AN ABSTRACT OF THE DISSERTATION OF

Shelby Armstrong for the degree of Doctor of Philosophy in Animal Science presented on November 22, 2016.

Title: The Effects of a Dietary Immunomodulatory Supplement on the Immune-Metabolic Axis in Growing Beef Cattle

Abstract approved:

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Gerd Bobe

Abstract

To evaluate the immunologic and metabolic effects of OmniGen-AF® (Phibro Animal Health corporation, Quincy, IL; OG) supplementation in growing beef cattle, we designed three studies using replacement beef heifers (8.5 and 10.5 months of age) and steers supplemented across backgrounding, transition, and finishing periods. In Study 1, the effect of OmnniGen-AF (OG) supplementation on expression of cytokines, chemokines, and associated receptors involved in the inflammatory response in whole blood cells of eight healthy purebred Angus heifers was evaluated during the priming phase (first 28d of supplementation). Heifers were trained to eat behind a Calan Broadbent system and then randomly assigned to control or supplemented daily with 56 g/hd/d OG group (n=4/group) to a basal diet consisting of grass hay, alfalfa hay, and ground corn. Blood was collected from the jugular vein before the study started (d0) and on days 3, 5, 10, 14, 21, and 28 of supplementation (priming phase). Genes coding for chemokine receptors (*CX3CR1, CXCR1*), stress

response (*NAMPT*), osteoclastogenesis (*TNFRSF11B*), and angiogenesis (*VEGFA*) were affected by treatment × time. Thirteen genes coding for interleukins and interleukin receptors (*IL1B*, *IL9*, *IL1RN*, *IL1R1*, *IL10RB*, *IL10RA*), chemokine ligand and receptors (*CCR2*, *CXCL2*, *CXCR1*, *CCL26*, *CCR1*), macrophage function (*CSF1*), and secondary immune response (*BMP2*) were down-regulated and *CCL1* was up-regulated by OG supplementation. Of the 20 receptors evaluated, 7 (35%) were influenced by OG supplementation, and both decoy receptors (*IL1RN* and *TNFRSF11B)* were regulated by OG supplementation. Results of Study 1 suggest that OG supplementation may induce a broad, regulatory effect on genes associated with immune cell communication in whole blood during the priming phase.

In Study 2, our objective was to evaluate the effect of OmniGen-AF® supplementation on immune, physiological and carcass ultrasound parameters in steers during backgrounding, transition and finishing periods. Nine purebred Angus half-sibling steers were divided into one of two treatment groups, Control (CNTL n=4) and OmniGen-AF® (OG; n=5), based on the mean of two consecutive fill body weights. Cattle were offered 0 g/hd/d (CNTL) or 56 g/hd/d of OG through a 28-d backgrounding period (limit-fed a predominantly forage diet), a 14-d transition period, and a 56-d finishing period on a high concentrate diet (104 days total). Whole blood was collected on days -4, 14, 21 and 28 of supplementation to evaluate markers of immune function in mRNA during the OG priming phase. Serum was collected on days 0, 14, 21, 28 (OG priming phase), 35, 43, 56, 70, 84, 98 and 104 (OG action phase) to assess markers of physiology. Body weights, average daily gain (ADG), and dry matter intake (DMI) were measured through the all phases; feed efficiency

was calculated bi-weekly; no difference was found between groups for any production parameter. Rib eye area (REA), 12th rib fat thickness (FT), rump fat (RF), REA/cwt and percent intramuscular fat (%IMF) were measured at 30-d intervals by ultrasound during the finishing phase. Predicted yield grade was completed using FT, REA, live body weight x 62% dressing percent and 2.5% Kidney Pelvic Heart fat (KPH); predicted quality grade was calculated using %IMF data. OG-supplemented cattle had a tendency to scan leaner over the 12th rib (P=0.06), had less rump fat (P=0.04), larger REA (P=0.009) and larger REA/cwt (P=0.03) which facilitated for a lower predicted numerical yield grade (P=0.03). %IMF and predicted quality grade were not significantly different between groups. IL10RB and CD80 were downregulated (P=0.02 and P=0.04, respectively), and CXCR2 and MAPK8 had a tendency to be downregulated during the backgrounding phase (P=0.08 and P=0.09, respectively). OG supplementation during the entire experiment increased serum chloride and haptoglobin concentrations and decreased serum NEFA concentrations. OG supplementation also attenuated the decrease in serum paraoxonase concentrations and ameliorated the increase in serum markers of liver cell damage (AST and GGT) at the end of the finishing period (compared to control cattle). Combined, these data suggest that OG may regulate immune system components during the OG priming phase and may act on the IMA during OG priming and action phases. During a high concentrate diet metabolic challenge, OG supplementation may prevent liver damage and improve predicted carcass grades by decreasing fat deposition and increasing REA.

In Study 3, we supplemented 8 purebred replacement Angus heifers (273.70 ± 8.88 kg) with 56 g/hd/day of OmniGen-AF® to a basal diet consisting of grass hay and alfalfa hay. The supplementation period was 28 days long (OG priming phase), during which body weights and blood collected on day 0, 7, 14, 21, and 28 of supplementation. Blood samples were collected for gene expression in whole blood (*CD80, CD62L, CXCR2, IL10RA, IL10RB, MAPK8, NOD2, TLR1*) and serum analysis of inflammatory markers (globulin, serum amyloid A, haptoglobin), markers of kidney function (BUN, creatinine), liver activity and liver cell damage (total protein, albumin, AST, GGT), metabolic markers (glucose, cholesterol, BHBA, NEFA) and serum mineral concentrations (Na, P, Cl, CA K). OmniGen-AF supplemented heifers (OG) were compared to non-supplemented controls (271.70±.88 kg; n=8) for each sample collection. Orts were collected daily and dry matter analysis was conducted twice per week for dry matter intake (DMI). No difference between groups was present in body weight, DMI, average daily gain, or feed efficiency. No differences in inflammatory markers, kidney function, liver activity, liver cell damage, or metabolism were observed between groups. The interaction of OG supplementation and time had an effect on serum albumin concentrations (P=0.003), such that OG heifers had increased serum albumin on day 7 and decreased serum albumin on day 28 (compared to controls, analyzed as change from baseline). Serum Na, P, Cl and Ca concentrations were not different between groups; however, OG supplemented heifers had lower phosphorus concentrations (control 0.69 ± 0.15 mg/dL vs. OG 0.10 ± 0.20 mg/dL; P=0.03), higher magnesium concentrations (control -0.08 ± 0.02 mg/dL from baseline vs. OG -0.17 ± 0.2 mg/dL

from baseline; P=0.005) and a treatment x time interaction was present for Na (P=0.0001), K (P=0.02), Cl (P=0.003), Ca (P=0.0002), P (P<0.0001) and Magnesium (P<0.0001). OG supplemented heifers had higher serum Na concentrations on day 7 (change from baseline) and lower serum Ca and Mg concentrations than controls on day 28 (change from baseline). OG supplementation did not influence *CD80, CD62L, CXCR2, IL10RB, MAPK8* or *TLR1* expression in whole blood. Conversely OG supplementation increased *IL10RA* and *NOD2* expression (P=0.02 and P=0.005) in whole blood and OG supplemented heifers had increased *IL10RA* expression on day 14 (P<0.001). When compared to previous results in beef cattle (using other sexes or animals in different stages of maturity), OG supplementation did not have consistent results on whole blood gene expression or serum indicators of animal health, suggesting that the effect OG supplementation on beef cattle may differ among ages and sexes.

The results of all three studies indicate OG supplementation may have a regulatory effect on markers of immune function during the OG priming phase, but these markers may not be consistent across sexes and/or stages of maturity. OG supplementation may also regulate serum mineral concentrations during the OG priming phase, but again these results are inconsistent. When cattle are subjected to a metabolic challenge, OG supplementation may support animal health through improved liver function and influencing the immune-metabolic axis. Additionally, OG supplementation during the finishing phase may improve potential carcass grades without altering body weight, average daily gain, dry matter intake or feed efficiency.

The Effects of a Dietary Immunomodulatory Supplement on the Immune-Metabolic Axis in Growing Beef Cattle

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Shelby Armstrong

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Shelby Armstrong, Author

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CONTRIBUTION OF AUTHORS

Gerd Bobe and Massimo Bionaz acted as advisors for all three studies. Experimental design for studies 1 and 2 was created by Shelby Armstrong. Data from experiment 1 was collected alongside another experiment conducted by Tyler Schell. Experimental design was co-developed by Gerd Bobe and Shelby Armstrong for experiment 3.

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CHAPTER 1

INTRODUCTION

The ultimate goal of the US beef cattle industry is to provide a consistent, affordable, nutritious product for the public. Over time, increased efficiency of beef cattle production in the feedyard can be contributed to advancements in nutrition including (but not limited to) adding increased concentrate for growth, subtherapeutic antibiotics and repartitioning agents. While all three of these methods have increased the efficiency and production of cattle fed through feedyards, they can also be met with resistance from consumers and policy makers. Public expectations for the beef industry are changing, with consumers and policy makers moving away from utilizing antibiotics and supraphysiological dietary supplements. Producers will be challenged to incorporate other methods to keep production consistent with current rates and affordable. This ultimately leaves an opportunity for new, natural-based immune function-promoting supplements to be incorporated into the beef industry to assist with metabolic regulation and animal health. The dairy industry has been successful utilizing these products to maximize animal health and production, especially yeast products (Avendaño-Reyes et al., 2006; Poppy et al., 2012; Zaworski et al., 2014; Broadway et al., 2015; Yuan et al., 2015) and blended products (Ryman et al., 2013; Playford et al., 2014; Brandão et al., 2016). However this is still a novel practice for beef producers, who could easily benefit from products that maximize animal health and immune function.

One blended product, OmniGen-AF® (OG; Phibro Animal Health Corporation, Quincy, IL) has been shown to augment markers of innate immune function, *CD62L* (Ryman et al., 2013; Nace et al., 2014; Playford et al., 2014) and *IL8R* (Playford et al., 2014), in dairy cattle when the product was supplemented longer than 45+ days. Company-produced product literature suggests a minimum priming period of 28 days; thus studies completed in dairy cattle (Y.-Q.Q. Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014; Playford et al., 2014; Brandão et al., 2016) evaluated endpoints when samples were collected after 28 days of OG supplementation. A recently published study in rodents evaluated markers of innate and adaptive immune function impacted by OG supplementation during the OG priming phase (d7, d14, d21, and d28 of supplementation; Branson et al., 2016). However, these results have not been validated in beef cattle.

Metabolic markers have been identified as participants in immune responses (Carroll and Sanchez, 2014), and it is well understood that nutrition interacts with cattle health and immune function (Galyean et al., 1999). Additionally, cattle often face metabolic and immunologic challenges during the finishing phase as high concentrate diets are used (B N Ametaj et al., 2009). Yet, the effect of a commercially available dietary supplement (OG) known to augment immune function has not been evaluated for its effects on blood markers of metabolic and immune function or animal health in beef cattle. The close relationship of metabolism and immune function may provide for a novel, joint platform in which dietary supplementation can be used to influence these two physiologic functions and ultimately increase production.

To advance knowledge regarding the immunologic-metabolic interaction in feedlot cattle, metabolic markers novel to this type of study such as NEFA, BUN, and glucose have been measured in response to an endotoxin challenge (Sanchez et al., 2014c); this is done in addition to the conventional immunological markers of cytokines and acute phase proteins commonly measured in similar LPS challenge models (Carroll et al., 2009b; a). Although the metabolic response to an immunologic challenge has been investigated in beef cattle, the reverse of examining the immunologic response to a metabolic challenge has not been extensively investigated in beef cattle. Finishing cattle frequently experience metabolic challenges in the form of high concentrate diets (Nagaraja and Chengappa, 1998; Owens et al., 1998) and immunologic challenges in the form of pathogen and endotoxin exposure. Liver function can also be impaired during an aggressive finishing phase with high concentrate diets, leading to liver abscesses and carcass condemnation (Nagaraja and Chengappa, 1998). A novel approach to the interaction of the immune-metabolic axis (IMA) would be to evaluate in the presence of a metabolic challenge, such as a high concentrate finishing diet, differential effects of dietary supplementation on markers of immune and liver function as well as metabolism. More knowledge surrounding this link between metabolism and immune function will be helpful to support animal health in challenging production situations, such as receiving, backgrounding and finishing, and improve profitability by minimizing liver function disruptions during the finishing phase.

The implications of this research extend beyond feedlot cattle into other phases of production. Breeding heifers and cows can also face metabolic challenges during grazing seasons due to daily fluctuations in feed and water availability. Additionally, cattle grazing on pasture are often exposed to severe environmental conditions without shelter, leaving them vulnerable to environmental pressures that can trigger an immunologic response. Female beef cattle who better tolerate environmental, immunologic, and metabolic challenges could be more profitable to beef producer.

Trace mineral supplementation of beef cattle has been extensively studied from an immunologic perspective (Galyean et al., 1999; Engle, 2001); providing trace minerals to range cattle is a common management practice. However, the effects of adding an immunomodulatory supplement to the diet of breeding beef cattle have not been investigated. Providing additional dietary support for metabolism and immune function may improve the health of rangeland cattle. Current industry practices already facilitate opportunities for administration of a dietary supplement. Despite the described potential applications, to our knowledge, no research has been done in regards to supplementing OG to growing beef heifers. A project which explores the IMA during OG supplementation in replacement heifers would ultimately add to our knowledge regarding the effect of dietary supplementation to enhance beef cattle production.

In the following three studies, our objective was to evaluate the previously unexplored immunologic and metabolic effects of OG supplementation during the OG priming phase (first 28 days), and action phase (after 28 days of

supplementation) in replacement heifers and finishing steers. Evaluating the IMA in replacement heifers and finishing steers will add to the current knowledge of physiology during these production phases. By gaining a deeper understanding of immunologic priming through dietary supplementation (first 28 days), we will add depth to current knowledge and explore a commonly ignored feeding period in which early changes in immunologic markers or metabolic markers may affect the IMA during the OG action phase. Additionally, evaluating the effect of OG supplementation in beef cattle will add to the nutritional tools available to producers to improve animal health and ultimately, profitability.

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CHAPTER 2

OMNIGEN-AF® ALTERS EXPRESSION OF IMMUNE-RELATED GENES IN WHOLE BLOOD OF HEALTHY ANGUS HEIFERS

Introduction

Animals in production agriculture often encounter stress through multiple phases of their productive life cycles. Examples include heat stress (Collier et al., 2008), shipping transport (Arthington et al., 2008), changes in nutrition, and parturition (Goff and Horst, 1997; Graugnard et al., 2012). Stress can lead to immunosuppression which may correspond to greater incidences of disease (Burton and Erskine, 2003). Cattle management can assist in limiting the effects of stressful events; however, dietary supplementation with products designed to support animal health has become a practical, efficient means of enhancing the immune system to combat these challenges (Brandão et al., 2016).

Dietary supplements for livestock may include a variety of products such as minerals, vitamins, fatty acids, yeast culture, live yeast, and mannan-oligosaccharides (MOS) intended to support production and immune function (Engle, 2001; Duff and Galyean, 2007; Broadway et al., 2015). One product available for use in the livestock industry, OmniGen-AF® (OG), provides evidence of a positive effect on innate immunity, predominantly an increase in L-selectin (*CD62L)* and interleukin 8 receptor (*IL8R*). While the induction of these genes represents a limited aspect of innate immune response, it is important to note that these genes are functional markers of neutrophil function and thus, provide a mechanism for the cell-mediated cascade leading to pathogen clearance also reported with OG supplementation (Forsberg et al., 2006; Forsberg et al.,

2007; Ortiz-Marty et al., 2012; Ryman et al., 2013). Specifically reported in dairy cattle, an increase in expression of CD62L and IL8R coincides with improved neutrophil-mediated killing capacity against pathogenic bacteria associated with mastitis (Rowson et al., 2009; Rowson et al., 2011; Ryman et al., 2013). Another aspect of the process of neutrophils or other immune cells responding to a pathogen challenge is the focal production of cytokines and chemokines for regulation. One cytokine (*IL1β*) and cytokine receptor (*IL8R*) are known to be regulated by OG (Wang et al., 2007); however, these two proteins do not provide a comprehensive understanding of the diverse class of cytokine and cytokine receptors that may be affected by OG supplementation. To increase our understanding of how OG supports the immune system beyond neutrophil function, further investigation of regulation of cytokines and cytokine receptors by OG supplementation is warranted. The intricate network of cytokines and cytokine receptors are responsible for far more than neutrophil function; thus, this investigation will provide new information regarding the holistic effects of OG supplementation.

While previous results offer intriguing insights into the biological effects of OG, they were obtained with animals experiencing controlled stress conditions. Limited investigation with regulatory biological molecules has been done in animals under non-stress conditions, an approach that excludes a large proportion of animals in production systems. In addition to the limited scope of biological action available, the previous database of research focuses on supplementation periods longer than 28 days and does not evaluate biological molecules that may

change in beef cattle production. More work needs to be done to 1) expand the scope of immune function gene markers, especially genes coding for cytokines that can be affected by OG supplementation, to better understand the mechanism of OG action; 2) investigate changes in immune function gene markers (coding for cytokines/chemokines and receptors) induced by supplementing OG in a non-stress model; 3) identify changes in immune function gene markers in beef cattle associated with OG supplementation; and 4) discover changes in immune function gene markers induced in the OG priming phase (first 28 days) of supplementation, compared to later OG feed supplementation phases.

