flaxseed diets. However, greater plasma n-3 FA concentrations were observed in flaxseed-fed cows due to a greater concentration of LNA in this source of supplemental fat. In addition, Petit (2002) fed diets containing Megalac® and micronized

soybean to lactating dairy cows during 16 wk post partum. On d 70, there were no treatment differences in plasma concentrations of LNA and EPA; however, greater plasma n-6 FA concentrations were suggested in micronized soybean-fed cows because of greater concentration of LA in micronized soybeans compared to Megalac®.

A negative relationship among plasma APP concentrations and ADG and DMI was observed in this study. Similar findings have been reported in weaned, transported calves (Arthington et al., 2005). These results are supportive of the link between inflammatory processes and feed intake and performance (Johnson, 1998). Growing evidence linking dietary PUFA to immune function, especially modulation of inflammatory processes (Calder et al., 2002), strengthens the concept for using dietary fats to modulate the acute phase reaction and improve livestock performance. Further studies are required to better understand the effects of PUFA on inflammatory responses of transport-stressed beef steers.

Table 3-1. Ingredients and nutrient composition of grain-based concentrate treatments fed to steers during pre- and post-shipping phase of the study. [1]

	Treatments[2]		
	CO	EN	MG
Ingredients (% as-fed)			
Wheat middlings	30.63	29.97	29.85
Cracked corn	20.00	19.58	19.50
Ground corn	20.00	19.58	19.50
Cottonseed meal	11.85	11.60	11.55
Cottonseed hulls	10.00	9.80	9.75
Cane molasses	6.25	6.12	6.10
Calcium carbonate	1.25	1.22	1.22
Mineral and vitamin mix[3]	0.03	0.03	0.03
Energy Booster 100®	…	2.10	...
Megalac®-R	...	...	2.50
Nutrient (DM basis)			
NE_g, Mcal/kg	1.17	1.24	1.24
TDN, %	72.97	75.42	75.37
CP, %	17.31	16.94	16.87
NDF, %	26.26	25.72	25.60
EE, %	3.93	5.91	6.02
Ca, %	0.68	0.67	0.90
P, %	0.64	0.62	0.62

[1] Steers were provided supplement daily (4.1 kg/steer) for a period of 40 d while grazing bahiagrass pastures (54.0 and 9.6% TDN and CP, respectively).

[2] CO = grain-based supplement non-fortified with a rumen-inert fat source; EN = grain-based supplement fortified with Energy Booster 100® (MSC Co, Carpentersville, IL), MG = grain based supplement fortified with Megalac®-R (Church & Dwight Co, Priceton, NJ).

[3] Cattle Select (Lakeland Animal Nutrition; Lakeland, FL); Ca (14%), P (9%), NaCl (64%), K (0.2%), Mg (0.3%), S (0.3%), Co (50 ppm), Cu (1,500 ppm), I (210 ppm), Mn (500 ppm), Se (40 ppm), Zn (3,000 ppm), F (800 ppm), Fe (800 ppm), Vitamin A (360,000 ppm).

Table 3-2. Fatty acid profile of supplemental fat sources used in the formulation of experimental diets (% of fatty acids).[1]

	Fat Supplements[2]	
	EN	MG
C12:0	0.1	0.1
C14:0	2.7	0.9
C16:0	41.5	36.3
C16:1	1.4	0.2
C18:0	38.7	3.9
cis-C18:1	12.6	26.7
trans-C18:1	0.1	01
C18:2	1.7	28.5
C18:3	0.1	3.0
Others[3]	1.1	0.3

[1] C12:0 = Lauric acid; C14:0 = Myristic acid; C16:0 = Palmitic Acid; C16:1 = Palmitoleic acid; C18:0 = Stearic Acid; *cis*-C18:1 = Oleic acid; *trans*-C18:1 = Vaccenic acid; C18:2 = Linoleic acid; and C18:3 = α-Linolenic acid.

[2] The fatty acid profile of the fat supplement was determined according to the manufacturer; EN = grain-based diet fortified with Energy Booster 100® (MSC Co, Carpentersville, IL); MG = Megalac®-R (Church & Dwight Co, Priceton, NJ).

[3] Others = Not detected.

Table 3-3. Nutrient composition of TMR fed to transport-stressed steers during the post-shipping phase of the study.

	Treatments[1]		
Nutrient (DM basis)[2]	CO	EN	MG
D 1 to 4[3]			
CP, %	14.1	13.9	13.9
NDF, %	58.6	58.4	58.4
EE, %	2.7	3.4	3.4
TDN, %	62.9	63.8	63.7
$NE_{g,}$, Mcal/kg	0.8	0.8	0.8
Ca, %	0.5	0.5	0.5
P, %	0.4	0.4	0.4
D 5 to 12[4]			
CP, %	14.8	14.6	14.5
NDF, %	46.5	46.2	46.1
EE, %	3.4	4.6	4.7
TDN, %	62.8	64.2	64.2
NE_g, Mcal/kg	0.8	0.9	0.9
Ca, %	0.6	0.5	0.7
P, %	0.5	0.5	0.5
D 13 to 29[5]			
CP, %	14.9	14.7	14.6
NDF, %	44.1	43.8	43.7
EE, %	3.6	4.8	4.9
TDN, %	62.5	64.1	64.1
NE_g, Mcal/kg	0.8	0.9	0.9
Ca, %	0.6	0.6	0.7
P, %	0.5	0.5	0.5

[1] CO = grain-based diet non-fortified with a rumen-inert fat source; EN = grain-based diet fortified with Energy Booster 100® (MSC Co, Carpentersville, IL); MG = grain-based diet fortified with Megalac®-R (Church & Dwight Co, Priceton, NJ).
[2] Except NEg which unit is Mcal/kg of DM.
[3] D 1 to 4 = free-choice of hay and 70:30 mixture of treatment concentrate:CSH[6].
[4] D 5 to 13 = free-choice of 60:25:15 mixture of treatment concentrate:CSH:hay.
[5] D 12 to 29 = free-choice of 65:28:7 mixture of treatment concentrate:CSH:hay.
[6] CSH = Cottonseed hulls.

Table 3-4. Effect of supplemental fat source on plasma fatty acid concentrations on d 0 and d 29 of the study[1].

	d 0		d 29			P-value[3]				SEM[4]	
Fatty acid[2]	CO	EN	CO	EN	MG	a	b	c	d	x	y
	----------mg/mL----------										
C14:0	0.0064	0.0073	0.0054	0.0067	0.0054	0.18	0.05	0.92	0.05	0.0006	0.0006
C16:0	0.0880	0.1050	0.1301	0.1611	0.1621	0.01	0.02	0.01	0.93	0.0064	0.0135
C16:1	0.0036	0.0035	0.0034	0.0035	0.0035	0.88	0.82	0.85	0.96	0.0003	0.0006
C17:0	0.0052	0.0061	0.0073	0.0089	0.0074	0.03	0.04	0.91	0.05	0.0004	0.0007
C18:0	0.1061	0.1367	0.1969	0.2344	0.2307	0.01	0.11	0.14	0.87	0.0100	0.0220
C18:1t	0.0080	0.0067	0.0087	0.0109	0.0113	0.11	0.29	0.21	0.85	0.0008	0.0021
C18:1c	0.0695	0.0849	0.0750	0.0850	0.0850	0.01	0.16	0.15	0.97	0.0054	0.0068
C18:2n-6	0.2226	0.2422	0.4422	0.5557	0.5656	0.45	0.04	0.03	0.85	0.0260	0.0550
C18:3n-3	0.0102	0.0130	0.0060	0.0073	0.0090	0.07	0.22	0.01	0.12	0.0010	0.0010
CLAc	0.0009	0.0009	0.0001	0.0001	0.0001	0.98	0.70	0.73	0.97	0.0001	0.0001
CLAt	0.0001	0.0002	0.0002	0.0002	0.0003	0.25	0.99	0.78	0.78	0.0001	0.0001
C20:4n-6	0.0143	0.0122	0.0177	0.0208	0.0183	0.07	0.20	0.79	0.29	0.0010	0.0020
C20:5n-3	0.0029	0.0033	0.0010	0.0010	0.0017	0.26	0.98	0.11	0.09	0.0004	0.0004
C22:5n-3	0.0048	0.0047	0.0019	0.0022	0.0036	0.70	0.74	0.08	0.15	0.0004	0.0010
C22:6n-3	0.0021	0.0018	0.0006	0.0005	0.0005	0.19	0.67	0.66	0.99	0.0001	0.0003
SFA[5]	0.2056	0.2547	0.3396	0.4110	0.4056	0.01	0.05	0.07	0.88	0.0200	0.0350
MUFA[6]	0.0810	0.0951	0.0870	0.0991	0.0997	0.02	0.15	0.12	0.94	0.0059	0.0080
PUFA[7]	0.2581	0.2783	0.4697	0.5879	0.5991	0.48	0.05	0.03	0.84	0.0280	0.0580
Total n-3[8]	0.0200	0.0230	0.0095	0.0110	0.0150	0.20	0.47	0.01	0.07	0.0022	0.0020
Total n-6[9]	0.2370	0.2540	0.4600	0.5800	0.5800	0.03	0.05	0.03	0.89	0.5100	0.0600
Total CLA[10]	0.0010	0.0010	0.0003	0.0003	0.0004	0.99	0.86	0.72	0.85	0.0001	0.0002
Total FA[11]	0.5447	0.6282	0.8963	1.0980	1.1044	0.09	0.05	0.04	0.94	0.0500	0.1000
Ratios											
SFA/UFA[12]	0.6185	0.7036	0.6240	0.6020	0.5840	0.01	0.33	0.08	0.44	0.0260	0.0225
n-3/n-6[13]	0.0870	0.0950	0.0200	0.0200	0.0250	0.18	0.83	0.13	0.07	0.0050	0.0030

[1] CO = grain-based diet non-fortified with a rumen-inert fat source. EN = grain-based diet fortified with Energy Booster® (MSC Co, Carpentersville, IL); MG = grain-based diet fortified with Megalac®-R (Church & Dwight Co, Priceton, NJ).

[2] C12:0 = Lauric acid; C14:0 = Myristic acid; C16:0 = Palmitic Acid; C16:1 = Palmitoleic acid; C18:0 = Stearic Acid; C18:1t = Vaccenic; C18:1c9 = Oleic acid; C18:2n-6 = Linoleic acid; C18:3n-3 = α-Linolenic acid; CLAc = *cis*-9, *trans*-11CLA; CLAt = CLA *trans*-10, *cis*-12; C20:4n-6 = Arachidonic acid; C20:5n-3 = eicosapentaenoic acid; C22:5n-3 = decosapentaenoic acid; C22:6 = docosahexaenoic acid.
[3] a = difference of LS means of CO x EN on d 0; b = difference of LS means of CO x EN on d 29; c = difference of LS means of CO x MG on d 29; d = difference of LS means of EN x MG on d 29.
[4] x = standard error of measurement on d 0; y = standard error of measurement on d 29.
[5] SFA = C14:0 + C16:0 + C17:0 + C18:0.
[6] MUFA = C16:1 + C18:1c + C18:1t.
[7] PUFA = C18:2n-6 + C18:3n-3 + CLAc + CLAt + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[8] Total n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[9] Total n-6 = C18:2n-6 + C20:4n-6.
[10] Total CLA = CLAc + CLAt.
[11] Total FA = Sum of all indentified fatty acids.
[12] SFA/UFA ratio = (C14:0 + C16:0 + C17:0 + C18:0) / (C16:1 + C18:1c + C18:1t + C18:2n-6 + C18:3n-3 + CLAc + CLAt + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3).
[13] n-3/n-6 ratio = (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C18:2n-6 + C20:4n-6).