Gene expression profiling array-based technology provides the means to investigate a relatively large number of immune function gene markers in a single sample, leading to greater insight into immunomodulatory effect of OG supplementation. The central hypothesis of this project is that OG will induce changes in gene expression of cytokines or cytokine receptors before 28 days of supplementation. The objective of this study was to monitor changes in expression of genes coding for cytokines and cytokine receptors in circulating blood cells in growing, purebred Angus heifers fed OG sampled on d 3, 5, 10, 14, 21 and 28 of supplementation.

Materials and Methods

Animal Care and Use

All animals were humanely treated and cared for in accordance with OmniGen Research Animal Handling Guidelines. These guidelines follow the

Guide for the Care and Use of Agricultural Animals in Research and Teaching (Mcglone et al., 2010).

Animal Housing and Feeding

Eight purebred Angus heifers were housed in a freestall barn (Corvallis, OR) with access to a Calan Broadbent Feeding System (American Calan, Northwood, NH) and fed a forage based diet comprised of grass hay and alfalfa. Cattle were allowed a 7-d acclimatization period then divided into two treatment groups: control animals (no nutritional supplement) and a nutritional supplement (56 g/hd/d; OmniGen-AF® Prince Agri Products, Quincy, IL). Feed was mixed in a custom mix wagon and offered to cattle twice daily; cattle enrolled in the treatment group were topdressed the product daily.

Blood collection, RNA purification and Reverse Transcription

Blood samples were collected on the first day of the trial (before supplementation began) and on d 3, 5, 10, 14, 21, and 28 of supplementation. Approximately 3 mL of whole blood was collected via jugular venipuncture into Tempus Blood RNA Tubes (Cat no 4342792, Life Technologies, Carlsbad, CA). Immediately after collection, tubes were shaken vigorously for 15 s and stored at -20°C until RNA purification (less than 2 mo). RNA was purified using the Tempus Spin RNA Isolation kit (Cat no 4380204, Life Technologies); upon completion, samples were stored at -80°C until reverse transcription.

RNA was evaluated for quality and concentration using a Thermo Scientific Multiskan Go microplate spectrophotometer (Cat no. 51119300; Thermo Fisher Scientific, Carlsbad, CA) and a µDrop Plate (cat no N12391,

Thermo Fisher Scientific). Only RNA with an absorbance ratio (260 nm : 280 nm) above 2.0 was used. One µg total RNA was used as a template for cDNA synthesis in a RT2 First Strand Kit (cat no 330401; QIAGEN, Valencia, CA). Upon completion, cDNA was stored at -20°C until use.

RTqPCR

One-hundred and two undiluted microliters of cDNA was combined with 1,350 µL RT2 SYBR Green qPCR Mastermix (cat no 330503; QIAGEN) and 1,248 µL molecular grade water (cat no 338132; QIAGEN) and thoroughly mixed. Twenty-five µL of the mixed solution was pipetted into each well of a RT2 Profiler PCR array- Cow Inflammatory Cytokines and Receptors plate (cat no 330231; QIAGEN). Well plates (one plate/sample; fifty-six plates total) were placed in a Bio-Rad C1000 series thermocycler (cat no 184-1100, Bio-Rad, Richmond, CA) and read using a CFX96 Real-Time PCR Detection System (cat no Bio-Rad 184-5097). Themocycling protocols were as follows: 10 min at 95°C, 40 cycles with 15 s at 95°C and 1 min at 60°C. A melt curve was completed directly after thermocycling (95°C to 65°C to 95°C in 0.5°C increments).

Data Analysis

Data were analyzed using LinReg software (Ruijter et al., 2009) to account for efficiency of amplification and normalized by a normalization factor calculated by geometrical mean of 3 internal control genes (*HPRT1, TBP,* and *YWHAZ*). Internal control genes were selected by testing with geNorm (Vandesompele et al., 2002). Briefly, all internal reference genes available in the plate (*ACTB*, *GAPDH*, *HPRT1*, *TBP*, *YWHAZ*) were analyzed and the mean

expression stability value (M value) was <1.5. The determination of the optimal number of control genes for normalization indicated that the use of three internal control genes offered the largest stability among the possible combinations (i.e., V-value = 0.326).

RTqPCR data were natural log-transformed prior statistical analysis. Presence of outliers was evaluated using the *Studentized* residuals; when studentized t > 2 samples were removed from the final dataset. The final data set (82 genes) was subjected to ANOVA analysis with treatment, time, and treatment × time as main effect and animal as random using JMP Genomics (SAS institute, NC, USA). Significance was deemed with a false discovery rate of < 0.10. Pathway analysis of the final dataset was performed using Dynamic Impact Approach (Bionaz et al., 2012) using as criteria: a) the whole dataset as background; b) differentially expressed genes with FDR of < 0.10, and c) pathways with at the least two genes in the whole dataset, and at the least two differentially expressed genes in each pathway. Further, we used Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang et al., 2009) using the full gene list as background and an EASE score of < 0.10 as cut-off to identify significantly enriched pathways.

Results

OG supplementation altered expression of 16 out of 82 measured genes during the OG priming phase; of those, 15 were down regulated and one (*CCL1*) was upregulated (Figure 2.1). Transcripts repressed by OG supplementation

included chemokines (*C5, CCL26, CXCL2*), chemokine receptors (*CXCR1, CCR1, CCR2*), interleukins (*IL1β, IL3, IL9*), interleukin receptors (*IL1R1, IL1RN, IL10RA, IL10RB*) and other cytokines (*CSF1, BMP2*) (Figure 2.2). Of the 20 receptors evaluated, 7 (35%) were influenced by OG supplementation. Additionally, both decoy receptors evaluated (*IL1RN* and *TNFRSF11B*) were regulated by OG supplementation. A time × treatment interaction was detected for *TNFRSF11B, VEGFA, CX3CR1, NAMPT* and *CXCR1* **(**Figure 2.3); however, changes in expression were not consistent among all genes, with mean separation occurring on different days and no recognized pattern apparent among all genes.

None of the pathways were enriched in DAVID by the differentially expressed genes. Using Dynamic Impact Approach, we detected an overall down-regulation of several KEGG pathways encompassing cell signaling and immune system development/response (Figure 4). The cellular signaling pathways with the most genes affected by OG supplementation were MAPK signaling (2 of 5 genes altered by OG supplementation), osteoclast differentiation (3 of 8 genes), TNF signaling (3 of 11 genes), and hematopoietic cell lineage (4 of 13 genes).

Discussion

CXCR1

*CXCR1***,** a gene coding for interleukin 8 receptor type 1 *(IL8R*), has been previously found to be upregulated in OG supplemented dairy cattle after two to

four months of supplementation (Ryman et al., 2013; Playford et al., 2014). In this study, the same gene was down-regulated by OG during the first 28 days of supplementation. Specifically, Ryman et al. (2013) found OG supplementation increased *IL8R* gene expression at four months of supplementation in Holstein heifers. Playford et al. (2014) also reported *IL8R* upregulation in lactating pasture-fed dairy cattle when OG supplementation lasted longer than 45 days. These researchers reported *IL8R* was upregulated on day 60 and 90 of supplementation but there was no difference between control and OG groups at 30 days of supplementation (Playford et al., 2014). Both Ryman et al. (2013) and Playford et al. (2014) focused on dairy cattle rather than beef cattle; both reported a longer supplementation period (greater than 45 days) compared to the study presented here for eventual *IL8R* gene expression induction. The downregulation of *CXCR1* reported in this study may be due to the biological action of OG, a difference in cattle type (dairy vs. beef), differences in RTqPCR data normalization, or statistical analysis rather than feeding duration.

CCL1

CCL1 was the only gene upregulated by OG supplementation during the 28d experimental period (Figure 2.1). Secreted by activated T cells, CCL1 binds to CCR8 (Louahed et al., 2003) and acts as a chemokine for monocytes, NK cells and dendritic cells (Miller and Krangel, 1992). The upregulation of *CCL1* could suggest an increased ability to attract monocytes into tissue for macrophage differentiation and ultimately improved wound clearing times. However, it is

important to note *CCL1* is not the only cytokine with monocyte chemotactic properties and that it is the only monocyte chemotactic agent upregulated in this study. To determine if OG supplementation increases monocyte migration, a functional cell assay needs to be conducted to assess diapedesis rates of OG supplemented cattle compared to their control counterparts.

Pathway Analysis

Supplementation of cattle with OG indicates repression of multiple genes associated with the inflammatory response. Two signaling pathways, determined by KEGG analysis, that were altered by OG supplementation include cytokine-cytokine receptor and the chemokine signaling pathways. The detection of changes in these biological pathways are to be expected due to the nature of the targeted array provided from QIAGEN; an array designed to evaluate differences in cytokines and cytokine receptors associated with the inflammatory response. The interaction of cytokines and chemokines is biologically relevant for studies of the immune system; however, the broad scope of the cell pathways identified here requires future research regarding the nature of specific cytokine-cytokine receptors and their impact on the whole biology of the animal during OG supplementation. Furthermore, no pathway was considered enriched according to DAVID analysis, limiting the conclusions which can be drawn regarding the effect of OG supplementation on immunological pathways during the first 28 d of supplementation in beef cattle.

Receptor Evaluation

Of important note in this study is the prominence of receptor gene expression regulated. A total of 35% of all gene receptors (7 of 20 analyzed receptor genes) evaluated on the array were down-regulated by OG supplementation. A closer look at the function of these receptors indicates that many have promiscuous binding patterns (Table 2.1). For example, *CCR2* and *IL1R1* are both capable of binding three unique cytokines, while *CCR1* can bind five unique cytokines, and *IL10RB* can bind seven unique cytokines as a part of a heterodimer receptor. The ligand receptor interaction of most cytokines results in an irreversible binding and leads to intracellular changes resulting in a biologic response (Bagley et al., 1997). The downregulation of numerous cytokine receptors in this study, which have the capability of initiating multiple, differentiated effects on the cellular signaling of inflammation could be crucial to regulating the immune system and preventing widespread, uncontrolled inflammation. Uncontrolled inflammation has been identified as a contributing factor to several infectious and metabolic diseases including mastitis, retained placenta, metritis, displaced abomasum and ketosis (Sordillo, 2016). It is possible that by limiting uncontrolled inflammation through dietary supplementation, decreased incidences of the aforementioned disorders and other health disruptions may occur.

Decoy Receptors

In addition to the multifunctional receptors regulated by OG supplementation, two 'decoy receptors', *IL1RN* and *TNFRSF11B* were also regulated by OG supplementation. *IL1RN* expression was downregulated by OG supplementation during the OG priming phase (Figure 2.2) and *TNFRSF11B* expression was dependent upon the interaction of time and OG supplementation (Figure 2.3). Decoy receptors bind ligands, preventing it from binding to its normal receptor and have been identified as a strategy to regulate inflammatory cytokines and chemokines (Mantovani et al., 2001). We speculate that by regulating decoy receptor expression, OG supplementation could be contributing to the regulation of inflammation during the OG priming phase.

Other Considerations

Although the discussion of data presented in this study is biologically interesting, the potential functional relevance is limited to a speculative nature due to 1) the nature of gene expression data, 2) the type of array used, and 3) the absence of differential cell counts. Gene expression data is useful for identifying potential mechanisms of action, but without functional immune cell data, interpretation is speculative. As previously mentioned, the array utilized here for gene expression profiling limits the data generated to aspects of immune cellular communications via cytokine and cytokine receptors, an area we wanted to focus on to detect novel response markers of OG supplementation. This targeted approach allows for a close investigation of cellular communication in circulating

immune cells; however, the absence of transcription factors or second messenger expression limits the clarity of data interpretation and usefulness of biological pathways involved in the immune cell response regulated by OG supplementation. Finally, whole blood is the tissue type used for this experiment. Although whole blood provides a great foundation for identifying novel response markers and examining immune cell communication, the lack of hematological data and differential cell counts greatly limits the biological interpretation. Biological implications of OG supplementation on cellular communication involved in the inflammatory response remain speculative, because differences seen in receptors or cytokines may be due to underlying differences in cellular populations.

Conclusion

With the exception of *CXCR1* and *IL1B*, the majority of genes discussed have not been previously associated with OG supplementation. These results indicate the methodology used in this study may provide opportunities to identify novel indicators and/or mechanisms involved in the regulation of the inflammatory response induced by OG supplementation during the growth phase in replacement beef heifers. Additionally, these date contribute novel findings regarding whole blood gene expression changes during the priming phase of OG supplementation in growing beef heifers.

Figure 2.1. Downregulation of *CCL1* expression is attenuated in OG-supplemented heifers during the first 28 days of OG supplementation. CCL1 gene expression is relative to *HPRT1*, *TBP*, and *YWHAZ.*

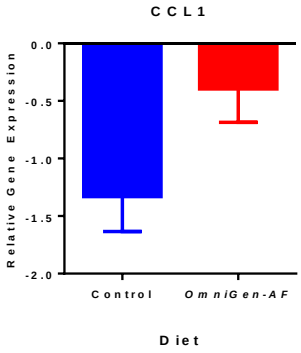

Figure 2.2. Genes suppressed by OG supplementation during the OG priming phase (first 28 days of supplementation). Gene expression is relative to *HPRT1*, *TBP*, and *YWHAZ*.

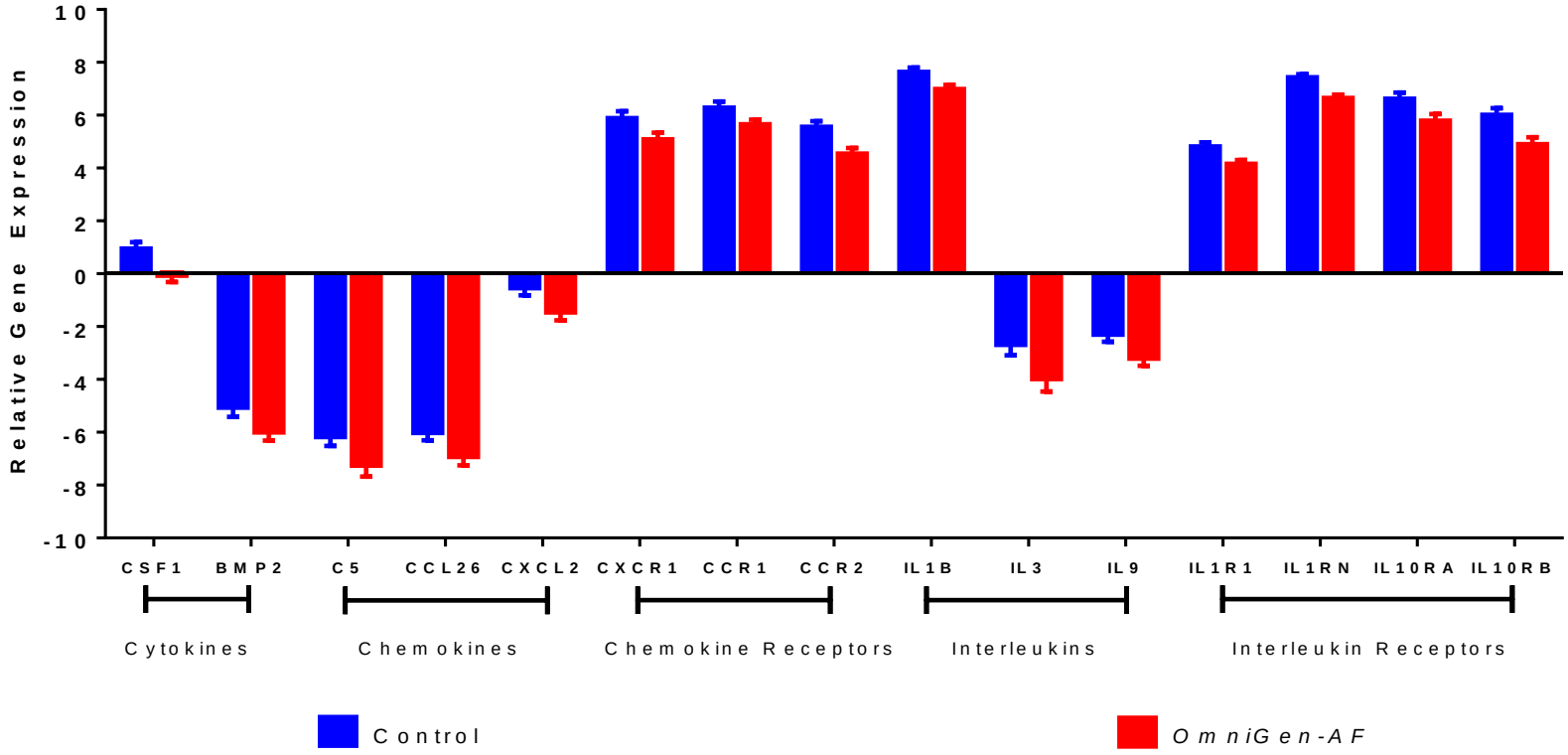

Figure 2.3. Effect of OG supplementation × time on expression of immune function-related genes in whole blood during the OG priming phase; *indicates comparison between control and OmniGen-AF is different (P<0.05) while # indicates comparison has tendency to be different (0.05<P<0.10). Gene expression is relative to *HPRT1*, *TBP*, and *YWHAZ*.

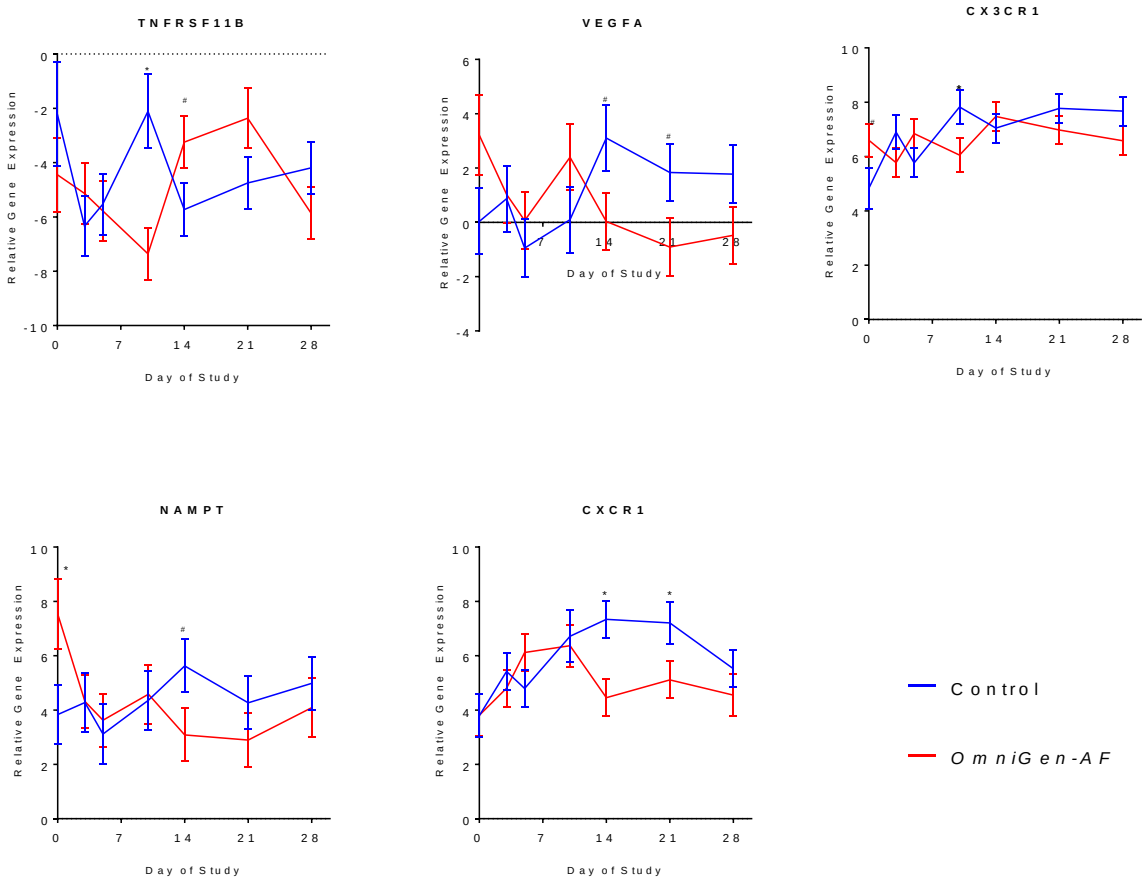

Figure 2.4. KEGG pathways (Kanehisa and Goto, 2000; Kanehisa et al., 2016) impacted by OmniGen-AF supplementation as determined using the Dynamic Impact Approach (Bionaz et al. 2012) .
*Number of transcripts in the pathway significantly affected by OmniGen AF treatment.

CATEGORY	PATHWAY	Impact	# DEG*
Environmental Information Processing	MAPK signaling pathway		2
	Jak-STAT signaling pathway		4
	Cytokine-cytokine receptor interaction		10
Organismal Systems	Osteoclast differentiation		3
	Chemokine signaling pathway		6
	TNF signaling pathway		3
	Hematopoietic cell lineage		4

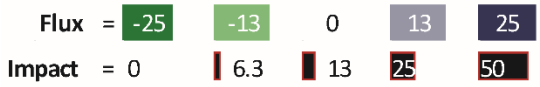

Table 2.1. Cytokine receptors regulated by OG supplementation (or the interaction of OG supplementation and time) during the OG priming phase can be promiscuous and bind to multiple cytokines.

Receptor[1]	Cytokine/chemokine
CCR1	MIP-1-alpha, MIP-1-delta, MCP-3, MIP-1-beta, MCP-1
CCR2	CCL2, CCL7, CCL13
CX3CR1	CX3CL1
CXCR1	IL8
IL1R1	IL1A, IL1B, IL1RN
IL10RA	IL10
IL10RB	IL10, IL22, IL26, IL28, IFNL1, IFNL2[2], IFNL3[2]

[1]Information acquired from Gene Cards (www.genecards.org)
[2]Requires IFNLR1 as co-receptor to mediate antiviral activity

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CHAPTER 3

DIETARY SUPPLEMENTATION OF A COMMERCIAL FEED ADDITIVE REGULATES IMMUNE FUNCTION, METABOLISM, LIVER FUNCTION AND IMPROVES PREDICTED CARCASS QUALITY

Introduction

Feedlot cattle experience a number of stressors and potentially immunocompromising situations from receiving to harvest time which can induce an immune or metabolic response. Specific examples include heat stress (Mitlohner et al., 2001; Mader and Davis, 2004; Collier et al., 2008; Gaughan et al., 2008), feed changes (B. N. Ametaj et al., 2009), housing conditions (Duff and Galyean, 2007; Arthington et al., 2008), and pathogen challenges (Burciaga-Robles et al., 2010). To introduce more control over the responses to these environmental factors, addition of feed supplements to the basal ration is common. Subtherapeutic antibiotic supplementation is common in feedlot rations (Alexander et al., 2008; Drake and Cooperative; Beauchemin et al., 2003; Callaway et al., 2003). However, increasing regulation by regulatory institutions forecasts a limitation on use for these types of supplements. As a result, the beef industry must look for other options to maintain efficiency and food safety, including blended products which maximize the use of multiple ingredients to improve animal health and safeguard against health events. One such product, which has already shown to improve the health of dairy cattle, is OmniGen-AF (OG). Decreased health events have been seen in multiple dairy production scenarios (Holland et al., 2013; Bewley et al., 2014; Holland et al., 2014). When pre-fed to beef cattle, steers and heifers mounted a stronger immune response to

an endotoxin challenge (Burdick et al., 2012; Sanchez et al., 2014a). While the results are promising for use in feedlot cattle when faced with a diet or immune challenge, detailed information regarding immunological or metabolic changes during a less intense challenge, such as diet change from low to high concentrate diet during the finishing phase, remains unknown.

Previously, OG supplementation has been shown to impact markers of immune function (*IL8R*; *CXCR1/CXCR2* and *L-Selectin CD62L*) in rats, mice (Ortiz-Marty et al., 2012), sheep, and dairy cattle (Y.Q. Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014; Playford et al., 2014). The focus of these studies was on the immune response elicited by a longer feeding period (45-60d); however, recent studies indicate that OG supplementation may initiate an immune response within the first 28 d of supplementation (Branson et al., 2014a; Branson et al., 2014b; Armstrong et al., 2015) in rats and Angus heifers, respectively (OG priming phase). An impact on metabolic status has also been identified during heat stress (Hall et al., 2014) and lipopolysaccharide challenge (Sanchez et al., 2014a; Sanchez et al., 2014b) in dairy and beef cattle, respectively. When OG was provided to replacement beef heifers and steers without an experimentally induced immune or metabolic challenge, carbohydrate metabolism was altered during a 49-d supplementation period and the 21 d following supplementation (Schell et al., 2016). Within this body of knowledge, the immunologic and metabolic effects of OG supplementation have not been extensively evaluated during multiple production phases in beef cattle, including the finishing phase.

To address these gaps in knowledge, we developed an experiment to profile the immunologic and metabolic changes in beef cattle when they transition from a primarily forage based, limit fed background diet to a high concentrate finishing diet. We hypothesize that at least one detectable change will be identified in metabolic or immunologic parameters as a result of OG supplementation without inducing any undesirable production or carcass characteristics. The objective of this study was to determine any change in immunologic, metabolic or production parameters induced by OG supplementation through the backgrounding or finishing phase.

Materials and Methods

Cattle and Diets

All animals were cared for under guidelines outlined in the Phibro Animal Health Animal Care and Use Policy. Nine half-sibling, purebred Angus steers were housed in a free stall barn (Corvallis, OR) with access to a Calan Broadbent Feeding System (American Calan, Northwood, NH). Cattle were allowed a 20-d training and acclimatization period and were then divided into two treatment groups: Control animals (no nutritional supplement; C; n=4), and a treatment group provided a nutritional supplement at 56 g/hd/d (n=5; OmniGen-AF® Phibro Animal Health Corporation, Quincy, IL). Feed was mixed in a custom mix wagon and offered to cattle twice daily. Orts were collected every 24 h, and dry matter analysis (Cunniff, 1998) was conducted to determine dry matter intake (DMI). Cattle were weighed weekly throughout the study.

Cattle were offered four different basal diets throughout the study: Priming (P), Transition 1 (T1), Transition 2 (T2; for analysis T1 and T2 are pooled into one phase identified as Transition or T), and Finishing (F; diet compositions found in Table 3.1). During the 28-d P phase, cattle were limit fed (10.5 kg, dry matter basis) a predominately forage-based ration. Those enrolled in the treatment group were supplemented 56 g/hd/d of OmniGen-AF (OG) top dressed into the top half of the feed bin. The second and third diet phases (T1 and T2) lasted a week each to facilitate an appropriate transition time between a forage-based diet and a high-concentrate diet (Figure 3.1). The F phase lasted for 62 d (Figure 3.1) and used a diet rich in fermentable carbohydrates (Table 3.1).

Ultrasound Data

Ultrasound data were collected on aSSD-500V ultrasound using a UST-5044-3.5 linear transducer (Aloka, Hitachi Aloka Veterinary, Ltd., Wallingford, CT) to scan for ribeye area (REA), relative ribeye area (REA/cwt), fat deposition over the 12th Rib (FT), rump fat (RF), and intramuscular fat (%IMF). Data were translated in real time using Designer Genes BIA Pro Plus Software (Designer Genes Technologies, Harrison, AR 72601). Theoretical yield grade was calculated using the formula described in Boggs et al. (2015); estimated hot carcass weights (eHCW) were calculated as 62% of the live body weight on the day of ultrasound data collection and eHCW was used in the yield grade equation.

Sample Collection, and Processes for qPCR Data

Blood samples were collected at baseline (before supplementation began) and on days 14, 21, and 28 of supplementation. Whole blood was collected via jugular puncture into Tempus Blood RNA Tubes (Cat no 4342792, ThermoFisher Scientific, Carlsbad, CA) with approximately 3 mL of blood. Immediately after collection, tubes were shaken vigorously for 15 s. Samples were stored at -20°C until RNA purification was complete. RNA was purified using the Tempus Spin RNA Isolation kit (Cat no 4380204, ThermoFisher); upon completion samples were stored at -80°C until reverse transcription was completed. RNA quality was determined using an Agilent 2100 bioanalyzer using the assay class eukaryote total RNA nano (version 2.6), all samples had an RIN greater than 8.3. Samples of sufficient quality were converted to cDNA using the iScript cDNA synthesis kit (Cat no. 1708890; BioRad Hercules, CA). Each sample was run in triplicate for each gene, reference and target. Target genes included markers of immune function: Cluster of differentiation 80 (*CD80*), Interleukin 8 Receptor B (*CXCR2*), Interleukin 10 receptor A and B (*IL10RA*, *IL10RB*), L-Selectin (*CD62L*), Mitogen-Activated Protein Kinase 8 (*MAPK8*), Nucleotide-Binding Oligomerization Domain Containing 2 (*NOD2*), and Toll-like Receptor 1 (*TLR1*). Multiple reference genes were evaluated including: (*GAPDH*), Hypoxanthine Phosphoribosyltransferase (*HPRT1*), Ribosomal Protein L-19 (*RPL19*), Ribosomal Protein Subunit 9 (*RPS9*), TATA-binding Protein (*TBP*), Tyrosine 3-monooxygenase/tryptophan5-monooxygenase activation protein (*YWHAZ*). All primers were ordered from the pre-designed catalog of bovine primers from

ThermoFisher Scientific; mastermix was created using 4 µL cDNA, 5 µL Taqman Gene Expression Master Mix (cat no 4369106, ThermoFisher scientific), 0.5 µL primer and 0.5 µL RNAse/DNase free water (all volumes per well). Samples were analyzed in triplicate and RT-qPCR was conducted in a 7900HT machine (Cat no 43290001, Thermofisher scientific, Frederick, MD, USA) with the following thermocycling conditions: 10 min at 95°C, 40 cycles with 15 s at 95°C and 1 min at 60°C. All genes (target and reference) were analyzed using LinReg Software (Ruijter et al., 2009) to account for efficiency of amplification. Reference genes were selected for stability using GeNorm (Vandesompele et al., 2002); TBP, RPL19 and RPS9 were considered the most stable (M=0.404 ,0.430, and 0.420 respectively) and thus, were used to normalize the data set; all data points were normalized to d -4.

Serum Collection and Analysis

Blood was collected via jugular puncture into a serum separator tube (SST) at baseline, and on d 7, 14, 21, 28, 35, 43, 56, 70, 84, 98, and 104 of supplementation. Samples were stored at 4°C until centrifugation at 3000 x *g* for 20 min. Serum was aliquoted in 1.25 mL amounts and frozen at -80°C until shipment to the Trevisi lab in Italy. Blood metabolites were analyzed at 37°C by a clinical auto-analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA). Glucose, total protein, albumin, total cholesterol, total bilirubin, creatinine, urea, Ca, P, Mg, aspartate aminotransferase (AST/ GOT), and γ-glutamyl transpeptidase (GGT) were determined using kits purchased from Instrumentation

Laboratory (IL Test, Bedford, MA, USA). Globulin was calculated as the difference between total protein and albumin. Electrolytes (Na+, K+, and Cl−) were detected by the potentiometer method (Ion Selective Electrode connected to ILAB 600). Zinc and NEFA were determined by commercial kits (Wako Chemicals GmbH, Neuss, Germany). Haptoglobin, SAA and BHBA were analyzed using methods described by Bertoni et al. (1998) that were adapted to the ILAB 600 conditions. Paraoxonase, SAA, NOx, NO_2, and NO_3 concentrations were determined as in (Osorio et al., 2014).

Statistical Analysis

Statistical analyses were performed using version 9.3 of SAS (SAS, Inc., Cary, NC, USA). Serum data were analyzed as change from baseline. All data was tested for normal distribution using the Shapiro-Wilk test in PROC UNIVARIATE; if necessary, data were natural log transformed to achieve normality. Data were analyzed using repeated-measures-in-time analysis in PROC MIXED. Repeated measures within animals were modeled using first order autoregressive variance-covariance matrix, which was the most parsimonious model based on the Akaike information criterion (AIC). To obtain the correct degrees of freedom, the Kenward Rogers degree of freedom adjustment was used. For production data, fixed effects of the statistical model were treatment (control and OG), phase (Priming, Transition and Finishing), and their interactions. For ultrasound data, fixed effects of the statistical model were treatment (control and OG), time (d49, 69, 104), and their interactions. For serum

data, fixed effects of the statistical model were treatment (control and OG), dietary phase (P- d-4, 14, 21, 28; T1/T2-d35 and F-d 43, 56, 70, 84, 98, 104), day (nested within phase), and their interactions. Due to a dramatic increase in AST and GGT at the end of the finishing phase, the variable of phase was redefined to allow for analysis. In the first analysis dietary phase was considered P- d-4, 14, 21, 28, T1/T2- d35 and F- d 43, 56, 70 and 84; the second analysis considered only d 98 and 104, diet phase was removed from the model.

PCR data was checked for analyze the outliers using SAS PROC GLM; any result with a studentized t-test greater 0.2 0was removed. The final data set was analyzed using PROC GLIMMIX of SAS with time and treatment as fixed effects, animal was used as random variable. All tests were two-sided. Significance was declared as $P<0.05$ and tendencies were defined as $0.05<P<0.10$ for all analyses.

Results

Production and Carcass Predictions

No significant difference was detected in production parameters including average daily gain, body weight, dry matter intake, or feed efficiency between control and OG supplemented animals ($P > 0.05$). Dietary phase influenced DMI ($P < 0.0001$), and had a tendency to influence body weight and average daily gain ($P = 0.08$). No significant interaction between OG supplementation and diet phase was observed for body weight, DMI, ADG or feed efficiency (Table 3.2).