Table 3-5. Correlations between plasma measurements, DMI and ADG of transport-stressed steers during post-shipping phase of the study.[1]

Item	Ceruloplasmin	Fibrinogen	ADG
Fibrinogen	0.25		
	0.05		
ADG	-0.26	-0.26	
	< 0.05	< 0.05	
DMI	-0.39	-0.24	0.76
	< 0.01	0.05	< 0.01

[1] Upper row = correlation coefficients; Lower row = *P*–values.

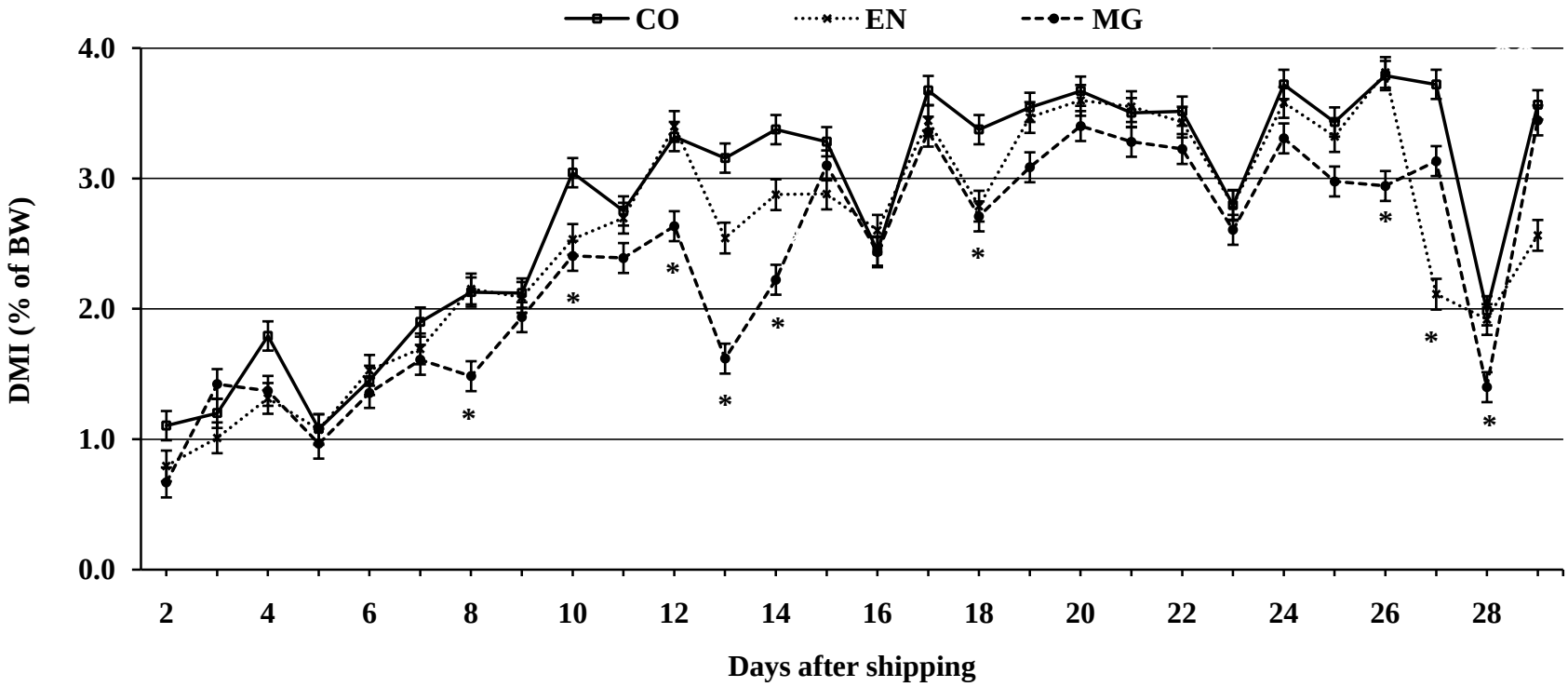

Figure 3-1. Least squares means of DMI of steers during the post-shipping phase of the study. Steers were fed diets containing Megalac®-R (MG), Energy Booster 100® (EN), or no supplemental fat (CO). The asterisks indicate when CO-fed steers had greater ($P \leq 0.05$) DMI compared to MG-fed steers (treatment x day interaction; $P \leq 0.05$). A day effect was observed ($P < 0.001$).

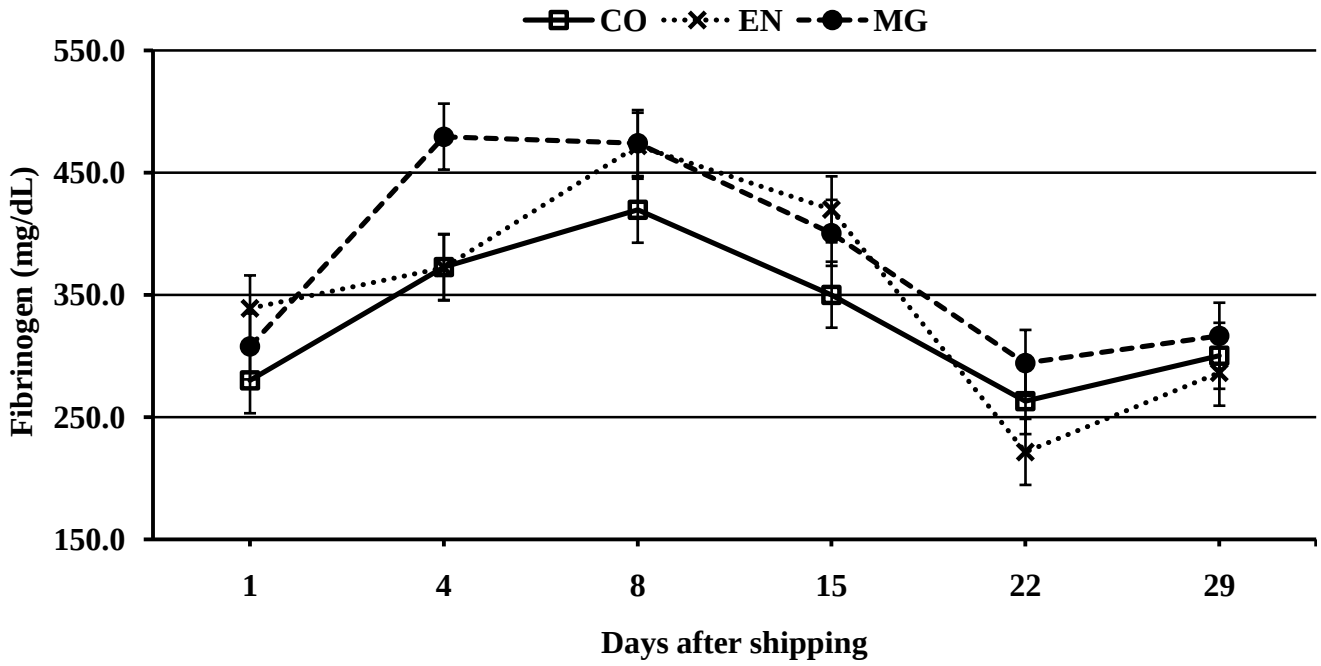

Figure 3-2. Least squares means of covariately adjusted plasma fibrinogen concentrations of steers during the post-shipping phase of the study. Steers were fed diets containing Megalac®-R (MG), Energy Booster 100® (EN), or no supplemental fat (CO). Steers arrived in the feedlot on d 1. A day effect was observed ($P < 0.01$).

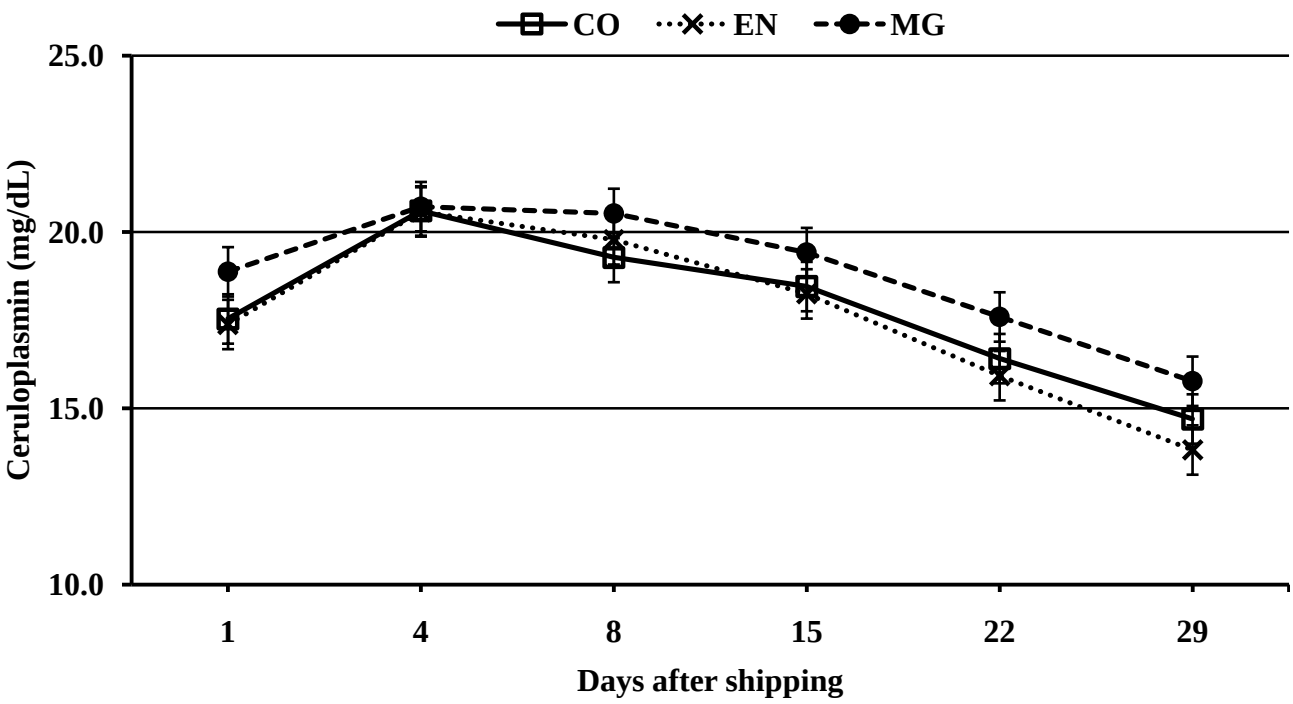

Figure 3-3. Least squares means of plasma ceruloplasmin concentrations of steers during the post-shipping phase of the study. Steers were fed diets containing Megalac®-R (MG), Energy Booster 100® (EN), or no supplemental fat (CO). Steers arrived in the feedlot on d 1. A day effect was observed ($P < 0.01$).