Close to harvest, cattle supplemented with OG during the finishing phase scanned with less rump fat (C- 0.73 ± 0.03 cm vs. OG- 0.61 ± 0.03 cm; $P = 0.04$) and had a tendency to have decreased fat deposition over the 12th rib (C- 0.70 ± 0.03 cm vs. OG- 0.61 ± 0.03 cm; $P = 0.06$). OG supplemented cattle also had increased REA (C- 60.16 ± 1.57 cm^2 OG- 66.74 ± 1.41 cm^2; $P = 0.009$), increased REA/cwt (C-9.32 ± 0.24 vs. OG- 10.35 ± 0.22; sq inch/100lbs BW; $P = 0.03$) and decreased predicted yield grade (C-3.52 ± 0.10 OG- 3.16 ±0.09; $P = 0.03$; Figure 3.2). %IMF, live body weight, and estimated hot carcass weight was not significantly altered by OG supplementation. Rump fat (P =0.01), FT, live body weight, estimated hot carcass weight, REA, REA/cwt ($P < 0.0001$) all increased over time whereas predicted yield grade decreased over time ($P = 0.0002$). No significant treatment x time interactions were present for FT, RF, live body weight, estimated hot carcass weight, REA, REA/cwt, YG, or %IMF (Figure 3.2).

Serum Analysis

Diet phase altered several serum mineral concentrations including Ca, P, Mg, Na, Cl, and Zn, protein (total protein, albumin, globulin), liver function markers (AST, GGT, paraoxonase), kidney function markers (creatinine and urea) and metabolic markers (NEFA and BHBA). Diet phase did not influence positive acute phase proteins (haptoglobin and SAA), cholesterol, potassium, NOx or NO_3 serum concentrations. Phase did have a tendency to influence NO_2 concentration (Table 3.2).

Overall NEFA concentrations were lower in OG supplemented cattle (P = 0.002; Figure 3.3) compared to control cattle. OG supplementation attenuated the decline in serum paraoxonase (P = 0.02; Figure 3.5) concentrations. Alternatively, cattle enrolled in the OG group had increased serum chloride concentrations (P = 0.02; data not shown) and haptoglobin concentrations (relative to starting values, P = 0.0002; Figure 3.4) over the course of the experiment compared to control cattle. There was a tendency for the interaction of dietary treatment and phase to influence changes in serum Mg concentration (P = 0.06), with the largest difference identified in the priming period (data not shown). Group x day interactions (nested within phase) were found for serum Mg, AST, NEFA, and creatinine. Day of sample collection was different for all mineral parameters except Ca and Mg, all protein markers, metabolic markers (BHBA, cholesterol, NEFA, glucose), markers of liver function (paraoxonase, AST, GGT), markers of kidney function (creatinine, urea), NO_2, and haptoglobin. Serum SAA had a tendency to be different between sample days.

qRT-PCR Data

Time, OG supplementation, or the interaction of the two did not significantly influence *CD62L* expression in whole blood. *IL10RA* and *NOD2* (both $P < 0.0001$) expression was different over time; *TLR1* gene expression had a tendency (P = 0.09) to be impacted by time, but was not significantly influenced by diet. *CXCR2* (P = 0.08) and *MAPK8* (P = 0.09) had a tendency to be

influenced by diet, and were influenced by time (both $P < 0.0001$). There was no significant diet by time interaction for *MAPK8*, however there was a treatment by time interaction for *CXCR2* ($P = 0.02$). OG supplementation downregulated *CD80* and *IL10RB* expression ($P = 0.004$ and $P = 0.02$ respectively). Time also influenced these two genes ($P = 0.001$ and $P = 0.0005$ respectively), but there was no significant treatment by time interaction for either gene (Figure 3.7).

Discussion

Production Parameters

OmniGen-AF had no detrimental effect on production parameters during the priming, transition, and finishing phase. Dietary phase did influence DMI; due to the dietary nature of the limit-fed OG priming phase, and the *ad libitum* transition and finishing phases; this result is logical and expected. Phase also had a tendency to increase body weight and average daily gain, again this is to be expected as finishing cattle should gain body weight during the finishing period, and average daily gain should increase with the higher plane of nutrition provided during the finishing phase compared to the limit-fed forage OG priming phase.

Carcass Predictions

Dietary supplementation during the finishing phase is a common practice to maximize profitability through harvest (Duff and Galyean, 2007; Leheska et al., 2009), especially when cattle are marketed on a grid, as diet components can be adjusted to finish cattle closer to the desired carcass grades. When cattle are marketed and sold on a grid, which offers higher payouts for leaner cattle who

offer a lower numerical yield grade, β-adrenergic agonists such as ractopamine-HCL (Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008; López-Carlos et al., 2010) or zilpaterol-HCL (Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008; Rathmann et al., 2009; López-Carlos et al., 2010) are used to control fat and lean deposition (Sillence, 2004) and to reduce days in the feedyard for cost reduction (Vasconcelos et al., 2008). However, this practice has recently come under scrutiny from regulatory institutions and consumers due to the negative side effects on animal health, leaving the market searching for an alternative dietary supplement that offers the same benefit without the potential adverse consequences. Issakowicz et al. (2013) investigated the influence of live yeast supplementation (*Saccharomyces cerevisiae*) on carcass parameters of feedlot lambs (as a model for beef cattle) and did not discover any differences in carcass ultrasound parameters in supplemented lambs compared to control lambs. Wang et al. (2011) used cinnamaldehyde supplementation in feedlot steers with no effect on any carcass parameter evaluated (HCW, BF, REA, marbling score, quality grade). The null effect of these two alternative diet supplements on ultrasound parameters in meat animals still leaves room for other products to impact production.

Serum Mineral Concentration

In this study, we detected a difference between control and OG steers in serum mineral concentrations during the finishing phase. Dietary mineral manipulation has previously been investigated to manipulate carcass performance.

Higher levels of dietary magnesium have been found to increase KPH (Ramirez and Zinn, 2000), but no other carcass parameters investigated (HCW, REA, FT, marbling score) were found to be different. In contrast, Spears and Kegley (2002) found Zinc increased marbling and quality grades, but also had a tendency to increase backfat. No previous experiments conducted with OG supplementation in feedlot cattle have investigated serum mineral parameters, and the results of this study do not produce the same results as studies which manipulated dietary mineral concentrations for enhanced carcass quality (Ramirez and Zinn, 2000; Spears and Kegley, 2002). This suggests that OG supplementation does not affect feedlot production or carcass quality in the same manner as dietary mineral supplementation, and the differences seen in serum mineral concentrations are not correlated.

In our study, the predicted effect on carcass quality more closely aligns with the results of the β-andrenergic agonists than supplementing live yeast, cinnamaldehyde, or manipulating dietary Zn or Mg levels. It is important to note that our study used potential carcass grades rather than post-mortem evaluation. Additionally, cattle genetics heavily influence carcass grades (predicted or actual; Marshall, 1994) and the half-sibling, purebred Angus cattle used in this study may heavily influence outcome. To see if these results hold true, a larger, more genetically diverse population of cattle should be supplemented with the product, with potential and post-mortem carcass grades evaluated through the finishing period.

Other Serum Parameters

Diet phase had the most profound impact on serum parameters with multiple markers of metabolism, inflammation, kidney function, liver activity, liver cell damage and serum mineral concentrations impacted by diet phase. Finishing phase diets similar to the one used in this study have been linked to liver abscesses (Nagaraja and Chengappa, 1998; Smith, 1998) and increased incidences of ruminal acidosis (clinical and subclinical; Galyean et al., 1999; Owens et al., 1998) as well as an increased general inflammatory status (B. N. Ametaj et al., 2009; Zebeli et al., 2012). The difference seen in serum parameters between phases is likely triggered by an increase in concentrate fed for the entire finishing period.

Cattle in the OG group had higher haptoglobin concentrations than control counterparts. Brandao et al (2016) found that lactating dairy cows supplemented with OG had higher haptoglobin concentrations than their control counterparts before, during, and after LPS infusion when supplemented with OG. Schell et al (2016) found growing beef steers held on a backgrounding diet had higher haptoglobin concentration when supplemented with OG relative to their control counterparts. Haptoglobin is often used as a general marker of inflammation (Galyean et al., 1999; Ceciliani et al., 2012; Graugnard et al., 2012; Carroll and Sanchez, 2014), and in this study the overall increase in haptoglobin could indicate a greater inflammatory response induced by OG supplementation. However, a closer look at the data (Figure 3.4) shows a peak in both groups on day 56, to the degree that cattle on d 56 had the greatest increase in serum

haptoglobin concentrations (relative to baseline) compared to any other day during the study ($P < 0.02$; data not shown). This spike in haptoglobin, and SAA concentration after an increase in dietary concentrate is consistent with Ametaj et al. (2009).

In this study, NEFA concentrations were lower in OG supplemented cattle than in the control group. During an LPS challenge, receiving beef steers supplemented with OG had lower NEFA concentrations than control steers (Sanchez et al., 2014a). Recent unpublished data shows beef heifers fed a finishing diet (70:30 concentrate: forage) and supplemented with OG had lower NEFA before, during and after a glucose tolerance test, suggesting less need to mobilize NEFA for energy, even during a metabolic challenge. It is possible that decreased NEFA concentrations are present during OG supplementation with or without a metabolic or immune challenge. High NEFA concentrations have been shown to inhibit immune cell function (Ster et al., 2012) and have been linked to an increase in liver-related disorders (Loor et al., 2005). A decrease of NEFA concentration in OG supplemented cattle suggests a metabolic regulation, leading to a potential decrease in liver-related disorders.

Liver Health Indicators in Serum

Toward the end of the finishing period, a marked increase in AST and GGT were seen in both groups (Figure 3.6); however, this rise is attenuated in OG supplemented steers. AST and GGT are released in response to liver cell damage (Bionaz et al., 2007). More specifically, AST has been shown to increase after

parturition in dairy cows (Hussein and Abd Ellah, 2008; Kabara et al., 2014), a time rife with physiologic changes (Goff and Horst, 1997) that can induce systemic inflammation and metabolic disorders involving the liver (Ametaj et al., 2005; Bertoni et al., 2008; Ceciliani et al., 2012; Sordillo and Raphael, 2013). This decrease in AST and GGT concentrations in the supplemented OG group may suggest a liver damage prevention of OG supplementation toward the end of an high-concentrate finishing period.

OG supplementation attenuated the decline in paraoxonase (PON) concentrations observed in similarly managed control cattle. PON is a negative acute phase protein (Rahman et al., 2010) which has been identified as a biomarker for inflammatory transition cows, when lower levels of PON were suggested to be an index of decreased liver function (Bionaz et al., 2007). With this in mind, an increase of PON in these cattle, in tandem with the GGT and AST results at the end of the finishing period as well as the decreased NEFA concentrations, may suggest an attenuation of liver damage after high concentrate feeding through dietary OG supplementation. However, before these conclusions can be considered resolute, a larger study that includes hepatic tissue collection is required to prove the suggested difference in liver function during OG supplementation.

Gene Expression

Previous work has shown an upregulation in *CD62L* and *CXCR2* gene expression (in WBC) when OG was supplemented to dairy cattle (Ryman et al.,

2013; Nace et al., 2014), especially after the 45 to 60 d of supplementation (Playford et al., 2014). This period is often referred to as the minimum time period required to prime the immune system to better handle an adverse health event. However, in this study we detected no difference in *CD62L* gene expression (whole blood) and a down regulation in *CXCR2* within the 28 day priming period. The different behavior in these two genes when compared to previous work may be attributed to many different factors including type (dairy vs. beef), sex (cows vs. steers), physiological state (production vs. maintenance), laboratory methods or statistical analysis. Of considerable note is the basal diet cattle were supplemented with: prior investigations of *CD62L* and *CXCR2* gene expression have predominantly been conducted in dairy cattle and Angus steers in this study were limit fed a predominately forage based diet for the first 28 days. Other studies have used a higher energy basal diet (Ryman et al., 2013; Nace et al., 2014) fed closer to *ad libitum* feeding rates. Previous research by Armstrong et. al (2015) conducted in beef heifers fed a lower-energy diet concluded *CXCR2* was down regulated within the first 28 days of feeding in whole blood. It is quite possible that if gene expression was followed past the 28 day supplementation period, the expression pattern may be similar to those of lactating dairy cattle found in Playford et al. (2013). Up regulation of both genes mentioned may not occur until after the conclusion of the priming period (45-60 days of supplementation), which was not followed to conclusion in this study.

Previous work conducted by Branson et al (2016) has also shown an increase in *Mapk8*, *Nod2*, *Tlr1* and *Cd80* gene expression in whole blood using a

rat model and a 28-d supplementation period. In order to examine the cross species effects of supplementation on these genes, we evaluated those parameters in backgrounded steers. Whereas OG did influence CD80 gene expression and had a tendency to influence MAPK8 gene expression, we found OG supplementation downregulated these genes in cattle, which is in contrast to their rodent counterparts. In these steers, OG supplementation had no impact on NOD2 or TLR1 gene expression. Much of these differences could be attributed to differences in the two species physiology.

Steers in this study were fed a similar diet to the heifers used in Armstrong et. al. (2015); *CXCR2* and *IL10RB* gene expression was similar in both studies. It is possible that these two genes could be candidates for molecular markers of immune function during early supplementation of OG in beef cattle. However, repeated studies with this same outcome and larger group numbers are required to make decisive conclusions regarding their use for future studies. Overall, gene expression data contributes to the body of knowledge surrounding immune system modulation induced by OG supplementation in growing beef cattle.

Conclusion

OG supplementation regulated the inflammation response during the OG priming phase (first 28 days of supplementation) and lowered predicted numerical yield grades during the finishing period by increasing REA and decreasing FT.

Additionally, providing OG in the basal diet during a metabolic challenge may limit hepatic cell damage and result in improved liver function.

Table 3.1. Diet composition for all diets used in finishing steer study. Ingredients are given as % of total diet.

	Priming	Transition 1	Transition 2	Finishing
Alfalfa	58.13	0	0	0
Oregon Hay	34.88	50	26	15
Corn	0	27.35	51.35	63.35
Cane Molasses	6.98	3	3	3
Soybean Meal 47.7%	0	1.5	1.5	1.5
Limestone 38%	0	1	1	1
ADE	0	0.15	0.15	0.15
Vitamin E	0	1	1	1
Urea	0	1	1	1
Distillers grain	0	15	15	15
CHS vitamin Premix[1]	0.15	0.15	0.15	0.15

[1] CHS vitamin premix contains the following (approximately): Calcium 12-13%, Phosphorus 6%, Salt 18-21.5%, Magnesium 6.75%, Sulfur 1.0%, Copper 3,500 ppm, Iodine 195 ppm, Manganese 3,300 ppm, Selenium 53-58 ppm, Zinc 7,500 ppm, Vitamin A 250,000IU/lb, Vitamin D 25,000 IU/lb, Vitamin E 250 IU/lb

Table 3.2. Production parameters of steers supplemented with, or without OG during multiple dietary phases. Data are LS Means ± SE.

		Priming				Transition				Finishing				Statistical Analysis		
Parameter	Unit	Control	OG		SE	Control	OG		SE	Control	OG		SE	Diet	Phase	Phase x Diet
Body Weight	kg	516.95	524.24	±	31.90	523.53	534.36	±	31.82	535.36	546.89	±	31.73	0.87	0.08	0.95
DMI	kg/day	10.26	10.31	±	0.22	12.35	12.18	±	0.31	13.03	12.62	±	0.16	0.29	<0.0001	0.70
ADG	kg	1.23	1.38	±	0.38	0.74	1.07	±	0.86	1.88	1.88	±	0.23	0.70	0.08	0.94
G:F	kg	0.12	0.16	±	0.02	0.09	0.11	±	0.05	0.16	0.16	±	0.02	0.27	0.78	0.62

Figure 3.1. Experimental design of diet phases including Priming (P), Transition (T1/T2) and Finishing (F) used during OG supplementation.

	Priming (P)					T1	T2	Finishing (F)								
Day	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	104

[1]-Blood and body weights were collected on all days listed
[2]-Ultrasound data was collected on day 49, 69 and 104 of supplementation

Figure 3.2. OmniGen-AF supplementation decreases fat deposition over the 12th rib and rump, increases ribeye area and relative ribeye area (measured by ultrasound), resulting in a lower numerical yield grade. Diet and Time effects are given on each graph; no treatment x time interactions were present, however due to the biological implications identified on day 104 of supplementation another analysis was conducted on day 104 alone. A * on this day indicates OG supplemented steers were significantly different than control. Data are means ±SE.