CHAPTER 4
EFFECTS OF MEGALAC®-R SUPPLEMENTATION ON MEASURES OF PERFORMANCE AND ACUTE-PHASE REACTION IN TRASPORTED BEEF HEIFERS

Material and Methods

This experiment was conducted from September to November 2007 at the University of Florida – IFAS, Range Cattle and Education Center, Ona, in 2 phases: pre-shipping (d -30 to 0), and post-shipping (d 1 to 29).

The animals utilized in this experiment were cared for in accordance with acceptable practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals and Facilities

Forty-eight Brahman-crossbred heifers (BW ± SD = 276 ± 32 kg; age ± SD = 330 ± 17 d) were utilized for this experiment. Prior to the start of the study, heifers were separated from dams and allowed to acclimate for a period of seven days from the stress of weaning. Following acclimation, heifers were stratified by BW and age, and randomly assigned to two supplementation treatments (pre-shipping-phase). Treatments consisted of a 1) grain-based supplement with Megalac®-R (MG), or 2) grain-based supplement without Megalac®-R (CO). Heifers were randomly allocated into six bahiagrass pastures (three pastures/treatment; eight heifers/pasture) and fed for a period of 30 days.

On d 0 the heifers were loaded onto a commercial livestock trailer and transported 1,600 km for a period of 24 h. Post-shipping, heifers were stratified by shrunk BW and assigned to one of the following two housing systems; 1) pasture-based system identical to the pre-shipping model (n = 24; three pastures / treatment; four heifers / pasture) or 2) individual feedlot facility (24 pens; twelve pens / treatment; one heifer / pen). Pre-shipping treatment allocations were continued in the 29-d post-shipping phase.

Pastures areas used during the pre- and the post-shipping phase were 1.07 ha in size. Individual pens used during the post-shipping phase had concrete floors and were 48 m^2 in size. Each pen contained a water source and two feed bunks (one for hay; one for concentrate).

Diets

Two grain-based supplements were utilized for this experiment, one with and one without the inclusion of Megalac-R® (150 g/heifer daily; Tables 4-1 and 4-2). Supplements were limit-fed at a rate of 3.0 and 2.5 kg of DM/heifer daily for CO and MG, respectively, during pre and post-shipping phases. Quality of bahiagrass pastures was determined to be 55.0% TDN and 8.7% CP (DM basis) from hand-plucked samples collected at the beginning of the trial and analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Samples were taken at 20 locations per pasture according to procedures determinated by Vendramini et al. (2006).

Pastures were not fertilized prior to or during the experimental period. Stargrass (*Cynodon nlemfuensis*) hay was offered to individually-fed heifers in the feedlot in amounts to ensure *ad libitum* consumption. Hay quality was calculated to be 53.5% TDN and 10.1% CP (DM basis) from hay samples collected weekly and analyzed by a commercial laboratory (Dairy One Forage Laboratory).

A complete commercial mineral and vitamin mix and water were offered *ad libitum* throughout both phases of the experiment (Table 4-3).

Sampling

During the pre-shipping phase, heifers shrunk BW (16 h of feed restriction) was recorded on d -30. In the post-shipping phase, heifers shrunk BW was recorded on d 1 (immediately following arrival at feeding facility) and at the end of the experiment (d 29). Full BW was recorded on d -15, 0, 4, 8, 15, 22, 28 in order to calculate DMI as a % of BW. Shrunk BW values were utilized to determine ADG during the pre- and post-shipping phases.

During the post-shipping phase, daily samples of offered and refused hay were collected, weighed, and analyzed for DM. Random representative samples of supplements ingredients were collected on a weekly basis for each phase of the trial for DM and nutrient determination.

Samples were dried in a 60^{o}C forced air oven for 96 h, ground through a 1-mm Wiley laboratory mill screen (Model 4, A. H. Thomas, Philadelphia, PA) and analyzed for nutrient composition according to analytical procedures of a commercial feed laboratory (Dairy One Forage Laboratory, Ithaca, NY).

Blood samples were collected from heifers on d 0, 1, 4, 8, 15, 22, and 28 and analyzed for plasma concentrations of ceruloplasmin, haptoglobin, and cortisol.

Blood Analysis

All blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, placed on ice immediately after sampling, and centrifuged (GPR Centrifuge, Model 349702; Beckman Instruments Inc., Fullerton, CA) at 2,000 x *g* for 30 min for plasma separation. The plasma of each sample was removed by transfer pipet to it respective vial and frozen at -20°C on the same day of collection.

A microplate spectrophotometer (PowerWave™ 340; BioTek Instruments, Inc., Winooski, VT) was used to determine plasma haptoglobin concentrations in duplicate samples by measuring haptoglobin/hemoglobin complexing (HpHbB) by the estimation of differences in peroxidase activity as described previously (Makimura and Suzuki, 1982). For haptoglobin concentrations ≤1.0 mg of HpHbB/100 mL, the intraassay CV was 12.6%, and for concentrations >1.0 mg of HpHbB/100 mL, the intraassay CV was controlled to values 7.1%. The interassay CV was controlled to values 8.9%.

A spectrophotometer (ThermoSpectronic™ Genesys™ 20; Thermo Fisher Scientific Inc., Waltham, MA) was used to determine plasma ceruloplasmin concentration. Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al. (1974). The intraassay and interassay CV were 4.6 and 8.8%, respectively. Ceruloplasmin concentrations were expressed as mg/dL, as described by King (1965).

Concentrations of plasma cortisol were determined using a Coat-A-Count Kit (DPC® Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I radioimmunoassay (RIA) via Gama Counter (Packard Cobra Auto Gamma 85005, GMI Inc., Ransey, MN). All samples were analyzed in duplicate with procedures previously validated for bovine samples (Eicher et al., 2000). Reference plasma samples containing 50 ng/mL of cortisol were analyzed for the calculation of intra- and interassay CV (5.5 and 6.4%, respectively). The minimum detectable concentration of cortisol was 5 ng/mL.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS, 2001).

The statistical model used for plasma measurements and DMI from individually-fed heifers was:

$$Y_{ijk} = \mu + TRT_i + D_j + TRTD_{ij} + HEIFER_{k(i)} + E_{ijk}$$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

D = fixed effect of day

TRTD = effect due to interaction of treatment and day

HEIFER = random effect of heifer within treatment

E = residual error

The statistical model used for ADG analysis for data obtained from individually-fed heifers was:

$Y_{ij} = \mu + TRT_i + HEIFER_{j(i)} + E_{ij}$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

HEIFER = random effect of heifer within treatment

E = residual error

The statistical model used for ADG analysis for data obtained from heifers on pasture during the pre-shipping phase was:

$Y_{ijk} = \mu + TRT_i + HEIFER_{j(k)} + PEN_{k(i)} + E_{ijk}$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

HEIFER = random effect of heifer within pen

PEN = random effect of pen within treatment

E = residual error

Results are reported as least square means. Means were separated using LSD. Significance was determined at $P \leq 0.05$ and tendencies include $P > 0.05$ and ≤ 0.10. Only

significant interactions were reported. Pearson correlation coefficients among plasma measurements, ADG, and DMI were generated using the CORR procedure of SAS (SAS, 2001).

Results

Measurements of Performance

No differences ($P = 0.99$) were reported in ADG between treatments in the pre-shipping phase (0.12 kg/d for both CO and MG treatments; SEM = 0.06). In addition, no post-shipping differences ($P \leq 0.30$) in ADG (0.78 and 0.66 kg/d for CO and MG treatments, respectively; SEM = 0.13) and G:F ratio (0.13 and 0.11 for CO and MG treatments, respectively; SEM = 0.01) among heifers maintained in the individual feeding facility were detected. No treatment effects ($P < 0.27$) or treatment x day interaction ($P < 0.77$) were detected for total DMI (2.01 and 1.92% of BW for CO and MG; SEM = 0.06; Figure 4-1) and voluntary hay DMI (1.03 and 1.10 % of BW for CO and MG, respectively; SEM = 0.04) of heifers following transportation. A day effect was observed ($P < 0.01$).

Plasma Measurements and Coefficients of Correlation

No treatment ($P \leq 0.54$) or treatment x day interactions ($P \leq 0.86$) for plasma ceruloplasmin (31.5 vs. 29.1 mg/dL for CO and MG, respectively; SEM = 1.40; Figure 4-2) and plasma cortisol (3.97 and 3.62 ug/mL for CO and MG, respectively; SEM = 0.39; Figure 4-3) concentrations were detected. A treatment ($P < 0.01$) effect was detected for plasma haptoglobin concentration (0.021 and 0.013 absorption at 450 nm x 100 for CO and MG, respectively; SEM = 0.002). Control-fed heifers had greater haptoglobin concentrations on d 1, 3 and 7 (Treatment x day interaction, $P < 0.002$; Figure 4-4) compared to MG-fed heifers. The effect of day was significant ($P < 0.01$) for all analyses completed. Pearson correlation coefficients among ceruloplasmin, haptoglobin, cortisol, DMI, and ADG are presented in Table 4-4. Significant positive ($P \leq 0.05$) correlations were detected between ceruloplasmin x cortisol, and haptoglobin

x DMI. The relationship between ADG x DMI tended ($P = 0.09$) to be positive. A negative ($P \leq 0.05$) correlation was observed between ceruloplasmin x ADG.

Discussion

Performance

The means between supplement treatments for ADG, DMI, and G:F were non-significant, even if though differences were numerically less for MG- than CO-fed heifers. These data are in agreement with Espinoza et al. (1995) and Kucuk et al. (2004) who suggested no effect of CSFA supplementation on the performance of growing and finishing steers. Differences in performance responses in Exp. 1 compared to Exp. 2 may be a result of adaptation to the supplemental fat. Heifers in Exp. 2 were offered MG supplements during the 30-d pre-shipping phase. This allowed for a period of adaptation to MG, which may have allowed for a more consistent DMI in the post-shipping phase of the study. In contrast, the steers in Exp. 1 were not provided a period of MG acclimation prior to the start of the post-shipping phase of the study. Further, in Exp. 2, heifers were provided their fat supplements in a limit-fed concentrate offered separately from the ground hay whereas fat was mixed in the TMR in Exp. 1. Complete consumption of the daily supplement was observed for all heifers, suggesting that the heifers were adapted to their fat treatment. According to Grummer et al. (1990) and Zinn (1988), a period of adaptation to fat supplementation is essential to maintain optimal feed intake; especially when CS of FA are the source of fat being supplemented.

Inflammatory Reaction

The significant day effect for plasma concentrations of Cp and cortisol was detected in the study, where independent of fat treatment; concentrations increased after shipping. According to The Merck Veterinary Manual (1997), normal values of Cp in cattle range from 16.8 to 34.2 mg/dL, and in this experiment Cp ranged from 17.8 to 45.8 mg/dL. The peaks at d 1

followed by a period of decreasing concentration of Cp confirm the presence of an acute-phase inflammatory reaction in heifers-fed treatments by d 1. This response is in agreement to a previous study by Arthington et al. (2008) that observed peak Cp during the first 72 h after transport followed by a gradual decrease in the concentration of this protein. In addition, Cullens (2005) observed no differences in plasma Cp concentrations in post-partum lactating dairy cows supplemented with CSFA (Megalac®-R) compared to cows receiving no supplement fat, although lactating heifers had lower concentration of plasma Cp when fed CSFA.

A treatment x day interaction was observed for Hp due to a greater concentration in CO-compared to MG-fed heifers on d 1, 3 and 7 after shipping. This response suggests that CO-fed heifers experienced a greater pro-inflammatory response to shipping compared with MG-fed heifers. Because in this experiment Hp was detected in plasma of all heifers suggesting that each was experiencing an acute-phase pro-inflammatory reaction. Supplementation with MG resulted in decreased Hp concentrations compared with CO-fed heifers. This decrease in Hp appears to be an anti-inflammatory response to the stress caused by shipping, and this type of response might be expected when animals are supplemented with an n-3 and not an n-6 (i.e. Megalac®-R) FA source.

The inclusion of PUFA into diets has been shown to modulate immune cell function (Calder et al., 2002). Schmitz & Ecker (2008) stated that the main difference between n-6 and n-3 FA-derived eicosanoids is that most of the mediators formed from ARA are pro-inflammatory; whereas those formed from EPA and DHA are anti-inflammatory. Megalac®-R is a CS of PUFA rich in LA (30% of total FA) which have been shown to stimulate cytokines (Farran et al., 2008) and other physiological mediators such as PGE_2 and LTB_4 (Yaqoob & Calder, 1993).

Lessard et al., (2004) suggested that the regulation of cytokine synthesis can be influenced by differences in the ratio of n-3 and n-6 PUFA in blood and other tissues. Lessard et al. (2003) fed whole flaxseed (16% LA; 57% LNA), CS of palm oil (8% LA; 0.3% LNA) and micronized soybeans (57% LA; 7% LNA) to lactating dairy cows from calving to 108 d of lactation. Twenty-one d after calving and 20 d after artificial insemination, serum concentrations of LNA and the n-3 to n-6 ratio was greater for cows fed whole flaxseed than cows fed micronized soybeans or CS of palm oil. Blood serum concentrations of PGE_2 were reduced in cows fed flaxseed for a minimum of 60 d, which is in agreement to the proposed anti-inflammatory effects of n-3 supplementation (Lessard et al., 2003).

Other research has shown conflicting results relative to the anti-inflammatory and inflammatory effects of n-3 and n-6 FA. Do Amaral (2008) reported lower plasma Cp concentrations in lactating heifers fed CS of palm and fish oils after calving compared to those fed an n-6 FA supplement. Greater plasma Fb concentrations were found in multiparous lactating cows fed an n-6 FA supplement compared to cows fed an n-3 FA supplement.

Yaqoob & Calder (2007) stated that DHA and LNA can be converted to EPA in animal cells. Because EPA is able to compete with ARA for the same enzymes and receptors, EPA can inhibit the production of eicosanoids such as PG-2 and LT-4 series from ARA (Calder, 1999); thereby reducing inflammation. The concentration of LNA and other n-3 FA in ruminant tissues is less compared with other FA, such as LA and stearic acid. Therefore, small tissue changes in n-3 FA's concentration may cause significant physiological transformation in cellular activation and communication, mainly due to synthesis of eicosanoids.

In the post-shipping phase of Exp. 2, 50% of the diet was stargrass hay, and during the pre-shipping phase, heifers were grazing bahiagrass pasture. In general, forages are recognized to

have greater n-3 FA concentration compared with grains which have greater n-6 FA content (Jurgens, 2002). The natural protection of the forage provided by cell wall components may be more adequate to prevent the release of fat and thus BH of PUFA, compared to the protection of other diet components such as grains and oils (Petit, 2002). Perhaps the combination of both factors: greater concentration of "protected" n-3 FA provided by forage, and greater changes in PUFA profile (mainly LA) caused by the ruminal BH, would allow increased absorption of n-3 FA in the small intestine by MG-fed heifers, and consequently, would influence the magnitude of an inflammatory response.

According to Farran et al. (2008), BH can be affected by many factors including: nature and amount of dietary lipid fed, type of protective treatment, and the nature and amount of forages and concentrates included in the diet. The reason to protect fat is to provide a greater amount of UFA in the small intestine, although CSFA are not completely inert to the actions of rumen bacteria (Wu et al., 1981). Sukhija & Palmquist (1990) suggested that the FA profile of CS may affect properties of inertness. According to the authors, CS of SFA are less dissociated than CS of UFA at any given ruminal pH. In agreement, Juchem (2007) observed BH rates greater than 85% for LA and 92% for LNA when fish oil were supplemented in CS- or oil-form. Despite BH, continuous, long-term PUFA supplementation has shown to modulate immune responses in cattle (Bilby et al., 2006; Silvestre, 2009), because certain amounts of PUFA are able to successfully escape microbial BH in the rumen, and be absorbed in the small intestine (Palmquist & Jenkins, 1980).

If BH occurs, the concentration of LA in the small intestine compared to that consumed by the animal will be less, and likely the concentrations of intermediate FA, such as *trans* C18:1 and CLA will be greater. A greater dietary supply of LA and LNA is known to increase milk

C18:1 concentrations (Dhiman et al., 1995) and CS of fish oil result in a greater concentration of trans-fatty acids in the rumen (Baumgard et al., 2000). Lundy, III et al. (2004) observed a greater concentration of *trans* C18:1 in the omasum of dairy cows supplemented with CS or amides of soybean oil (56% LA) than cows supplemented with unprotected soybean oil. This observation is in agreement with Harfoot & Hazlewood (1978) who suggested that greater concentrations of PUFA in ruminal contents may cause the accumulation of *trans* C18:1 by inhibiting the conversion of *trans* C18:1 to C18:0 (stearic acid), especially when PUFA are present as free acids. If this theory is applied to the current experiment, MG-fed heifers would have had a greater absorption of trans-fatty acids and CLA, which may affect FA composition, especially the n-3 to n-6 ratio, of cellular membranes.

Monounsaturated fatty acids, such as oleic acid (C18:1), are non-essential since they can be synthesized *de novo*. In vitro studies have shown that oleic acid is able to suppress lymphocyte inflammation (Miles & Calder, 1998), additionally, Jeffery et al. (1996) observed that feeding diets containing high-oleic sunflower oil and olive oil (80% C18:1) decreased the proliferation of spleen lymphocytes in rats compared with feeding low-fat or safflower oil (75% LA) diets. Besler & Grimble (1995) demonstrated that feeding rats for eight wk with diets containing 5 or 10% butter (rich in oleic acid) or olive oil completely suppressed increases of tissue zinc content, liver protein synthesis, and serum Cp concentrations in response to subcutaneous *Escherichia coli* endotoxin, when compared with maize oil (47% LA) diet. These studies are in agreement with Yaqoob (2002) who stated that MUFA-rich oils have physiological effects which are similar to fish oils. Pariza et al. (2000) demonstrated that CLA supplementation to LPS-challenged rats appeared to stimulate an anti-inflammatory response. Additionally, Cook et al. (1993) observed that CLA-derived eicosanoids could affect synthesis of PGs negatively;

therefore decreasing synthesis of TNF-α. Therefore, it is suggested that increased availability of MUFA, mainly *trans* C18:1, and CLA may modulate anti-inflammatory immune responses in mammals.

The APP are often used as a marker of stress and which is commonly confused with an undesirable response (Silvestre, 2009). However, the acute-phase reaction provides an early non-specific defense mechanism against insult before specific immunity is achieved (Petersen et al., 2004). In turn, Calder et al. (2002) suggested controversy exists in the literature concerning the effects of n-6 and n-3 FA on the immune response, and the discrepancies among experiments could be due to differences among species, source of PUFA added to diets, time of supplementation, and physiological status of the animals. The concentrations of cytokines in blood or in neutrophills were not evaluated in this study. However, several authors (Johnson, 1997; Spurlock, 1997; Calder, 1999) have reported that synthesis of APP, such as Hp, is directly stimulated by cytokines, mainly IL-6 and TNF-α. In conclusion, it is suggested that MG-fed heifers experienced decreased blood Hp concentrations due to a reduced inflammatory reaction may caused in response to PUFA supplementation, even if LA is one of the FA present in more abundance in the MG product.

Table 4-1. Ingredient and nutrient composition of grain-based supplements fed heifers during the pre- and post-shipping phases of the study.

	Treatments[1]	
	CO	MG
Ingredient (% of dietary DM)		
Soybean Hulls	74.3	72.6
Cracked Corn	10.3	…
Cottonseed Meal	14.1	21.5
Megalac-R	…	5.9
Limestone	1.2	…
Component (DM basis)		
$NE_{g,}$ Mcal/kg	0.8	0.85
TDN, %	63.3	67.1
CP, %	16.9	19.4
NDF, %	54.9	55.0
EE, %	3.0	7.9
Ca, %	0.5	1.2
P, %	0.3	0.3

[1] CO = grain-based supplement without Megalac®-R (Church & Dwight Co, Priceton, NJ); MG = grain-based supplement with Megalac®-R.

Table 4-2. Fatty acid profile of supplemental fat source used in the formulation of experimental MG supplement (% of fatty acids).[1]

	Fat Supplement[2]
	MG
C12:0	0.1
C14:0	0.9
C16:0	36.3
C16:1	0.2
C18:0	3.9
cis-C18:1	26.7
trans-C18:1	01
C18:2	28.5
C18:3	3.0
Others[3]	0.3

[1] C12:0 = Lauric acid; C14:0 = Myristic acid; C16:0 = Palmitic Acid; C16:1 = Palmitoleic acid; C18:0 = Stearic Acid; cis-C18:1 = Oleic acid; trans-C18:1 = Vaccenic acid; C18:2 = Linoleic acid; and C18:3 = α-Linolenic acid. The fatty acid profile of the fat supplement was determined according to the manufacturer.
[2] MG = Megalac®-R (Church & Dwight Co, Priceton, NJ).
[3] Others = Not detected.

Table 4-3. Nutrient composition of mineral and vitamin mix supplement.[1]

	Amount
Macro elements (%)	
Calcium (Ca)	14.0
Phosphorus (P)	9.0
Sodium chloride (NaCl)	64.0
Potassium (K)	0.2
Magnesium (Mg)	0.3
Sulfur (S)	0.3
Micro elements (ppm)	
Cobalt (Co)	50
Copper (Cu)	1,500
Iodine (I)	210
Manganese (Mn)	500
Selenium (Se)	40
Zinc (Zn)	3,000
Fluorine (F)	800
Iron (Fe)	800
Vitamins (IU/kg)	
Vitamin A	360,000

[1] The nutrient composition of the mineral and vitamin mix supplement was supplied by the manufacturer. (Cattle Select; Lakeland Animal Nutrition; Lakeland, FL).

Table 4-4. Correlations between plasma measurements, DMI and ADG of transport-stressed heifers during post-shipping phase of the study.[1]

Item	Haptoglobin	Ceruloplasmin	Cortisol	DMI
Ceruloplasmin	0.32			
	0.13			
Cortisol	0.10	0.40		
	0.65	0.05		
DMI	0.51	-0.03	-0.03	
	0.01	0.90	0.88	
ADG	0.14	-0.40	-0.18	0.35
	0.51	0.05	0.39	0.09

[1] Upper row = correlation coefficients. Lower row = *P*–values.