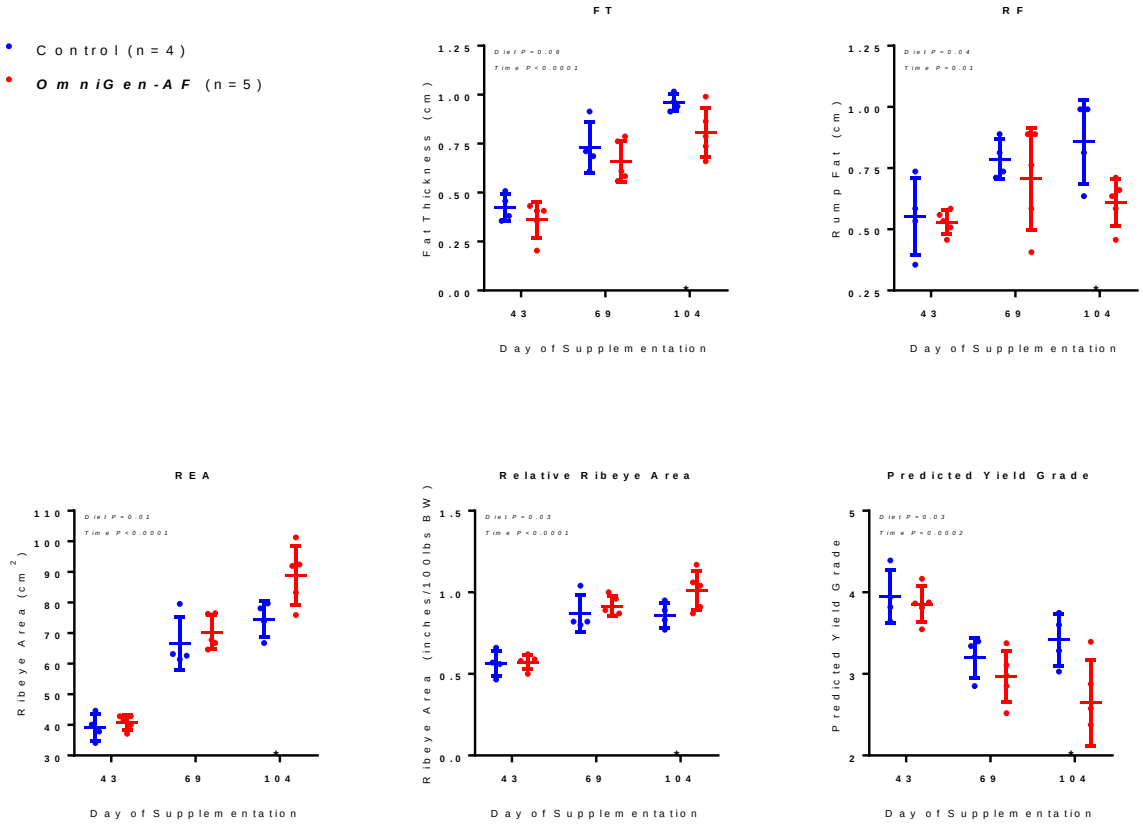


Figure 3.3. OG Supplementation decreases serum NEFA concentrations; data are presented as means ± standard deviation.

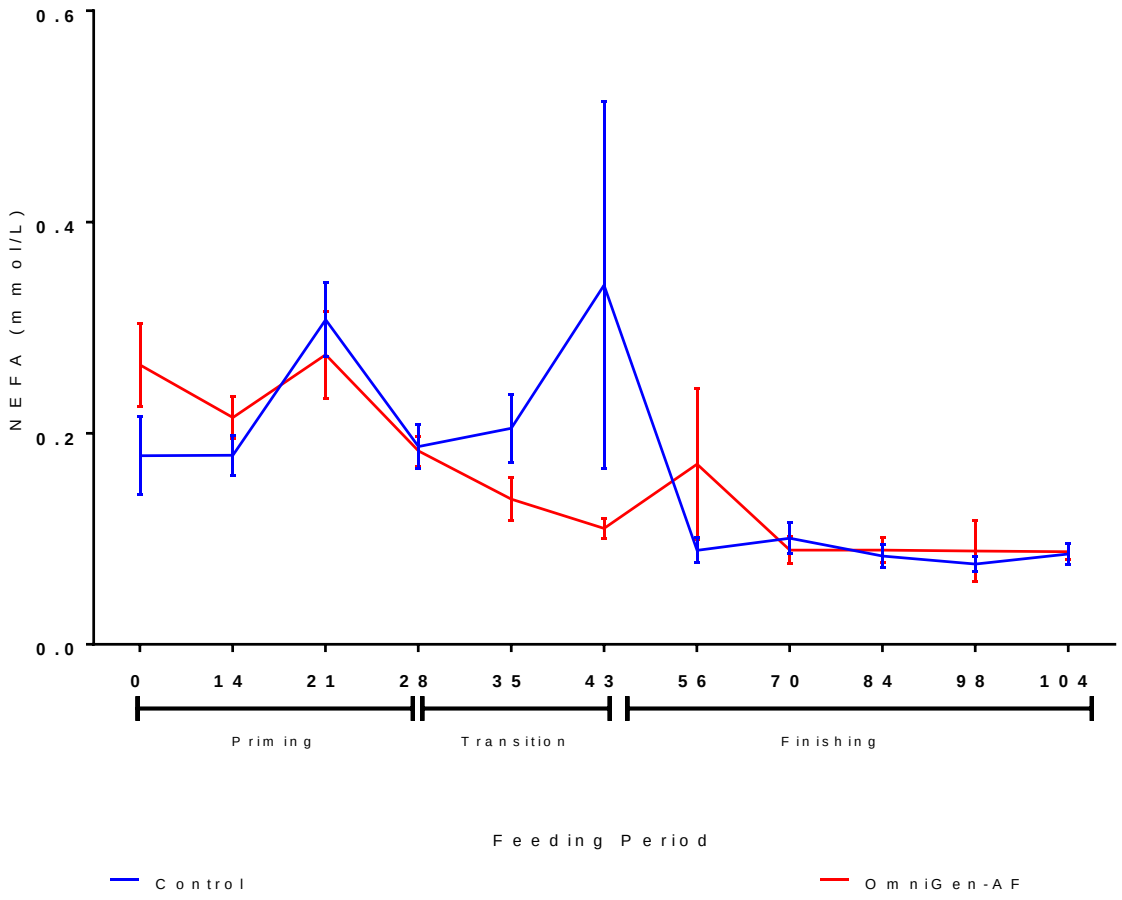


Figure 3.4. Serum haptoglobin concentrations in steers through multiple diet phases; data are presented as means ± standard deviation.

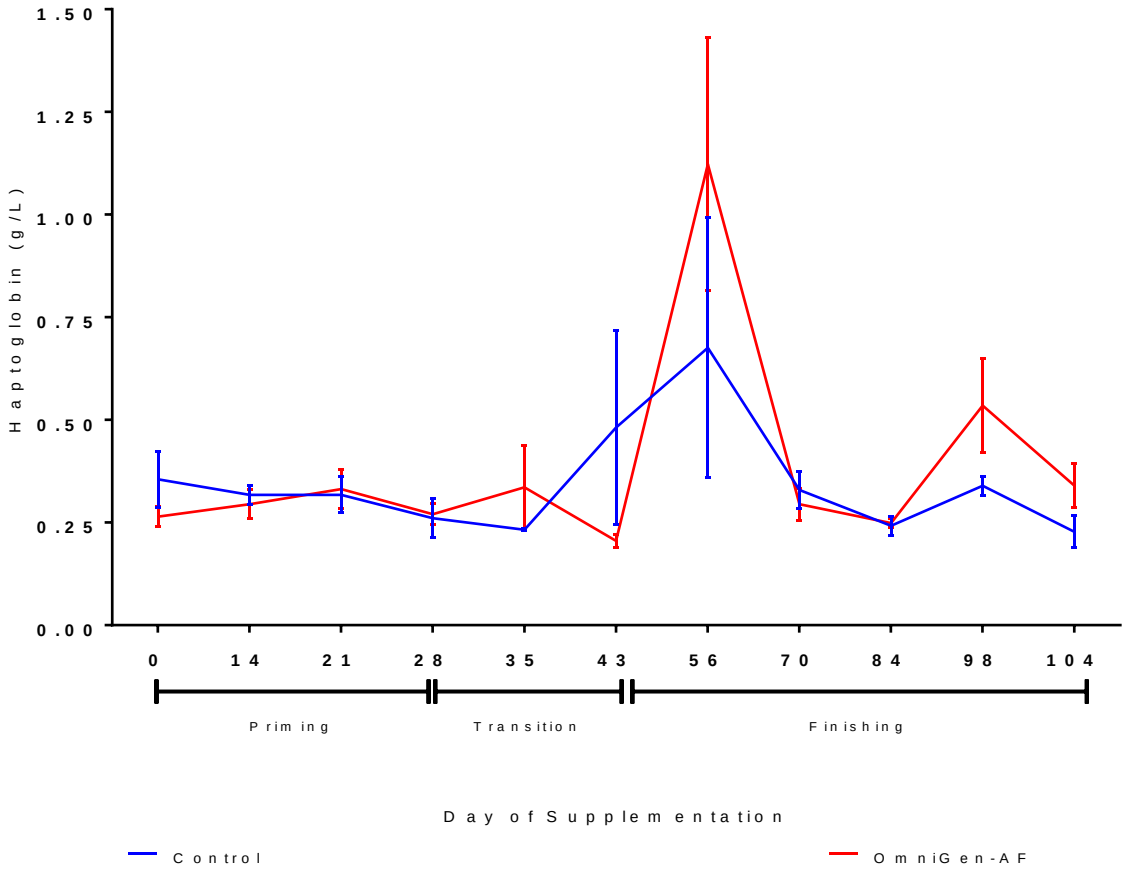


Figure 3.5. OG Supplementation attenuates the decline in serum paraoxonase (PON). Data are shown as change from baseline (LS means ±SE)

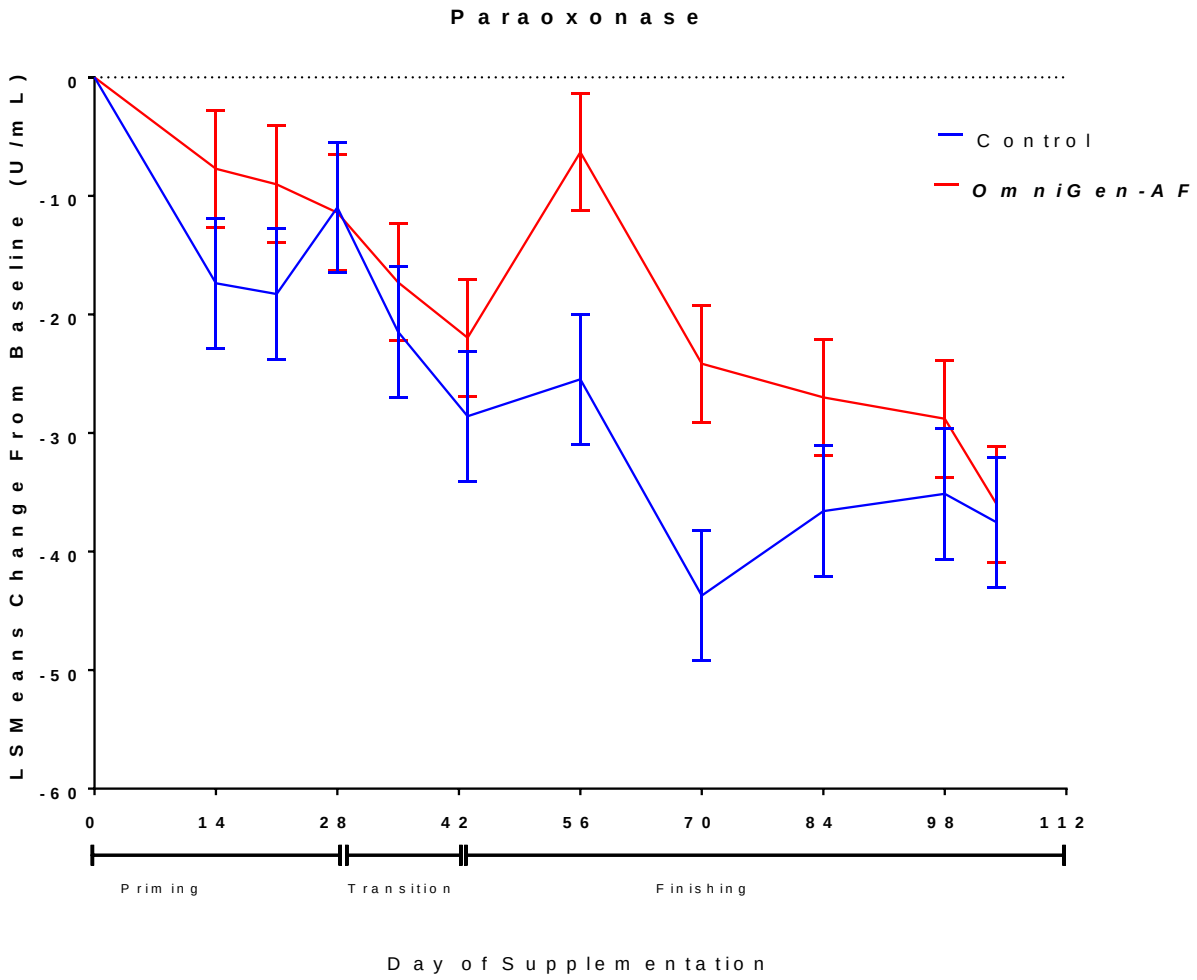


Figure 3.6. OG supplementation attenuates liver cell damage induced by high concentrate feeding as indicated by AST (A) and GGT (B) concentrations. Data are presented as means ± standard deviation.

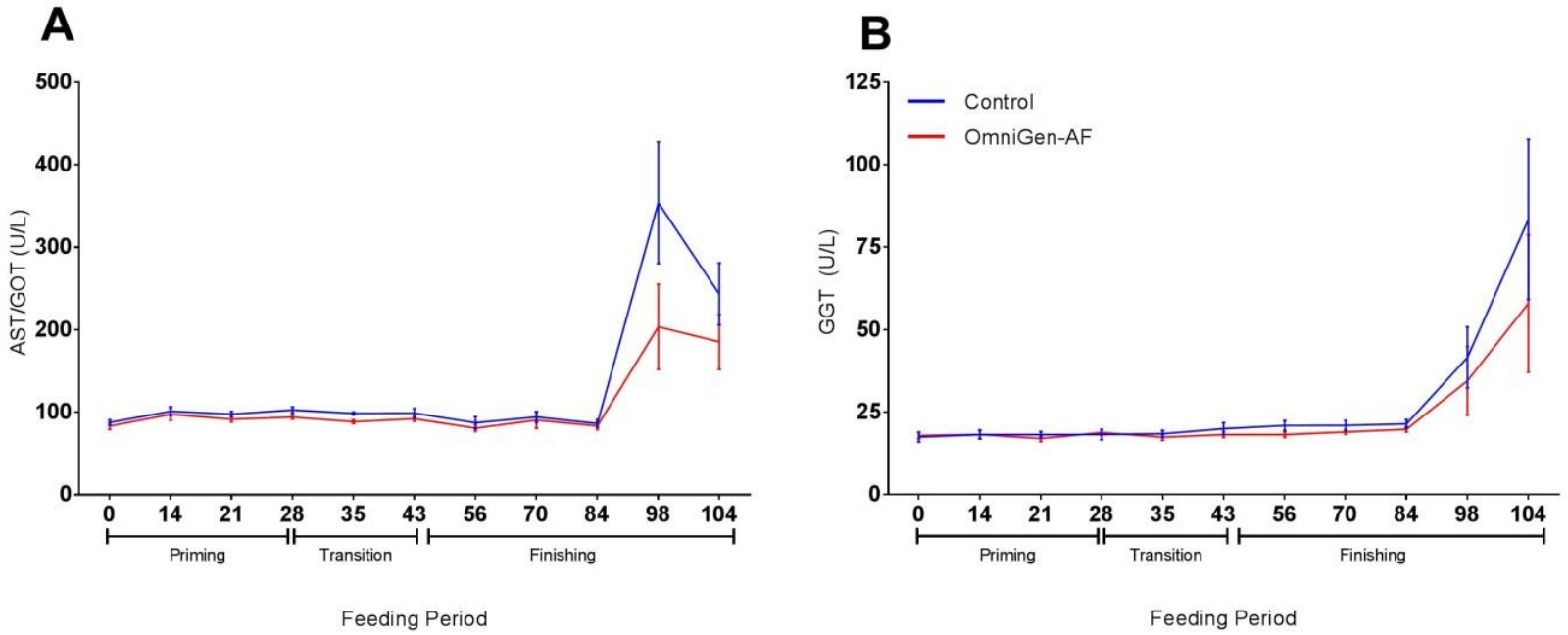
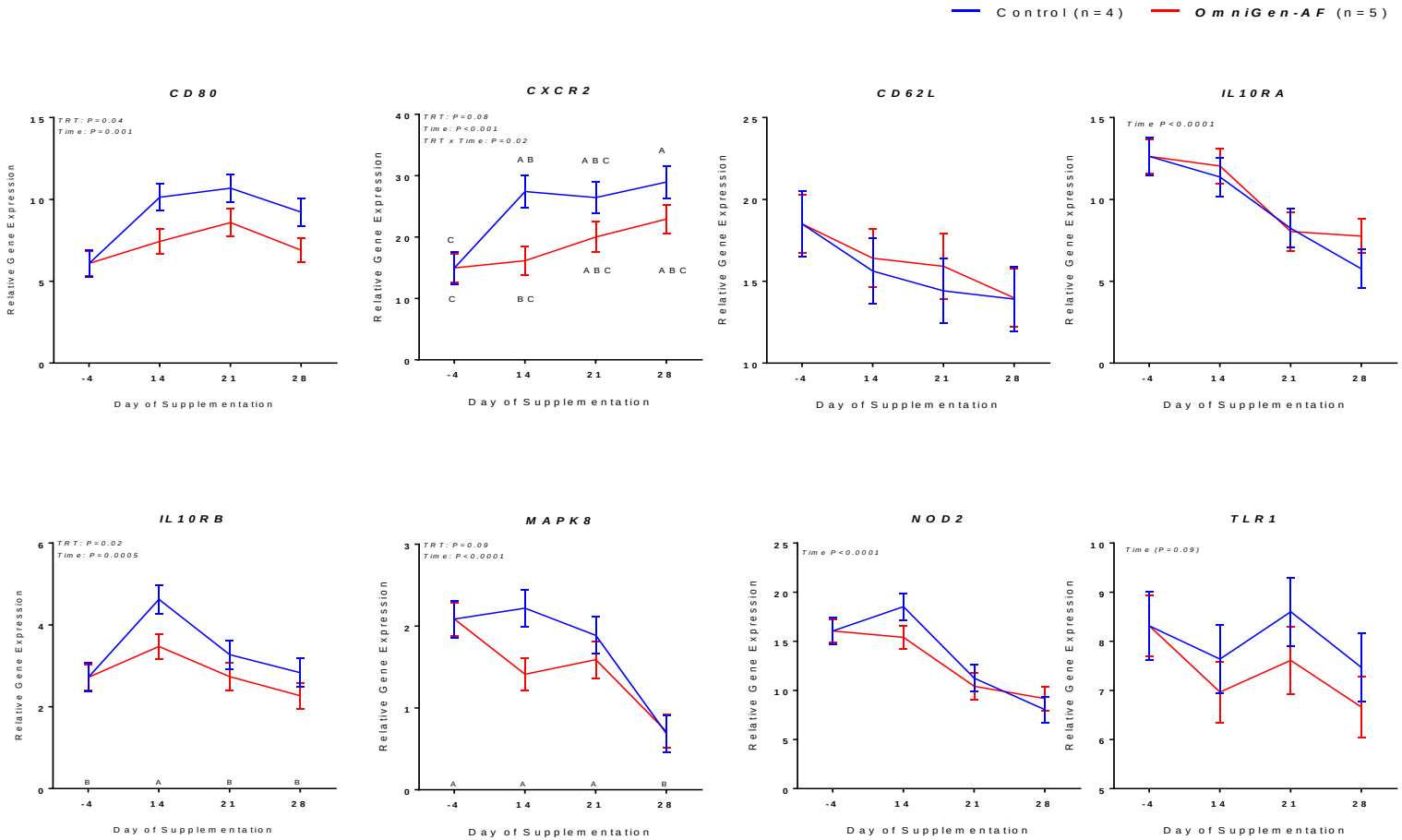