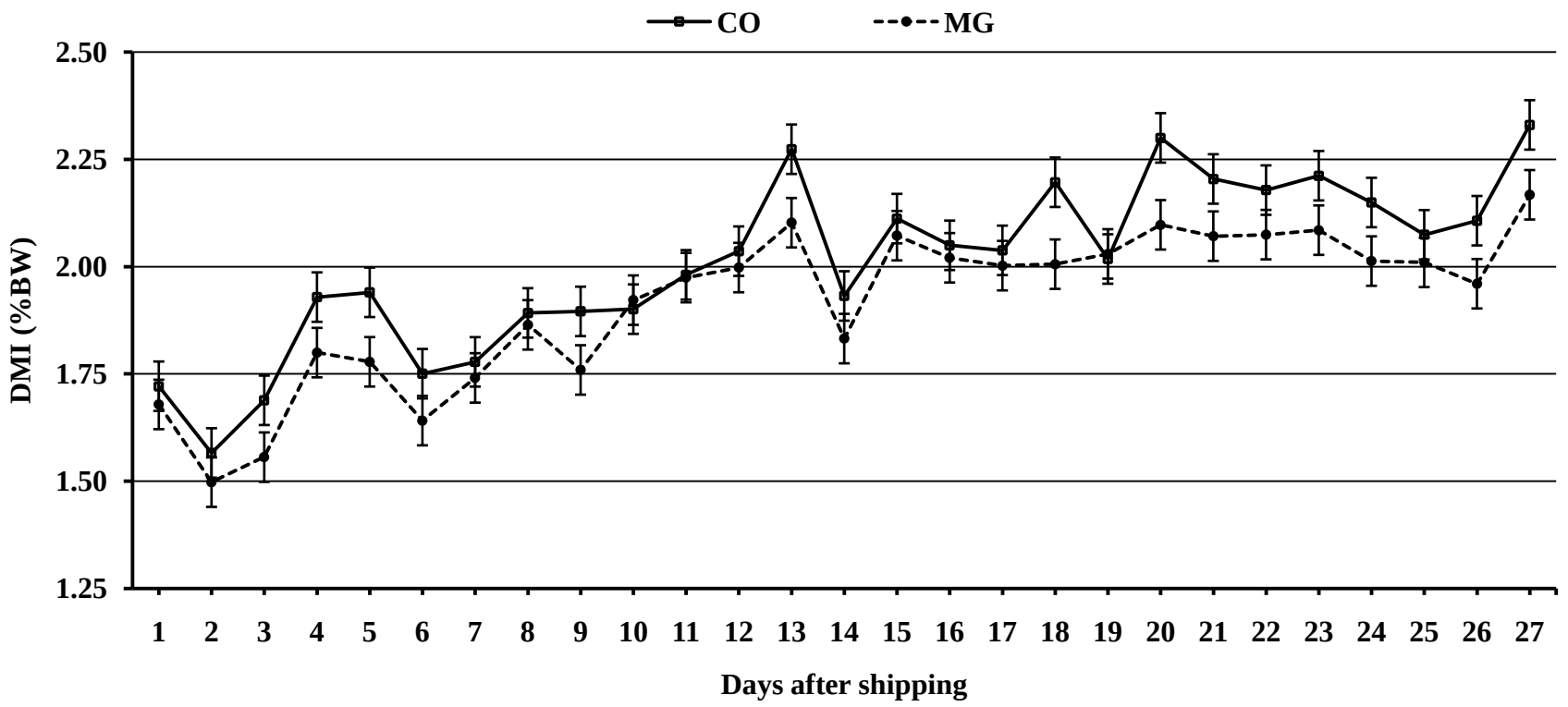

Figure 4-1. Least squares means for DMI of pen-fed heifers during the post-shipping phase of the study. Heifers were fed grain-based supplements containing Megalac®-R (MG) or no supplemental fat (CO) from 30 d before to 27 d after shipping. A day effect was detected ($P < 0.001$).

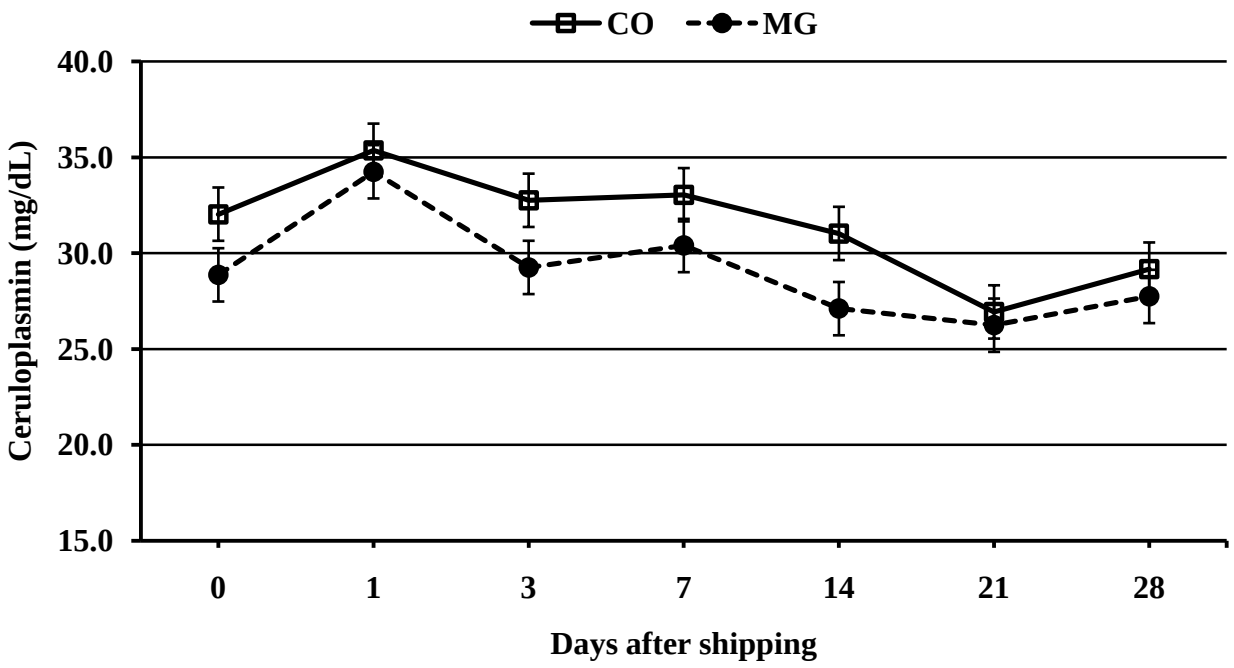

Figure 4-2. Post-shipping concentrations of plasma ceruloplasmin of heifers fed grain-based supplements containing Megalac®-R (MG) or no supplemental fat (CO) from 30 d before to 27 d after shipping. Heifers were loaded onto a trailer on d 0 and arrived in the feedlot on d 1. A day effect was observed ($P < 0.001$).

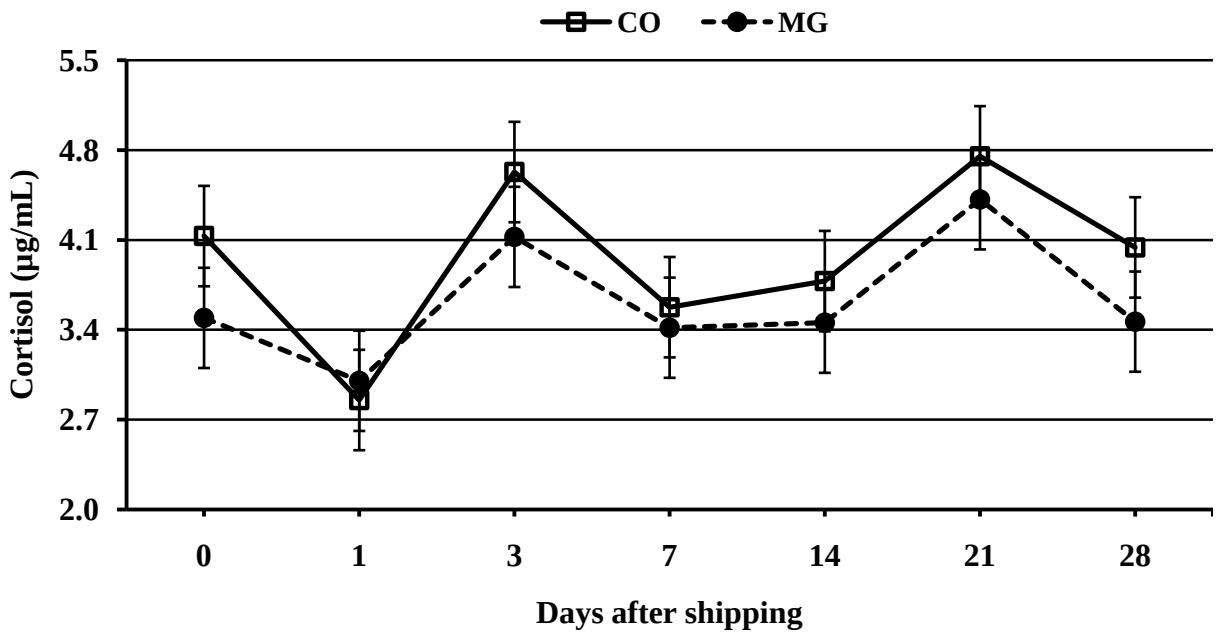

Figure 4-3. Post-shipping concentrations of plasma cortisol of heifers fed grain-based supplements containing Megalac®-R (MG) or no supplemental fat (CO) from 30 d before to 27 d after shipping. Heifers were loaded onto a trailer on d 0 and arrived in the feedlot on d 1. A day effect was observed ($P < 0.001$).

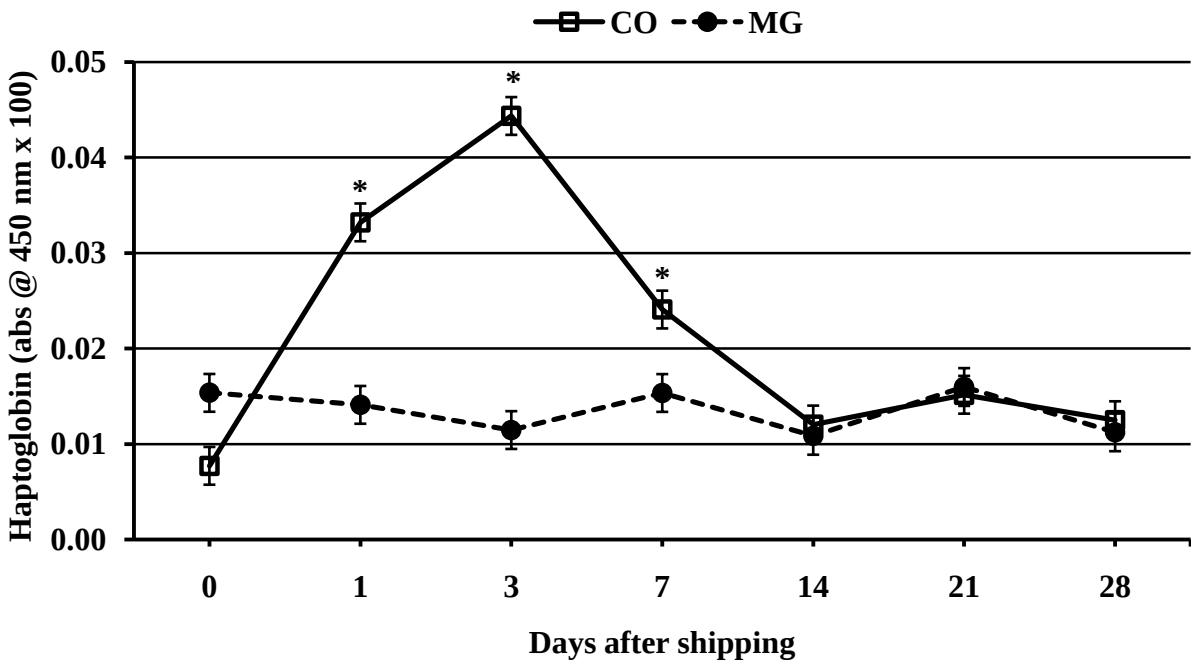

Figure 4-4. Plasma concentrations of haptoglobin of heifers fed grain-based supplements containing Megalac®-R (MG) or no supplemental fat (CO) from 30 d before to 27 d after shipping. Heifers were loaded onto a trailer on d 0 and arrived in the feedlot on d 1. The asterisks at d 1, 3 and 7 indicate that CO-fed heifers had greater plasma concentration of haptoglobin compared to MG-fed heifers (Treatment x d interaction; $P < 0.001$). A d effect was observed ($P < 0.001$).

CHAPTER 5
GENERAL CONCLUSION

The data from these experiments suggest MG supplementation to growing cattle during the feedlot receiving period negatively affects ADG, DMI, and G:F negatively, mainly if they have not been exposed to it previously. One potential explanation for this response is palatability. A poor acceptability of CS of PUFA by cattle may impact DMI, particularly over a short-term, feedlot receiving period (approximately 30 d). A period of adaptation to MG may be a useful prior to transport and feedlot entry.

Supplementation of MG appears to impact the inflammatory reaction of transport-stressed cattle. However, it appears likely that supplementation of MG, prior to the immune-challenge, is required to illicit this response. This supposition is derived from the current studies, where heifers supplemented with MG for 30 d prior to shipping experienced a decreased acute-phase reaction compared to CO-fed heifers. In contrast, the same results were not observed in transported steers that started MG supplementation only after shipping stress and received into the feedlot.

The reason why MG-fed heifers experienced a reduced inflammatory response is unknown. However, one explanation might be related to the concentration of MUFA and LA in the diet. In addition, the ruminal BH rate may influence the amount and type of FA absorbed in the small intestine. Further research is required to understand the effects of the supplemental PUFA sources, as well as the timing of PUFA supplementation on measures of performance and inflammation of transport-stressed beef calves.

LIST OF REFERENCES

Agnes, F., P. Sartorelli, B. H. Abdi, and A. Locatelli. 1990. Effect of transport loading or noise on blood biochemical variables in calves. Am. J. Vet. Res. 51: 1679-1681.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

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

Arthington, J. D., J. W. Spears, and D. C. Miller. 2005. The effect of early weaning on feedlot performance and measures of stress in beef calves. J. Anim. Sci. 83:933-939.

Arthington, J. D., X. Qiu, R. F. Cooke, J. M. B. Vendramini, D. B. Araujo, C. C. Chase Jr., and S. W. Coleman. 2008. Effects of pre-shipping management on measures of stress and performance of beef steers during feedlot receiving. J. Anim. Sci. 86:2016-2023.

Atkinson, R. L., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, B. W. Hess, and D. C. Rule. 2006. Site and extent of digestion, duodenal flow, and intestinal disappearance of total and esterified fatty acids in sheep fed a high-concentrate diet supplemented with high-linoleate safflower oil. J. Anim. Sci. 84:387-396.

Baldwin, D. E., R. G. Marshall, and G. E. Wessman. 1967. Experimental infection of calves with myxovirus parainfluenza-3 and Pasteurella haemolytica. Am. J. Vet. Res. 28:1773-1782.

Ban, E., F. Haour, and R. Lenstra. 1992. Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. Cytokines 4:48-54.

Bateman, H. G., II, J. N. Spain, M. R. Ellersieck. 1996. Influence of by-product feeds and tallow on lactation performance of Holstein cows during two seasons. J. Dairy Sci. 79:114-120.

Bauchart, D., F. Legay-Carmier, M. Doreau, and B. Gaillard. 1990. Lipid metabolism of liquid-associated and solid-adherent bacteria in rumen contents of dairy cows offered lipid-supplemented diets. Br. J. Nutr. 63:563-578.

Baumann, H. and J. Gauldie. 1994. The acute phase response. Immunol. Today 15:74-80.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278: R179-R184.

Beam, S. W. and W. R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81:121-131.

Besler, H. T. and R. F. Grimble (1995). Comparison of the modulatory influence of maize and olive oils and butter on metabolic responses to endotoxin in rats. Clin. Sci. 88: 59-66.

Bilby, T. R., T. Jenkins, C. R. Staples, and W. W. Thatcher. 2006. Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: III. Fatty acid distribution. J. Dairy Sci. 89: 3386-3399.

Breazile, J. E. 1996. The physiology of stress and its relationship to mechanisms of disease and therapeutics. Vet. Clin. N. Am. 4:441-480.

Brokaw, L., B. W. Hess, A. D. Schleicher, E. J. Scholljegerdes, L. M. Yun, J. D. Hegg, and J. Bratka. 2000. Evaluation of supplemental high oil corn for heifers grazing summer pasture. Proc. West. Sect. Am. Soc. Anim. Sci. 51:290-294.

Brokaw, L., B. W. Hess, and D. C. Rule. 2001. Supplemental soybean oil or corn for beef heifers grazing summer pasture: effects on forage intake, ruminal fermentation, and site and extent of digestion. J. Anim. Sci. 79:2704-2712.

Brokaw, L., B. W. Hess, S. J. Bartle, R. D. Landeis, B. M. Alexander, and G. E. Moss. 2002. Effects of hand-fed versus self-fed fat supplementation on growth and reproductive performance of developing beef heifers. Prof. Anim. Sci. 18:38-43.

Bull, L. S., L. J. Bush, J. D. Friend, B. Harris, and E. W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. J. Dairy Sci. 48:1459-1466.

Calder, P. C. 1999. Dietary fatty acids and the immune system. Lipids 34(Suppl.): S137-S140.

Calder, P. C. and R. F. Grimble. 2002. Polyunsaturated fatty acids, inflammation and immunity. Eur. J. Clin. Nutr. 56(Suppl 3):S14-S19.

Calder, P. C., P. Yaqoob, F. Thies, F. A. Wallace, and E. A. Miles. 2002. Fatty acids and lymphocyte functions. Br. J. Nutr. 87(Suppl. 1):S31-S48.

Caughey, G. E., E. Mantzioris, R. A. Gibson,L. G. Cleland, and M. J. James. 1996. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 FA from vegetable oil and fish oil. Am. J. Clin. Nutri. 63:116-122.

Chalupa, W. and A. J. Kutches. 1968. Biohydrogenation of linoleic-1-14C acid by rumen protozoa. J. Dairy Sci. 27:1502-1508.

Chalupa, W., B. Rickabaugh, D. S. Kronfeld, D. Sklan. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. J. Dairy Sci. 67: 1439-1444.

Chalupa, W., B. Vecchiarelli, A. E. Elser, D. S. Kronfeld, D. Sklan, and D. L. Palmquist. 1986. Ruminal fermentation in vivo as influenced by long-chain fatty acids. J. Dairy Sci. 69:1293-1301.

Chang, H. R., D. Arsenijevic, J. C. Pechère , P. F. Piguet, N. Mensi, L. Girardier, and A. G. Dulloo. 1992. Dietary supplementation with fish oil enhances in vivo synthesis of tumor necrosis factor. Immunol. Lett. 34: 13-17.

Cheng, K. J., T. A. McAllister, J. D. Popp, A. N. Hristov, Z. Mir, and H. T. Shin. 1998. A review of bloat in feedlot cattle. J. Anim. Sci. 76: 299-308.

Choi, B., and D. L. Palmiquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. 126:2913-2919.

Clapham, W. M., J. G. Foster, J. P. S. Neel, and J. M. Fedders. 2005. Fatty acid composition of traditional and novel forages. J. Agric. Food Chem. 53: 10068-10073.

Cole, N. A., T. H. Camp, L. D. Rowe, D. G. Stevens, and D. P. Hutcheson. 1988. Effect of transport on feeder calves. Am. J. Vet. Res. 49:178-183.

Cole, N. A. and D. P. Hutcheson.1985. Influence of pre-fast feed intake on recovery from feed and water deprivation by beef steers. J. Anim. Sci. 60:772-780.

Cook, J. A., J. Geisel, P. V. Halushka, and H. D. Reines.1993. Prostaglandins, thromboxanes, leukotrienes, and cytochrome P-450 metabolites of arachidonic acid. New. Horiz. 1:60-69.

Cullens, F. M. 2005. Effects of the timing of initiation of fat supplementation on productive and reproductive responses of periparturient dairy cows during summer. M.S. Thesis, University of Florida, Gainesville, FL.

Dantzer, R. and K. W. Kelley. 1989. Stress and immunity: an integrated view of relationships between the brain and the immune system. Life Sci. 44:1995-2008.

Dawson, R. M., N. Hemington, and G. P. Hazlewopd. 1977. On the role of higher plant and microbial lipases in the ruminal hydrolysis of grass lipids. Br. J. Nutr. 38:225-232.

Demetriou, J. A., P. A. Drewes, and J. B. Gin, 1974. Ceruloplasmin. In Clinical Chemistry: Principles and Techniques. Pages 857-846. 2nd Edition. Harper and Row, Hagerstown, MD.

Devendra , C. and D. Lewis. 1974. The interaction between dietary lipids and fibre in the sheep. II. Digestability studies. Anim. Prod. 19:67-76.

Dhiman, T. R., G. R. Anand, L. D. Satter, and M. W. Pariza. 1999. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82:2146-2156.

Dhuyvetter, K. C., A. M. Bryant, and D. A. Blasi 2005. Case of study: Preconditioning beef calves: Are expected premiums sufficient to justify the practice? Prof. Anim. Sci. 21:502-514.

Do Amaral, B. C. Effect of supplemental fat source on production, immunity, hepatic gene expression, and metabolism of periparturient dairy cows. Doctorate Dissertation. University of Florida, Gainesville, FL.

Doreau, M. and A. Ferlay. 1994. Digestion and utilization of fatty acids by ruminants. Anim. Feed Sci. Tech. 45:379-396.

Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517-1526.

Eicher, S. D., J. L. Morrow-Tesch, J. L. Albright, J. W. Dailey, C. R. Young, and L. H. Stanker. 2000. Tail-docking influences on behavioral, immunological, and endocrine responses in dairy heifers. J. Dairy Sci. 83: 1456-1462.