Figure 3.7. Investigation of 8 immune markers in purebred Angus steers limit-fed a backgrounding diet and supplemented with OmniGen-AF compared to non-supplemented controls. Significant effects and effects which tend toward significance are present in the upper left hand corner of the graph. When a treatment x time interaction occurs (CXCR2), time points with dissimilar letters were identified as different comparisons (post-hoc analysis). Gene expression is relative to *RPL19*, *RPS9*, and *TBP* expression; data are LSMeans ±SE.



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CHAPTER 4

THE EFFECT OF SUPPLEMENTING AN IMMUNOMODULATORY FEED ADDITIVE TO PUREBRED REPLACEMENT BEEF HEIFERS

Introduction

Immune supplementation with feed additives is a concept used in dairy cattle (Caroprese et al., 2009; Zaworski et al., 2014; Sordillo, 2016) and poultry (Lee et al., 2008; Lee et al., 2011) industries, but is rather novel in the beef industry. Transition cows supplemented with OmniGen-AF® (OG) and challenged with an intravenous LPS challenge presented enhanced immunocompetence and milk yield relative to their control counterparts (Brandão et al., 2016). In periparturient heifers, OG supplementation amplified leukocyte function consistent with antibacterial activity and enhanced mammary gland health (Nace et al., 2014). When supplemented to lactating, pasture-fed dairy cattle, *IL8R* and *CD62L* were upregulated (in whole blood compared to controls) on days 60 and 90 of supplementation (Playford et al., 2014). Heifers supplemented with the product have displayed increased *CD62L* expression and improved mammary health (Ryman et al., 2013). OG-supplemented rats showed a potential increase in recognition and responses to bacterial pathogens, an increase in T-cell activation and differentiation during the first 28 days of supplementation (Branson et al., 2016). To validate, translate, and extend the results of the rat model in growing Angus heifers, we designed the study at hand. We hypothesized that supplementation of purebred Angus replacement heifers with OmniGen-AF for 28 d, results in changes in the metabolic and immune profile.

Materials and Methods

Cattle and Diets

All animals were cared for under guidelines outlined in the Phibro Animal Health Animal Care and Use policy. Sixteen purebred Angus heifers were blocked by body weight (the mean of two pre-feeding body weights on consecutive days) into one of two dietary groups; Control (0 g OG/hd/d) and OG (OG, 56 g/hd/day). Product (for the treatment group) was top-dressed at every feeding. Cattle were trained to eat behind Calan Gates (American Calan, Northwood, NH) and housed in a freestall barn. Cattle were allowed a 14d training period and 7d acclimatization period before diets were supplemented. Feed was mixed in a custom mix wagon and offered to cattle once daily. Orts were collected every 24 hours and dry matter analysis was conducted to determine DMI (as outlined in Cunniff, 1998). Diets were collected twice throughout the study and sent to SureTech laboratories (Indianapolis, IN 46221, USA) for analysis (**Table 4.1**). Cattle were offered a traditional replacement heifer diet *ad libitum* (**Table 4.1**) and weighed weekly throughout the study, and average daily gain (ADG), feed efficiency (F:G) were calculated based off these figures.

Blood Collection, RNA Purification

Blood samples were collected twice for baseline analysis (d -4 and 0) and on d 7, 14, 21, and 28 of supplementation. Whole blood (approximately 3 mL) was collected via jugular puncture into Tempus Blood RNA Tubes (Cat no 4342792, Life Technologies, Carlsbad, CA). Immediately after collection, tubes were shaken vigorously for 15 s. Samples were stored at -20°C until RNA purification was complete. RNA was purified

using the Tempus Spin RNA Isolation kit (Cat no 4380204, Life Technologies); upon completion samples were stored at -80°C until reverse transcription was completed. Blood for serum analysis was collected via jugular puncture BD serum separator tubes (367988 Cat no. BD967988 VWR, Visalia, CA 93291, USA) and spun at 3,000 x *g* for 20 min at 4°C. Samples were aliquoted into 1 mL volumes and stored at -80°C until use. Blood samples for plasma analysis was collected in potassium K_2EDTA tubes and processed in the same manner as serum separator tubes.

Serum Analysis

Serum indicators of kidney function (BUN, creatinine), liver activity (total protein and albumin), liver cell damage (AST, GGT), metabolism (glucose, cholesterol, BHBA and NEFA) and mineral concentrations (Na, K, Cl, Ca, P, and Mg) were measured on a Beckman-Culture AU480 with on-board reagents, calibrated for bovine blood parameters. Haptoglobin was analyzed in duplicate using the bovine haptoglobin kit from MyBioSource (cat no MBS4640002 San Diego, CA USA) according to manufacturer's instructions. Detection limit was 15.6 ng/mL. Interassay CV was 8.4% and intraassay CV was 5.2%. Serum amyloid A was analyzed as per Zaworski (2014) in duplicate using Trideltakit KAA0021 (Invitrogen, Camarillo, CA 93012 USA). Lower end detection limit was 1.25 ng/mL; interassay CV was 18% and intra-asssay CV was 6.1%.

RNA Quality, Reverse Transcription, Thermocycling conditions

RNA quality was determined using an Agilent 2100 bioanalyzer using the assay class eukaryote total RNA nano (version 2.6), all samples had an RIN greater than 8.3.

Samples of sufficient quality were converted to cDNA using the iScript cDNA synthesis kit (Cat no. 1708890; BioRad Hercules, CA). Each sample was run in triplicate for each gene, reference and target. Target genes included markers of immune function: Cluster of differentiation 80 (*CD80*), Interleukin 8 Receptor B (*CXCR2*), Interleukin 10 receptor A and B (*IL10RA, IL10RB*), L-Selectin (*CD62L*), Mitogen-Activated Protein Kinase 8 (*MAPK8*), Nucleotide-Binding Oligomerization Domain Containing 2 (*NOD2*), Toll-like Receptor 1 (*TLR1*). Multiple reference genes were evaluated including: (*GAPDH*), Hypoxanthine Phosphoribosyltransferase (*HPRT1*) Ribosomal Protein L-19 (*RPL19*), Ribosomal Protein Subunit 9 (*RPS9*), TATA-binding Protein (*TBP*), Tyrosine 3-monooxygenase/tryptophan5-monooxygenase activation protein (*YWHAZ*). All primers were ordered from the pre-designed catalog of bovine primers from ThermoFisher Scientific; mastermix was created using 4 µL cDNA, 5 µL Taqman Gene Expression Master Mix (cat no 4369106, ThermoFisher scientific), 0.5 µL primer and 0.5 µL RNAse/DNase free water (all volumes per well). Samples were analyzed in triplicate and RT-qPCR was conducted in a 7900HT machine (Cat no 43290001, Thermofisher scientific, Frederick, MD, USA) with the following thermocycling conditions10 min at 95°C, 40 cycles with 15 s at 95°C and 1 min at 60°C. All genes (target and reference) were analyzed using LinReg Software (Ruijter et al., 2009) to account for efficiency of amplification. Reference genes were selected for stability using GeNorm (Vandesompele et al., 2002); *TBP*, *RPL19* and *RPS9* were the most stable and therefore were used to normalize the data set. All data points were normalized to day 0.

Statistical Analysis

Statistical analyses were performed using version 9.3 of SAS (SAS, Inc., Cary, NC, USA). Serum data were analyzed as change from baseline. All data was tested for normal distribution using the Shapiro-Wilk test in PROC UNIVARIATE, if necessary, serum data were naturally log-transformed and gene data were log2-transformed to achieve normality, respectively. Serum and performance data were analyzed using repeated-measures-in-time analysis in PROC MIXED. Fixed effects of the statistical model were treatment (control and OG), and day of supplementation and the interaction of the two. Repeated measures within animals were modeled using first order autotoregressive variance-covariance matrix, which was the most parsimonious model based on the Akaike information criterion (AIC). Gene expression data were analyzed using PROC GLIMMIX of SAS with time and treatment as fixed effects, animal was used as random variable. To obtain the correct degrees of freedom, the Kenward Rogers degree of freedom adjustment was used. All tests were two sided; significance was declared at $P < 0.05$, tendencies as $0.05 < P < 0.10$.

Results

DMI, Body Weight and ADG

There was no significant difference in DMI between control and OG supplemented heifers. DMI increased over time ($P < 0.0001$); there was no significant treatment x time interaction. Average daily gain (ADG), feed efficiency (Gain:Feed kg), and body weight were not influenced by OG supplementation (**Table 4.2**). Time increased body weight, and ADG (both $P < 0.001$; data not shown).

Serum Analysis

OG supplementation decreased serum phosphorus and magnesium concentrations (compared to baseline). Time influenced serum mineral concentrations (Na, K, Cl, Ca, P, Mg), serum markers of liver cell damage (AST and GGT), liver activity markers (albumin, total protein), and some serum markers of metabolism (cholesterol and NEFA), inflammation (globulin) and kidney function (BUN; Table 4.3*).*

Serum albumin (diet x time $P = 0.003$) concentrations in the OG group initially spiked (D7) compared to baseline (increase of 0.025 g/L ± 0.04 vs. cntl -0.10 ± 1.70 g/L; $P = 0.03$), but was decreased compared to control group (OG -0.16 g/L ± -0.04, CNTL -0.00 ± 0.04 g/L; $P = 0.0007$) by the end of the 28-d supplementation period (Figure 4.1). The interaction of diet and time also influenced (or had a tendency to influence) serum mineral concentrations for Ca (diet x time $P = 0.005$) and Na and Mg (P=0.07). Serum Ca were initially increased in the OG group, (D7-OG 0.075± 0.12 mg/dL vs. CNTL -0.18 ± 0.09 mg/dL; $P = 0.09$) but decreased by d28 (OG -0.56± 0.12 mg/dL vs. CNTL -0.07 ± 0.09 mg/dL; $P = 0.001$). The drop in serum Na concentrations had a tendency to be ameliorated in OG supplemented heifers at d 7 (OG -0.25±1.23 mEq/L vs. CNTL -3.5±0.95 mEq/L; $P = 0.09$), but no other days produced a diet x time interaction. Serum Mg concentrations were decreased in OG supplemented animals on d 14 (OG -0.26± 0.05 mg/dL vs. CNTL -0.08 ±0.03 mg/dL; $P = 0.002$) and 28 (OG -0.30 ± 0.04 mg/dL vs. CNTL -0.18 ± 0.03 mg/dL; P=0.03; **Figure 4.1***).*

Addition of OG to the basal diet had no significant effects on serum markers of inflammation (globulin, serum amyloid A, haptoglobin), kidney function (BUN, creatinine), liver activity (total protein, albumin), hepatocyte damage (AST, GGT),

metabolism (glucose, cholesterol, BHBA, NEFA), and some serum mineral concentrations (Na, K, Cl, Ca). Time did not impact serum concentrations of inflammatory markers (serum amyloid A and haptoglobin), creatinine (kidney function), and metabolism (glucose and BHBA). The interaction or diet x time had no effect on serum concentrations of markers of inflammation, kidney function, hepatocyte damage, liver metabolism, liver activity (total protein) and some serum mineral concentrations (K, Cl, P).

Gene Expression

Analysis of gene expression focused on previously identified OG-regulated genes in rats and cattle. OG supplementation, time or the interaction of the two had no significant effect on *CXCR2*, *CD62L* or *TLR1* gene expression in whole blood of healthy Angus heifers. Time increased *CD80 (*$P = 0.02$), *IL10RA* ($P < 0.0001$), *IL10RB* ($P = 0.0003$), *MAPK8* ($P < 0.0001$) and *NOD2* ($P < 0.0001$) gene expression. OG supplementation increased *IL10RA* and *NOD2* gene expression ($P = 0.02$ and $P = 0.005$ respectively; **Figure 4.2**). A treatment x time interaction was present for *IL10RA* and OG supplemented heifers expressed more *IL10RA* than control counterparts on d 14.

Discussion

Production Parameters

OmniGen-AF supplementation did not affect DMI, body weight, average daily gain or feed efficiency in this study. This concurs with previous work done in OG supplemented beef cattle (Schell et al., 2016; Armstrong et al., 2016b; Reuter, 2007).

Serum Mineral Concentration

Serum Mg concentrations had a tendency to be regulated by the interaction of OG supplementation and diet phases (backgrounding, transition and finishing) in a previous feedlot steer study (Chapter 3), in which OG supplemented cattle had increased Mg serum concentration (relative to baseline) during the OG priming phase (a diet which mimics the one used in this study). In that study, the change of serum Mg concentrations was measured as a change from baseline on d 14, 21 and 28 of OG supplementation when fed a predominantly forage based diet and countered the results of the study at hand, when on day 14 OG supplemented cattle had increased serum Mg concentrations (relative to baseline) compared to control counterparts. Cattle in both studies were comparable in age; it is possible that OG supplementation could have a differential sex response when fed to growing beef cattle. This would encourage the conclusions of Schell et al (2016) who found that the effects of OG supplementation are differentially regulated by sex in growing beef cattle.

Metabolic Response

OG supplementation did not regulate NEFA concentrations, in contrast to the work of Sanchez et al. (2014b) where OG supplemented cattle had lower NEFA concentrations than their control counterparts. Likely this is due to the interaction of difference in basal diet and supplementation; cattle on a finishing diet have more need for metabolic regulation than do cattle fed at maintenance, or slightly above maintenance.

Acute Phase Protein Response

Acute phase proteins were not influenced by OG supplementation in this group, in contrast to previous (unpublished) data in which OG supplementation during the finishing phase in feedlot cattle was higher. This supports previous conclusions that haptoglobin concentrations are not different in growing Angus heifers without an induced, controlled stress (Schell et al., 2016). Additionally, liver enzymes AST and GGT were not impacted. A previous study conducted with OG-supplemented finishing steers (unpublished data) predict a liver rescue effect during the final weeks of a high concentrate diet; however, in this study no high concentrate diet was used in these heifers. We logically draw the conclusion that in order to see the liver rescue effect, a stress must be present.