Elliot, J. P., J. K. Drackley, C. G. Aldrich, and N. R. Merchen. 1997. Effects of saturation and esterification of fat sources on site and extent of digestion in steers: Ruminal fermentation and digestion of organic matter, fiber, and nitrogen. J. Anim. Sci. 75:2803-2812.

Elsasser, T. H., T. S. Rumsey, A. C. Hammond and R. Fayer**.** 1988. Influence of parasitism on plasma concentrations of growth hormone, somatomedin-C and somatomedin-binding proteins in calves. J. Endocrinol. 116:191-200.

Espinoza, J. L., J. A. Ramirez-Godinez, J. A. Jimenez, and A. Flores. 1995. Effects of calcium soaps of fatty acids on postpartum reproductive activity in beef cows and growth of calves. J. Anim. Sci. 73:2888-2892.

Farran, T. B., C. D. Reinhardt, D. A. Blasi, J. E. Minton, T. H. Elsasser, J. J. Higgins, and J. S. Drouillard. 2008. Source of dietary lipid may modify the immune response in stressed feeder cattle. J. Anim. Sci. 86:1382-1394.

Farrar, W. L., M. Korner, and K. A. Clouse .1991. Cytokine regulation of human immunodeficiency virus expression. Cytokine 3: 531-542.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. Edition. Federation of Animal Science Societies, Savoy, IL.

Finck, B. N., K. W. Kelley, R. Dantzer, and R. W. Johnson. 1998. In vivo and in vitro evidence for the involvement of tumor necrosis factor-alpha in the induction of leptin by lipopolysaccharide. Endocrinology 139:2278-2283.

Fluharty, F. L., and S. C. Loerch. 1997. Effects of concentration and source of supplemental fat and protein on performance of newly arrived feedlot steers. J. Anim. Sci. 75:2308-2316.

Garcia, M. R., M. Amstalden, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2003. Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age. J. Anim. Sci. 81:261-268.

Gassman, K. J., D. C. Beitz, F. C. Parrish, and A. Trenkle. 2000). Effects of feeding salts of CLA to finishing steers. Abstracts. J. Anim. Sci. 78(Suppl. 1):S85.

Gibb, D. J., F. N. Owens, P. S. Mir, Z. Mir, M. Ivan, and T. A. McAllister. 2004. Value of sunflower seed in finishing diets of feedlot cattle. J. Anim. Sci. 82:2679-2692.

Gillis, M. H., S. K. Duckett, J. R. Sackman, C. E. Realini, D. H. Keisler, and T. D. Pringle. 2004. Effects of supplemental rumen-protected conjugated linoleic acid or linoleic acid on feedlot performance, carcass quality, and leptin concentrations in beef cattle. J. Anim. Sci. 82:851-859.

Grovum, W. L. 1981. Factors affecting the voluntary intake of food by sheep: III. The effect of intravenous infusions of gastrin, cholecystokinin and secretin on motility of the reticulo-rumen and intake. Br. J. Nutr. 45:183-201.

Grummer, R. R.1988. Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci. 71: 117-123.

Grummer, R. R., M. L. Hatfield, and M. R. Dentine. 1990. Our industry today: Acceptability of fat supplements in four dairy herds. J. Dairy Sci. 73:852-857.

Grummer, R. R. and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838-3852.

Grunfeld, C., R. Gulli, A. H. Moser, L. A. Gavin, and K. R. Feingold. 1989. Effect of tumor necrosis factor administration in vivo on lipoprotein lipase activity in various tissues of the rat. J. Lipid Res. 30: 579-585.

Gulati, S. K., M. R. Garg, and T. W. Scott. 2005. Rumen protected protein and fat produced from oilseeds and/or meals by formaldehyde treatment; their role in ruminant production and product quality: A review. Aust. J. Exp. Agric. 45:1189-1203.

Haaland, G. L., J. K. Matsushima, D. E. Johnson, and G. M. Ward (1981). Nutritive value of protected tallow product fed with hay to beef cattle as assessed by respiration calorimetry. J. Anim. Sci. 52:394-397.

Haddad, S. G. and H. M. Younis. 2004. The effect of adding ruminally protected fat in fattening diets on nutrient intake, digestibility and growth performance of Awassi lambs. Anim. Feed Sci. Tech. 113:61-69.

Hallas, J L., K. S. Gajinala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546.

Harfoot, C. G. 1978. Lipid metabolism in the rumen. Prog. Lipid Res. 17:21-54.

Harrison, J. H., R. L. Kincaid, J. P. McNamara, S. Waltner, K. A. Loney, R. E. Riley, and J. D. Cronrath. 1995. Effect of whole cottonseeds and calcium salts of long-chain fatty acids on performance of lactating dairy cows. J. Dairy Sci. 78:181-193.

Hazlewood, G. P. and R. M. Dawson. 1975. Intermolecular transacylation of phosphatidylethanolamine by a Butyrivibrio sp. Biochem. J. 150:521-525.

Heinrich, P. C., J. V. Castell, and T. Andus. 1990. Interleukin-6 and the acute phase response. Biochem. J. 265:621-636.

Hess, B. W., G. E. Moss, D. C. Rule. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86 (E. Suppl.):E188-E204.

Jeffery, N. M., P. Sanderson, E. J. Sherrington, E. A. Newsholme, and P. C. Calder. 1996. The ratio of n-6 to n-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. Lipids 31:737-745.

Jenkins, T. C., and D. L. Palmquist. 1982. Effect of added fat and calcium on invitro formation of insoluble fatty acid soaps and cell wall digestibility. J. Anim. Sci. 55:957-963.

Jenkins, T. C., and B. F. Jenny. 1992. Nutrient digestion and lactation performance of dairy cows fed combinations of prilled fat and canola oil. J. Dairy Sci. 75:796-803.

Jenkins, T. C. 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76:3851-3863.

Jerred, M. J., D. J. Carroll, D. K. Combs, and R. R. Grummer. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on lactation performance of dairy cattle. J. Dairy Sci. 73: 2842-2854.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci. 75:1244-1255.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest. Anim. Endocrinol. 15: 309-319.

Jolly, C. A., Y. H. Jiang, R. S. Chapkin, and D. N. McMurray. 1997. Dietary (n-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion and the formation of dicylglicerol and ceramide. J. Nutr. 127:37-43.

Juchem, S.O. (2007). Lipid digestion and metabolism in dairy cows: Effects on production, reproduction and health. Doctorate Dissertation. University of California, Davis, CA.

Juchem, S., R. Cerri, M. Villaseñor, K. N. Galvão, R. G. S. Bruno, H. M. Rutigliano, E. J. DePeters, F. T. Silvestre, W. W. Thatcher. 2008. Supplementation with Calcium Salts of Linoleic and trans-Octadecenoic Acids Improves Fertility of Lactating Dairy Cows. Reprod. Dom. Anim. 10.1111/j.1439-0531.2008.01237.x

Jurgens, M. H. 2002. Review of Nutrients and Digestion. Pages 17-24. In Animal feeding and nutrition. 9th Edition. Dubuque, IA, Kendall/Hunt Pub.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. y. Chouinard, M. E. Van Amburg, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128: 881-885.

Kepler, C. R., K. P. Hirons, J. J. McNeill, and S. B. Tove. 1966. Intermediates and products of the biohydrogenation of linoleic acid by Butyrinvibrio fibrisolvens. J. Biol. Chem. 241:1350-1354.

Khansari, D. N., A. J. Murgo, and R. E. Faith. 1990. Effects of stress on the immune system. Immunol. Today 11:170-175.

King, P. 1965. Infection in General Practice. Appl. Ther. 7:490-495.

Kinouchi, K., G. Brown, G. Pasternak, and B. Donner. 1991. Identification and characterization of receptors for tumor necrosis factor-alpha in the brain. Biochem. Biophys. Res. Commun. 18:1532-1538.

Klasing, K. C. 1988. Nutritional aspects of leukocytic cytokines. J. Nutr. 118: 1436-1446.

Klasing, K. C. and B. J. Johnstone. 1991. Monokines in growth and development. Poult. Sci. 70:1781-1789.

Klasing, K. C. and D. R. Korver. 1997. Leukocytic cytokines regulate growth rate and composition following activation of the immune system. J. Anim. Sci. 75:58-67.

Kramer, J. K., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossola, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 32:1219-1228.

Kucuk, O., B. W. Hess, and D. C. Rule. 2004. Soybean oil supplementation of a high-concentrate diet does not affect site and extent of organic matter, starch, neutral detergent fiber, or nitrogen digestion, but influences both ruminal metabolism and intestinal flow of fatty acids in limit-fed lambs. J. Anim. Sci. 82:2985-2994.

Lefcourt, A. M. and T. H. Elsasser. 1995. Adrenal responses of Angus x Hereford cattle to the stress of weaning. J. Anim. Sci. 73: 2669-2676.

Nelson, D. L., and M. M. Cox. 2005. Storage lipids. Pages 343-346. In Lehninger principles of biochemistry. 4th Edition. New York, NY, W. H. Freeman and Company.

Lessard, M., N. Gagnon, H. V. Petit. 2003. Immune response of postpartum dairy cows fed flaxseed. J. Dairy Sci. 86: 2647-2657.

Lessard, M., N. Gagnon, D. L. Godson, and H. V. Petit. 2004. Influence of parturition and diets enriched in n-3 or n-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period. J. Dairy Sci. 87:2197-2210.

Leupp, J. L. G. P. Lardy, S. A. Soto-Navarro, M. L. Bauer, and J. S. Caton.2006. Effects of canola seed supplementation on intake, digestion, duodenal protein supply, and microbial efficiency in steers fed forage-based diets. J. Anim. Sci. 84: 499-507.

Lloyd, K. E., C. S. Whisnant, G. W. Huntington, and J. W. Spears. 2002. Effects of calcium salts of long-chain fatty acids on growth, reproductive performance, and hormonal metabolite concentrations in pubertal beef heifers and postpartum cows. Prof. Anim. Sci. 18:66-73.

Loerch, S. C., and F. L. Fluharty. 1999. Physiological changes and digestive capabilities of newly received feedlot cattle. J. Anim. Sci. 77:1113-1119.

Lucy, M. C., C. R. Staples, F. M. Michael, W. W. Thatcher, and D. J. Bolt. 1991. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin F2 alpha, luteinizing hormone, and follicular growth. J. Dairy Sci. 74:483-489.

Lundy, III, F. P., E. Block, W. C. Bridges Jr., J. A. Bertrand, T. C. Jenkins. 2004. Ruminal biohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci. 87: 1038-1046.

Makimura, S. and N. Suzuki. 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. Jpn. J. Vet. Sci. 44: 15-21.

Mattos, R., C. R. Staples, and W. W. Thatcher. 2000. Effects of dietary fatty acids on reproduction in ruminants. Rev. Reprod. 5: 38-45.

Mattos, R., C. R. Staples, A. Arteche, M. C. Wiltbank, F. J. Diaz, T. C. Jenkins, and W. W. Thatcher. 2004. The effects of feeding fish oil on uterine secretion of PGF2alpha, milk composition, and metabolic status of periparturient Holstein cows. J. Dairy Sci. 87:921-932.

Memon, R. A., C. Grunfeld, A. H. Moser, and K. R. Feingold. 1994. Fatty acid synthesis in obese insulin resistant diabetic mice. Horm. Metab. Res. 26: 85-87.

The Merck Veterinary Manual. 1997. 8th Edition. Merck and Co., Inc., Whitehouse, NJ.

Miles, E. A. and P. C. Calder. 1998. Modulation of immune function by dietary fatty acids. Proc. Nutr. Soc. 57: 277-292.

Miller, W. J., J. L. Carmon, and H. L. Dalton. 1958. Influence of high levels of plant and animal fats in calf starters on growth, feed consumption, and palatability. In Journal Paper 71. College of Agriculture Experiment Station, University of Georgia, Athens, GA.

Moore, J. A., R. S. Swingle, W. H. Hale. 1986. Effects of whole cottonseed, cottonseed oil or animal fat on digestibility of wheat straw diets by steers. J. Anim. Sci. 63:1267-1273.

Moran, T. H., P. J. Ameglio, G. J. Schwartz, and P. R. McHugh. 1992. Blockade of type A, not type B, CCK receptors attenuates satiety actions of exogenous and endogenous CCK. Am. J. Physiol. 262: R46-R50.

NASS. The National Agriculture Statistical Service. Livestock National Statistics. 1996.

Ngidi, M. E., S. C. Loerch, F. L. Fluharty, D. L. Palmquist. 1990. Effects of calcium soaps of long-chain fatty acids on feedlot performance, carcass characteristics and ruminal metabolism of steers. J. Anim. Sci. 68:2555-2565.

Oldick, B. S., C. R. Staples, W. W. Thatcher, and P. Gyawu. 1997. Abomasal infusion of glucose and fat-effect on digestion, production, and ovarian and uterine functions of cows. J. Dairy Sci. 80:1315-1328.

Palmquist, D. L. and T. C. Jenkins.1980. Fat in lactation rations: Review. J. Dairy Sci. 63:1-14.

Palmquist, D. L. and E. A. Moser. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J. Dairy Sci. 64:1664-1670.

Palmiquist, D. L. 1994. The role of dietary fats in efficiency of ruminants. J. Nutr. 124(Suppl.):S1377-S1382.

Pariza, M. W., Y. Park, M. E. Cook. 2000. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc. Soc. Exp. Biol. Med. 223:8-13.

Pariza, M. W., Y. Park, M. E. Cook. 2001. The biologically active isomers of conjugated linoleic acid. Prog. Lipid. Res. 40:283-298.

Park, Y., K. J. Albright, J. M. Storkson. M. E. Cook., and M. W. Pariza. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids 32:853-858.

Pavan, E., S. K. Duckett, and J. G. Andrae. 2007. Corn oil supplementation to steers grazing endophyte-free tall fescue. I. Effects on in vivo digestibility, performance, and carcass traits. J. Anim. Sci. 85:1330-1339.

Peck, M. D. 1994. Interactions of lipids with immune function II: Experimental and clinical studies of lipids and immunity. J. Nutr. Biochem. 5:514-521.

Pepys, M. B. and M. L. Baltz. 1983. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. 34:141-212.

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

Petit, H. V. 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. J. Dairy Sci. 85:1482-1490.

Pomposelli, J. J., E. A. Mascioli, B. R. Bistrian, S. M. Lopes, and G. L. Blackburn.1989. Attenuation of the febrile response in guinea pigs by fish oil enriched diets. J. Parenter. Enteral Nutr. 13:136-140.

Purasiri, P., A. Murray, S. Richardson, S. D. Heys, D. Horrobin, and O. Eremin. 1994. Modulation of cytokine production in vivo by dietary essential fatty acids in patients with colorectal cancer. Clin. Sci. 87:711-717.

Qiu, X., J. D. Arthington, D. G. Riley, C. C. Chase Jr., 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: 2367-2374.

Reidelberger, R. D. 1994. Cholecystokinin and control of food intake. J. Nutr. 124(Suppl. 8):S1327-S1333.

Reidelberger, R. D., G. Varga, and R. M. Liehr. 1994. Cholecystokinin suppresses food intake by a nonendocrine mechanism in rats. Am. J. Physiol. 267:R901-R908.

Richards, C., J. Gauldie, and H. Baumann. 1991. Cytokine control of acute phase protein expression. Eur. Cytokine Netw. 2: 89-98.

Santos, J. E. P., T. R. Bilby, W. W. Thatcher, C. R. Staples, F. T. Silvestre. 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Dom. Anim. 43(Suppl. 2):23-30.

SAS Software Statistics. Version 8.2 Edition. 2001. SAS inst. Inc., Cary, NC.

Schauff, D. J. and J. H. Clark. 1989. Effects of prilled fatty acids and calcium salts of fatty acids on rumen fermentation, nutrient digestibilities, milk production, and milk composition. J. Dairy Sci. 72: 917-927.

Schauff, D. J. and J. H. Clark. 1992. Effects of feeding diets containing calcium salts of long-chain fatty acids to lactating dairy cows. J. Dairy Sci. 75:2990-3002.

Schmitz, G. and J. Ecker. 2008. The opposing effects of n-3 and n-6 fatty acids. Prog. Lipid Res. 47:147-155.

Spallholz, J. E., L. M. Boylan, and J. A. Driskell. 1999. Lipids. Pages 23-40. In Nutrition : Chemistry and Biology. 2nd Edition. Boca Raton, FL, CRC Press.

Sprecher, H. 1981. Biochemistry of essential fatty acids. Prog. Lipid Res. 20:13-22.

Selye, H. 1976. Stress in Health and Disease. Butterworth Inc., Boston, MA.

Silvestre, F. T., T. S. M. Carvalho, C. Crowford, N. Francisco, J. E. P. Santos, S. C. Kim, T. Jenkins, C. R. Staples, and W. W. Thatcher. 2009. Feeding n-6 and n-3 fatty acids to dairy cows: Effects on immunity, fertility and lactation. Proc. FL Rum. Nutr. Symp. 20:75-90.

Simas, J. M., J. T. Huber, Z. Wu, K. H. Chen, S. C. Chan, C. B. Theurer, and R. S. Swingle. 1995. Influence of steam-flaked sorghum grain and supplemental fat on performance of dairy cows in early lactation. J. Dairy Sci. 78: 1526-1533.

Sklan, D., U. Moallem, Y. Folman. 1991. Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J. Dairy Sci. 74:510-517.

Sonti, G., S. E. Ilyin, and C. R. Plata-Salaman. 1996. Anorexia induced by cytokine interactions at pathophysiological concentrations. Am. J. Physiol. 270:R1394-R1402.

Sprecher, H. (1981) Biochemistry of essential fatty acids. Prog. Lipid Res. 20:13-22.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. J. Anim. Sci. 75:1773-1783.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81: 856-871.

Sukhija, P. S., and D. L. Palmquist. 1990. Dissociation of calcium soaps of long-chain fatty acids in rumen fluid. J. Dairy Sci. 73: 1784-1787.

Thatcher, W. W., M. Binelli, J. M. Burke, C. R. Staples, J. D. Ambrose, and S. Coelho. 1995. Antiluteolytic signals between conceptus and endometrium. Theriogenology 47:131-140. USDA. The United States Department of Agriculture; the Consensus of Agriculture Statistics. 2002.

USDA – NASS. The United States Department of Agriculture; National Agriculture Statistical Service. Florida Agricultural Statistics Districts. 2008.

Vendramini, J. M. B., L. E. Sollenberger, J. C. B. Dubeux Jr., S. M. Interrante, R. L. Stewart Jr., and J. D. Arthington. 2006. Concentrate supplementation effects on forage characteristics and performance of early weaned calves grazing rye-ryegrass pastures. Crop Sci. 46:1595-1600.

Williams, P. P. 1993. Immunomodulating effects of intestinal absorbed maternal colostral leukocytes by neonatal pigs. Can. J. Vet. Res. 57:1-8.

Wu, Z., and D. L. Palmquist. 1991. Synthesis and biohydrogenation of fatty acids by ruminal microorganisms in vitro. J. Dairy Sci. 74:3035-3046.

Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025-3034.

Yaqoob, P. and P. C. Calder. 1993. The effects of fatty acids on lymphocyte functions. Int. J. Biochem. 25: 1705-1714.

Yaqoob, P. 2002. Monounsaturated fatty acids and immune function. Eur. J. Clin. Nutr. 56(Suppl. 3): S9-S13.

Yaqoob, P. 2004. Fatty acids and the immune system: From basic science to clinical applications. Proc. Nutr. Soc. 63:89-104.

Yaqoob, P. and P. C. Calder. 2007. Fatty acids and immune function: new insights into mechanisms. Br. J. Nutr. 98(Suppl.):S41-S45.

Zinn, R. A. 1988. Comparative feeding value of supplemental fat in finishing diets for feedlot steers supplemented with and without monensin. J. Anim. Sci. 66:213-227.

Zinn, R. A. 1989. Influence of level and source of dietary fat on its comparative feeding value in finishing diets for feedlot steers: Feedlot cattle growth and performance. J. Anim. Sci. 67:1038-1049

BIOGRAPHICAL SKETCH

Davi Brito de Araujo was born in Mogi Mirim, a small town located in the Citrus Belt of the State of São Paulo/Brazil in 1981. He is the oldest son of Eduardo Netto de Araujo and Arlete Aparecida Brito de Araujo; and brother of Marília Brito de Araujo. He is also the oldest grandson of Eivany Julianetti de Brito, who owns a cattle ranch in Aquidauana / MS, and an orange grove and feedlot ranch in Mogi Mirim /SP, where he spent all his childhood and when his background in cattle began.

Davi started the School of Veterinary Medicine at the São Paulo State University (UNESP) - Botucatu / SP, in 2000. The UNESP - Botucatu Vet School has been ranked among the Top 2 programs in the country during the last 15 years. During the five years of the vet school program, Davi participated at the Student Enterprise of Beef and Dairy Production (CONAPEC Jr.) with his first advisor Dr. José Luiz Moraes Vasconcelos, who offered the opportunity for Davi to study as an intern in the USA during his last two semesters of his Vet School program.

In January 2005, Davi started an internship at UC Davis – VMTRC (Tulare, CA) advised by Dr. José Eduardo Portela Santos, where he had the chance to work with nutrition, reproduction, and health of dairy cattle. On July 2005, he moved to Ona, Florida, where he started another internship, advised by Dr. John Arthington at the Range Cattle REC.

Davi received his DVM degree in December of 2005, and came back to the RCREC in March of 2006 to work on another internship; as a result he started a MS degree in the summer of 2007. In 2008, Davi started a MAB, which is combined with his first MS, in the Department of Food Resource and Economics, advised by Dr. Allen Wysocki.

At Ona, Davi have worked with Dr. Arthington on evaluation of strategies to improve performance and health of immune-challenged beef cattle. Fatty acids and trace minerals supplementation to calves during the receiving period of feedlot are the highlights of his research. During his four years in the US, Davi has already published with other authors, three manuscripts in refereed journals and 16 abstracts.