Immune Function (mRNA expression from whole blood)

OG supplementation did not have the same effect on markers of immune function in whole blood as seen in previous studies. Previous studies with OG supplementation in beef cattle have not detected any regulation of *NOD2;* however, this upregulation has been found in rats (Branson et al., 2016). Previous downregulation of *IL10RA* has been found in OG supplemented cattle (Armstrong et al., 2015); however, in this set of heifers upregulation of this gene occurred. Even though the diet effect of *IL10RB* is not significant, the trend of expression closely follows it's dimer pair. Previous studies in beef cattle indicate OG supplementation may downregulate the IL10 receptor complex in the first 28 d on product (Armstrong et al., 2015, 2016a); however, results of heifers in this study contradict that conclusion. It is important to note that heifers in this study were

smaller than those used in Armstrong et al. (2015), and of a different sex than those used in Armstrong et al. (2016). Physiology, age/ maturity or sex could be contributing to the different response in gene expression seen in this study.

Conclusion

In conclusion, supplementing healthy, growing Angus heifers in this study did not regulate metabolism, impact liver function, and acute phase proteins and did not impact growth. Gene expression in whole blood did not follow existing trends established in our previous studies. Some serum mineral concentrations were altered. When compared with existing knowledge regarding the product, we conclude that the effect of OG supplementation on serum response markers may be modified by gender, age, and background diet. Additionally, more meaningful differences in performance, metabolism and immune function are seen when the product is fed during times of stress.

Table 4.1. Basal replacement heifer diet offered *ad libitum* to growing purebred Angus heifers.

	Unit	Dry Matter Basis	As-Fed
Moisture	(%)		25.155
Dry Matter	(%)		74.845
Protein	(%)	13.16	9.835
FAT	(%)	2.645	1.98
ADF	(%)	34.625	25.915
NDF	(%)	55.175	41.295
NDICP	(%)	2.635	1.97
NFC	(%)	26.29	19.68
ASH	(%)	5.365	4.03
Ca	(%)	0.87	0.655
P	(%)	0.27	0.205
Mg	(%)	0.245	0.185
K	(%)	1.825	1.37
S	(%)	0.255	0.19
Na	(%)	0.255	0.19
Cl	(%)	0.535	0.4
TDN		64.5	47.5

Table 4.2. Production parameters of replacement heifers during a one month product supplementation of OmniGen-AF. Data are LSMeans ±SE.

	Control	OmniGen-AF	SE	P-Value
n	8	8		
Body weight (kg)				
d0	273.18	271.70	8.88	0.90
d7	281.14	277.73	8.88	0.79
d14	281.82	279.43	8.88	0.85
d21	288.52	285.91	8.88	0.84
d28	293.18	284.66	8.8	0.51
DMI (kg)	6.78	6.65	0.06	0.12
ADG (kg)	0.71	0.46	0.14	0.79
G:F (kg)	0.10	0.09	0.01	0.96

Table 4.3. Results of statistical analysis of serum parameters in growing purebred Angus heifers. Diet (Control or OmniGen-AF), and time (change from baseline on day 7, 14, 21, and 28) were analyzed as fixed factors with animal as the random, repeated variable.

		Mean (Δ from baseline)				P-Value		
	Unit	Control	OmniGen-AF		SE	Diet	Time	Diet x Time
Inflammation								
Globulin	g/dL	-0.09	-0.12	±	0.08	0.82	0.002	0.16
SAA	ng/mL	0.47	0.82	±	0.26	0.32	0.44	0.98
Haptoglobin	ng/mL	1.94	3.56		0.82	0.12	0.86	0.34
Kidney Function								
BUN	mg/dL	1.23	0.49	±	0.38	0.2	<0.0001	0.34
Creatinine	mg/dL	-0.05	-0.03	±	0.03	0.75	0.11	0.58
Liver Activity								
Total Protein	g/dL	-0.12	-0.19	±	0.08	0.57	<0.0001	0.45
Albumin	g/dL	-0.03	-0.08	±	-0.03	0.25	0.002	0.003
Liver cell damage								
GGT	U/L	-0.73	0.21	±	0.61	0.3	0.06	0.35
AST	U/L	-3.85	-4.28	±	2.22	0.89	0.02	0.53
Metabolism								
Glucose	mg/dL	-1.08	-0.53	±	1.27	0.77	0.84	0.51
Cholesterol	mg/dL	5.35	4.94	±	1.77	0.87	<0.0001	0.75
BHBA	mg/dL	0.26	0.31	±	0.11	0.75	0.18	0.55
NEFA	mEq/L	0.85	0.8	±	0.09	0.71	0.001	0.93
Mineral								
Sodium	mEq/L	-2.48	-2.11	±	0.57	0.66	0.0001	0.07
Potassium	mEq/L	-0.02	0.07	±	0.08	0.42	0.02	0.26
Chloride	mEq/L	-2.95	-2.18	±	0.41	0.20	0.003	0.11
Calcium	mg/dL	-0.07	-0.13	±	0.06	0.50	0.0002	0.005
Phosphorus	mg/dL	0.69	0.1	±	0.17	0.03	<0.0001	0.13
Magnesium	mg/dL	-0.08	-0.17	±	0.02	0.005	<0.0001	0.07

Control: n=8; OG n=8. Data are LSMeans±SE

Figure 4.1. The interaction of OG supplementation and time influences (or have a tendency to influence) the change in serum albumin, sodium, calcium and magnesium (compared to baseline). Control (n=8) heifers are represented in blue while heifers supplemented with OmniGen-AF (n=8) are represented in red. A dotted line represents baseline values, lines are the LS means of change from baseline ± SED. *Indicates mean separation between diet groups on given day is significant ($P \leq 0.05$); # indicates comparison is trending toward significance ($0.05 \leq P \leq 0.10$).

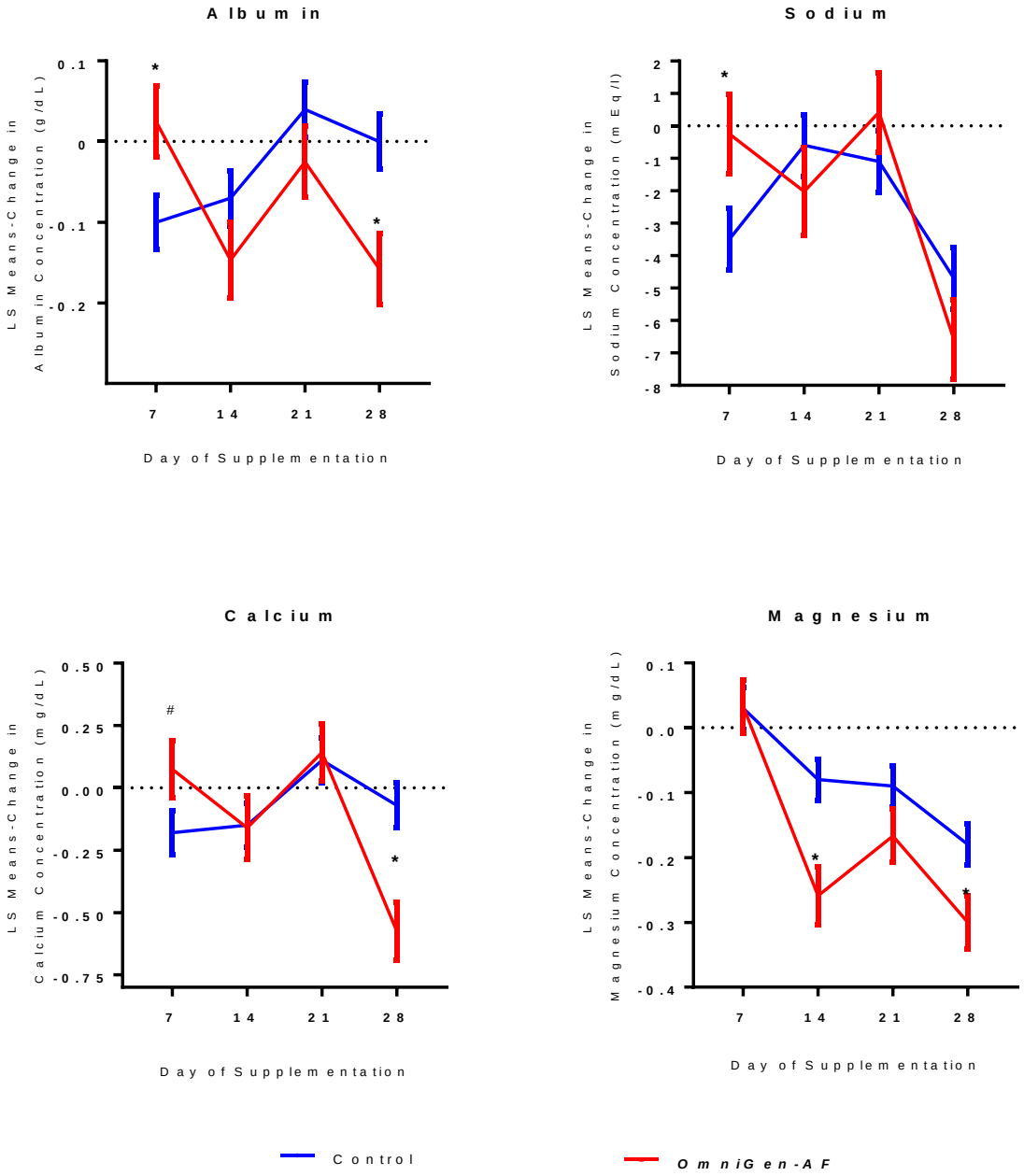
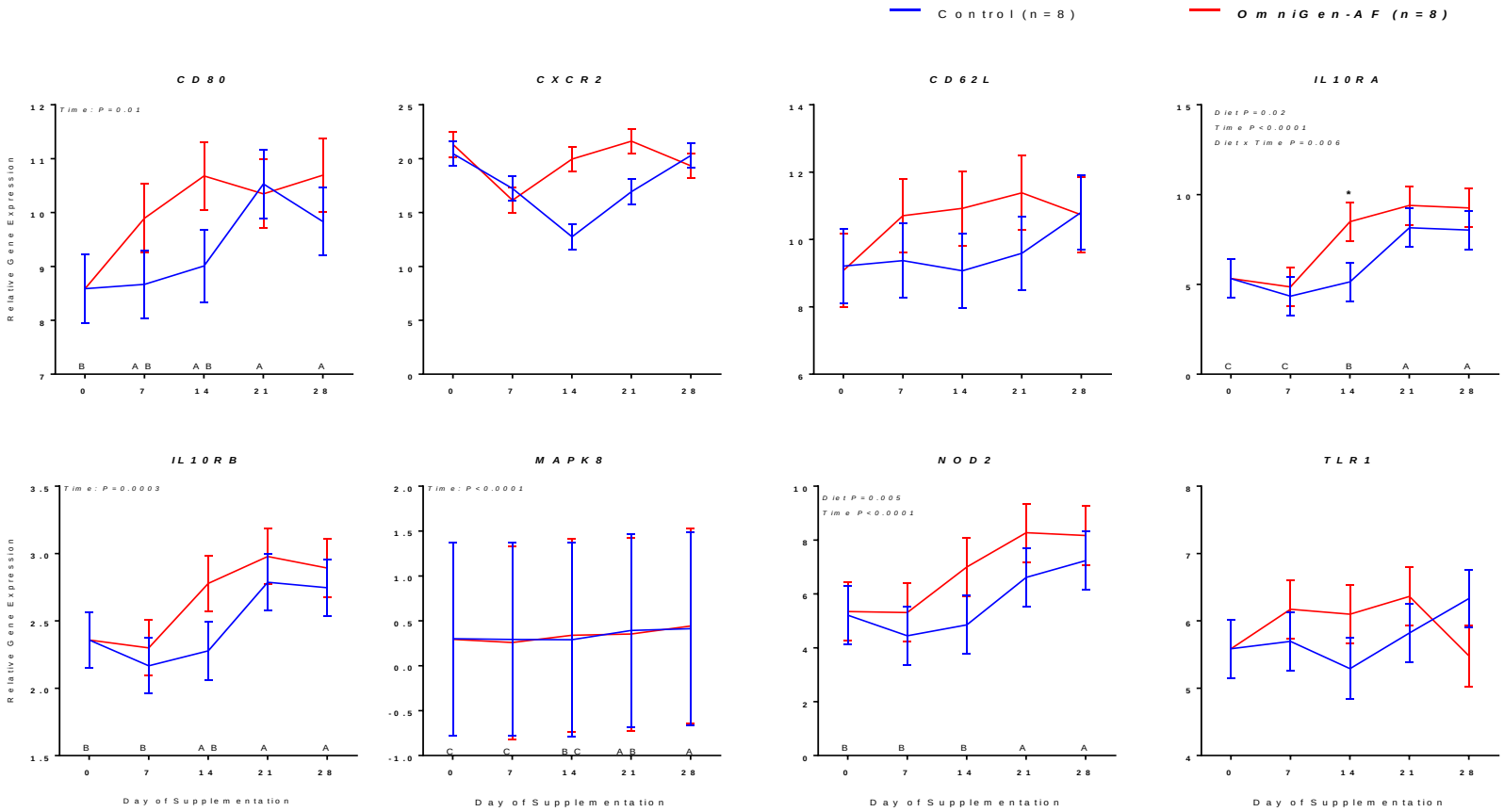


Figure 4.2. Investigation of 8 immune markers in purebred Angus heifers supplemented with OmniGen-AF compared to non-supplemented controls. When present, values representing significant differences are located in the upper left hand corner of the graph. When a OG supplementation x time interaction occurs, time points with a * indicate mean separation between groups; time points with dissimilar letters were identified as different comparisons (post-hoc analysis). Gene expression is relative to *RPL19*, *RPS9* and *TBP* expression.



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CHAPTER 5

CONCLUSION

Immune supplementation is a novel management tool for beef production systems, and, to our knowledge, no other studies have previously reported on the effects of supplementing OG. OG is an immune supplement used in dairy cows, which improved animal health by augmenting the immune system (Wang et al., 2007). Beef cattle experience metabolic and immunologic challenges during backgrounding, pasturing and finishing such as diet changes, unstable environmental conditions and pathogen challenges and, thus, would benefit from dietary supplementation. Immune supplementation may support the IMA to better handle these conditions. In three studies, we explored regulation of metabolism and the immune system through dietary OG supplementation in replacement beef heifers and finishing steers. The results from all three studies do not only complement our knowledge of dietary immune system supplementation in other models (ruminant and non-ruminant); they also provide novel data for beef replacement heifers, a largely unexplored area of research, and finishing cattle experiencing metabolic stress.

In eight 10.5-mo old purebred Angus replacement heifers, OG supplementation altered gene expression of 35% of receptors and 100% of decoy receptors evaluated during the priming phase (**Study 1**). Receptors, altered by OG supplementation, were promiscuous in their binding capabilities, and multifunctional in the immune system. KEGG pathway analysis identified MAPK signaling, Jak-STAT signaling, cytokine-cytokine receptor interaction, osteoclast differentiation, chemokine signaling, TNF signaling and hematopoietic cell lineage pathways to be

downregulated by OG supplementation; in contrast, DAVID analysis did not identify any of these pathways as enriched. To truly recognize the impact OG dietary supplementation to beef heifers during the OG priming phase, these data will need to be considered with other future studies to determine functional impact of OG on the IMA.

In nine purebred Angus steers supplemented with OG expressed less *CD80* and *IL10RB* and had a tendency to express less *CXCR2* and *MAPK8* in whole blood than their control counterparts during the OG priming phase (**Study 2**). *IL10RB* and *CXCR2* downregulation is consistent with results of the first study; however, *CD80* and *MAPK8* regulation was different than previously conducted rodent models (Branson et al., 2016). In these steers, *CXCR2* expression was also inconsistent with previous reports in dairy cattle supplemented with OG for longer than 28 days (Playford et al., 2014). The differences in these results could lie in the difference in feeding duration (OG priming vs. action phase), or physiology (beef cattle vs. rats or dairy cattle).

After the OG priming phase, introducing a metabolic challenge (high concentrate diet throughout finishing) induced inflammation (indicated by an increase in haptoglobin); furthermore, serum markers of hepatic function indicate a decrease in liver function. However, the increase of liver cell damage serum markers AST/GGT was attenuated in OG supplemented cattle and the decline in protective enzyme paraoxonase in serum was also attenuated compared to non-supplemented controls. OG-supplemented cattle also had decreased serum NEFA concentrations during the finishing period. All of these serum markers of cattle health occurred in tandem with

increased ribeye area, decreased 12th rib fat deposition and rump fat, ultimately leading to a decreased predicted numerical yield grade with no effect on quality grade. These predicted advantages at harvest are valuable from an industry standpoint, because they will help improve carcass grades and, ultimately, carcass value. All of these results, when taken together result in the following conclusions: OG supplementation regulated the inflammation response during the OG priming phase, limited hepatocyte damage, improved markers of immune function and lowered predicted numerical yield grades.

In sixteen 8.5-mo old purebred replacement Angus heifers, OG supplementation did not induce any changes in serum markers of metabolism, liver function, or acute phase proteins (**Study 3**). *IL10RA* and *NOD2* expression in whole blood was increased in OG supplemented heifers during the OG priming phase. *NOD2* expression has been shown to be increased in whole blood of rats during the OG priming phase (Branson et al., 2016) , but this effect has never been demonstrated in cattle studies before. OG supplementation downregulated *IL10RA* expression in whole blood in the first study, had no effect in the second study, but was upregulated in this study. It is possible that cattle physiology and maturity may be interacting with OG supplementation to produce different results across different studies. Heifers in the first study were more mature (older and heavier) than those in the third study, and the second study was conducted with steers. These factors could contribute to the differences in gene expression we saw, and explain the differences between studies.

With the results from studies 1, 2 and 3 considered, the following conclusions can be drawn from our novel studies in beef cattle: OG supplementation may differentially regulate markers of immune function during the OG priming phase in growing beef cattle (sex and maturity dependent). When a chronic metabolic stress is introduced, OG supplementation may improve liver cell function. OG supplementation can increase predicted ribeye area, and decreased fat deposition (rump and 12^{th} rib) in finishing steers, leading to lower predicted numerical yield grades without influencing intake, gain or efficiency. These results indicate a benefit of providing OG in replacement heifer and finishing steer diets to help homeorhetic adaptation of the IMA.

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APPENDIX I

BLAST identification, gene symbols, names and functions for a targeted qPCR array designed to evaluate the cytokine inflammatory response in whole blood of healthy Angus heifers

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001035018	AIMP1	Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	MGC128105, SCYE1, p43	Protein coding gene induced by apoptosis, involved in the control of angiogenesis, inflammation and wound healing.
NM_001099141	BMP2	Bone morphogenetic protein 2	MGC159940	Protein coding gene which belongs to the transforming growth factor-β superfamily. Acts as a disulfide-linked homodimer and induces bone and cartilage formation.
NM_001166616	C5	Complement component 5	-	Fifth component of a complement. Plays a role in inflammation and cell killing.
XM_001253011	CCL1	Chemokine (C-C motif) ligand 1	-	Chemotactic for monocytes, NK cells, immature B cells and dendritic cells.
NM_205773	CCL11	Chemokine (C-C motif) ligand 11	MGC137967	Binds to CCR3 and displays chemotactic activity for eosinophils, thought to be involved in atopic dermatitis, allergic rhinitis, asthma and parasitic infections.
XM_002695627	CCL16	Chemokine (C-C motif) ligand 16	-	Displays chemotactic activity for lymphocytes and monocytes. Potent myelosuppressive activity. Suppresses proliferation of myeloid progenitor cells. Unregulated by IL-10.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
XM_001788943	CCL17	Chemokine (C-C motif) ligand 17	-	Displays chemotactic activity for T lymphocytes. The product of this gene binds to chemokine receptors CCR4 and CCR8. Plays roles in T cell development in thymus and trafficking and activation of mature T cells.
NM_174006	CCL2	Chemokine (C-C motif) ligand 2	MCP-1, MCP-1A, MCP1, MCP1A, SCYA2	Chemotactic activity for monocytes and basophils. Has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates (I.e. psoriasis, rheumatoid arthritis and atherosclerosis). Binds to chemokine receptors CCR2 and CCR4.
NM_174263	CCL20	Chemokine (C-C motif) ligand 20	-	Chemotactic attracts lymphocytes, sometimes neutrophils. Inhibits the proliferation of myeloid progenitors. Involved in the formation and function of the mucosal lymphoid tissues by attracting lymphocytes and dendritic cells toward epithelial cells. Possesses antibacterial activity for *E. coli*.
NM_001099162	CCL22	Chemokine (C-C motif) ligand 22	MGC152608	Displays chemotactic activity for monocytes, dendritic cells, natural killer cells and chronically activated T lymphocytes. Binds to CCR4.
NM_001046596	CCL24	Chemokine (C-C motif) ligand 24	MGC137287	Chemotactic for resting T-Lymphocytes and eosinophils. Offers low capacity for neutrophil chemotactic activity. Strong suppressor of colony formation by a multipotential hematopoietic progenitor cell line. Binds to CCR3.
NM_001205635	CCL26	Chemokine (C-C motif) ligand 26	-	Chemotactic for eosinophils and basophils. Binds to CCR3.
NM_174511	CCL3	Chemokine (C-C motif) ligand 3	CCL3L1, MIP1A, SIS-beta	Monokine with inflammatory and chemokinetic properties. Binds to CCR1 and CCR5. Major HIV suppressive factors produced by CD8+ T-cells.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001075147	CCL4	Chemokine (C-C motif) ligand 4	MIP1B ACT2 AT744 G-26 SCYA2	Induces a dose-dependent inhibition of different strains of HIV-1, HIV-2 and SIV. Retains abilities to induce down-modulation of CCR5 surface expression. Can inhibit the CCR5 mediated entry of HIV-1 in T-Cells Major HIV suppressive factors produced by CD8+ T-cells.
NM_175827	CCL5	Chemokine (C-C motif) ligand 5	MGC127014, RANTES	Chemoattractant for blood monocytes, memory T-helper cells, and eosinophils. Causes release of Histamine from basophils, activates eosinophils. Binds to CCR1, CCR3, CCR4 and CCR5. Major HIV-suppressive factors produced by CD8+ T Cells. Induces a dose-dependent inhibition of some viruses.
NM_174007	CCL8	Chemokine (C-C motif) ligand 8	SCYA8	Chemotactic factor which attracts monocytes, lymphocytes, basophils and eosinophils. Can bind heparin. Can inhibit chemotactic effect of CCL7, CCL2, CCL5, and CCL8.
NM_001077839	CCR1	Chemokine (C-C motif) receptor 1	MIP1aR RANTES receptor	Binds to MIP1a, MIP1delta, RANTES and MCP-3 also binds (to a lesser degree) MIP-1β or MCP-1. After binding, subsequently transduces a signal by increasing the intracellular calcium ions level. Responsible for affecting stem cell proliferation.
NM_001194964	CCR10	Chemokine (C-C motif) receptor 10	-	Transduces a signal by increasing the intracellular calcium ions level and stimulates chemotaxis in a pre-B cell line after binding to CCL27 and CCL28.
NM_001194959	CCR2	Chemokine (C-C motif) receptor 2	MCP-1 Receptor	Receptor for CCL2, CCL7 and CCL13 chemokines. Mediates agonist-dependent calcium mobilization and inhibition of adenylyl cyclase.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001194960	CCR3	Chemokine (C-C motif) receptor 3	-	Predicted to be a seven transmembrane protein. Contributes to proper positioning of activated T cells within antigenic challenge sites and specialized areas of lymphoid tissues.
NM_001100293	CCR4	Chemokine (C-C motif) receptor 4	-	Receptor for MIP1, RANTES, TARC and MCP-1.
NM_001011672	CCR5	Chemokine (C-C motif) receptor 5	MGC137803	Receptor for many inflammatory chemokines. Transduces a signal by increasing intracellular calcium ion levels. Plays a role in the control of granulocytic lineage proliferation and differentiation.
NM_001194961	CCR6	Chemokine (C-C motif) receptor 6	LARC Receptor	Binds to MIP3-alpha/LARC. Transduces signal by altering intracellular Calcium ion levels. Important for B- lineage maturation and antigen-driven B-cell differentiation. May regulate dendritic/T Cell migration and recruitment.
NM_001194962	CCR8	Chemokine (C-C motif) receptor 8	-	Chemokine receptor predicted to be a seven transmembrane protein similar to G-Protein coupled receptors.
NM_174624	CD40LG	CD40 ligand	TNFSF5	Mediates B-cell proliferation in the absence of co-stimulus as well as IgE production in the presence of IL-4. Involved in immunoglobulin class switching.
NM_174026	CSF1	Colony stimulating factor 1 (macrophage)	Lanimostim MCSF1	Plays essential role in the regulation of survival, proliferation and differentiation of hematopoietic precursor cells especially macrophages and monocytes. Stimulates the release of pro-inflammatory cytokines. Regulates osteoclast proliferation and differentiation, the regulation of bone resorption and is required for correct bone development. Regulates cell adhesion and cell migration, plays a role in lipoprotein clearance.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_174027	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	CSF, GM-CSF, GMCSF	Stimulates the growth and differentiation of hematopoietic precursor cells from various lineages including granulocytes, macrophages, eosinophils and erythrocytes.
NM_174028	CSF3	Colony stimulating factor 3 (granulocyte)	GCSF	Controls the production, differentiation and function of granulocytes.
NM_001102558	CX3CR1	Chemokine (C-X3-C motif) receptor 1	MGC140194	Acts as a receptor for the CX3C chemokine fractalkine and mediates both its adhesive and migratory functions.
NM_001046551	CXCL10	Chemokine (C-X-C motif) ligand 10	MGC137238	Chemotactic for monocytes and T-lymphocytes. Binds to CXCR3. Binding results in pleiotropic events such as stimulation of monocytes, NK cell migration, T-cell migration and modulation of adhesion molecule expression.
NM_001113173	CXCL11	Chemokine (C-X-C motif) ligand 11	-	Chemotactic for interleukin-activated T-cells but not unstimulated T-cells, neutrophils or monocytes. Induces calcium release in activated T-cells. Binds to CXCR3, may play an important role in CNS diseases where T-cell recruitment is involved. Also thought to play a role in skin immune responses.
NM_001113174	CXCL12	Chemokine (C-X-C motif) ligand 12	MGC139895, SDF1	Chemoattractant active on T-Lymphocytes and monocytes. Activates CXCR4 to induce rapid increase in levels of intracellular calcium ions. Binds to ACKR3 to active the bet-arrestin pathway. Acts as a positive regulator of monocyte migration, and a negative regulator of monocyte adhesion. Decreases monocyte adherence to surfaces coated with ICAM-1.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001015576	CXCL13	Chemokine (C-X-C motif) ligand 13	MGC134170, Angie	B lymphocyte chemoattractant, preferentially promotes the migration of B lymphocytes (compared to T cells and macrophages) by stimulating calcium influx of cells expressing BLR-1. Functions in the homing of B lymphocytes to follicles.
NM_175700	CXCL2	Chemokine (C-X-C motif) ligand 2	CXCL1, GRO1, MGSA	Produced by activated monocytes and neutrophils at the site of inflammation. Suppresses hematopoietic progenitor cell proliferation.
NM_174300	CXCL5	Chemokine (C-X-C motif) ligand 5	CXCL6, GCP-2, GCP2, SCYB6	Involved in neutrophil activation and recruitment.
NM_001113172	CXCL9	Chemokine (C-X-C motif) ligand 9	HuMIG Crg-10 MIG CMK	Cytokine which affects growth, movement or activation state of T cells. Binds to CXCR3. Most of the functions are unknown.
NM_001105038	CXCR1	Chemokine (C-X-C motif) receptor 1	IL8RA IL8R1	Receptor for IL8 (powerful neutrophil chemotactic factor). Upon binding IL8, neutrophils can be activated.
NM_174360	CXCR2	Interleukin 8 receptor, beta	IL8R2, IL8RB	Receptor for IL8 (powerful neutrophil chemotactic factor). Upon binding IL8, neutrophils can be activated. Also binds with high affinity to CXCL3 and NAP-2.
NM_001011673	CXCR3	Chemokine (C-X-C motif) receptor 3	Mig-R GPR9 IP10	Isoform 1: Receptor for CXCL9, CXCL10, CXCL11. Promotes cell chemotaxis response. Isoform 2: receptor for CXCL4, mediates the inhibitory activities of CXCL9, CXCL10, CXCL11 through a cAMP mediated signaling pathway. Does not promote cell chemotaxis response. Isoform 3: CXCL11.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001011675	CXCR5	Chemokine (C-X-C motif) receptor 5	BLR1	Binds to B-lymphocyte chemoattractant (BLC) and involved in B-cell migration into B-cell follicles of spleen and peyers patches. Splice variants encoding different isoforms have been reported for CXCR5.
NM_001098859	FASLG	Fas ligand	TNFSF6 CD95L APT1LG1	Transmembrane protein critical for triggering apoptosis of some cells such as lymphocytes. May be involved in T cell development.
NM_174086	IFNG	Interferon, gamma	IF1	Soluble cytokine with antiviral, immunoregulatory and anti-tumor properties. Potent activator of macrophages.
NM_001205757	IL10RA	Interleukin 10 receptor, alpha	IL10R1 CD210	Receptor for IL10. Structurally related to interferon receptors. Has been shown to mediate the immunosuppressive signal of IL10. Inhibits the synthesis of pro-inflammatory cytokines. Activation of this receptor leads to tyrosine phosphorylation of JAK1 and TYK2 kinases.
NM_001076975	IL10RB	Interleukin 10 receptor, beta	IL10R2	Shared surface cell receptor required for the activation of the following class 2 cytokines: IL10, IL22, IL26, IL28 and IFNL1. Co-expression of IL10RA required for IL10 induced signal transduction.
NM_174089	IL13	Interleukin 13	-	Immunoregulatory cytokine produced primarily by activated Th2 cells. Involved in several stages of B-cell maturation and differentiation. Upregulates CD23 and MHC class II expression. IL-13 promotes IgE isotype switching of B cells and down-regulates macrophage activity. In doing so, pro-inflammatory cytokines and chemokine production is inhibited.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_174090	IL15	Interleukin 15	-	Cytokine which stimulates the proliferation of T-Lymphocytes. Requires interaction with IL-2Rgamma and IL2beta.
NM_001075253	IL16	Interleukin 16	NIL16 PRIL16 LCF	Stimulates a migratory response in CD4+ lymphocytes, monocytes and eosinophils. Primes CD4+, T-cells for IL-2, and IL15 responsiveness. Induces T-lymphocyte expression of IL2R.Isoform1: Scaffolding protein which anchors ion channel in membrane. Isoform 3: cell progression in T-cells. Involved in transcriptional regulation of SKP2, probably part of transcriptional repression complex on the core promoter of the SKP2 gene. Acts as scaffold for the DNA binding subunit of GABP transcriptional factor complex. Maintains transcriptional repression and blocking cell cycle progression in resting T-cells.
NM_001008412	IL17A	Interleukin 17A	CTLA8	Pro-inflammatory cytokine produced by activated T cells. Regulates NFκB and mitogen-activated protein kinases. Can stimulate the expression of IL6 and cyclooxygenase-2, and enhance nitric oxide production. High levels are involved with chronic inflammatory diseases.
NM_001192045	IL17B	Interleukin 17B	IL20 NIRF ZCYT07	Stimulates the release of TNFα and IL1β from THP1. Primarily localized to neuronal cell bodies
NM_001192082	IL17F	Interleukin 17F	ML1 CANDF6	Expressed by activated T cells. Stimulates IL6, IL8, CSF2 production. Found to inhibit the angiogenesis of endothelial cells and induce endothelial cells to produce IL2/ TGFB1 and MCP1.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_174092	IL1A	Interleukin 1, alpha	-	Produced by activated macrophages. Stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation and fibroblast growth factor activity.
NM_174093	IL1B	Interleukin 1, beta	-	Produced by activated macrophages. Induces IL-2 release, B-cell maturation and proliferation and fibroblast growth activity. Involved in the inflammatory response. Reported to stimulate the release of prostaglandin and collagenase from synovial cells.
NM_001206735	IL1R1	Interleukin 1 receptor, type I	-	Receptor for IL1A, IL1B and IL1RN. After binding to IL1, it associates with the co-receptor IL1RAP to form the high affinity IL1 receptor complex. This complex mediates IL1 dependent activation of NFκB, and MAPK. Signaling involves the recruitment of adapter molecules such as TOLLIP, MYD88 and IRAK1 or IRAK2.
NM_174357	IL1RN	Interleukin 1 receptor antagonist	-	Inhibits IL1 by binding to IL1R1 and preventing association with coreceptorIL1RAP. No IL1 like activity. Decoy receptor.
NM_198832	IL21	Interleukin 21	Za214	Prominent immunoregulatory activity. Promotes the transition between innate and adaptive immunity. Induces IgG1 and IgG3 production from B-cells. Synergistically plays a role of proliferation and maturation of NK cells in synergy with IL15. May regulate proliferation of mature B and T cells (with stimuli). Synergistically stimulates IFNG production in T and NK cells (with IL15 and IL18). During a T-cell mediated response, may inhibit dendritic cells.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001164653	IL27	Interleukin 27	IL27A IL27p28	Pro and anti-inflammatory properties. Can regulate T-Helper cell development, suppress T-cell proliferation, stimulate cytoxic T-cell activity, induce isotype switching in B-cells and has diverse effects on innate immune cells. Strong synergistic effect with Il12 to trigger IFN gamma production of naïve T cells.
XM_002687690	IL2RB	Interleukin 2 receptor, beta	IL15RB P75	IL2 receptor. β subunit is involved in receptor mediated endocytosis and transduces the mitogenic signals of IL2.
NM_174359	IL2RG	Interleukin 2 receptor, gamma	-	Common subunit of the IL2 receptor. Used for a variety of interleukins (IL2, IL4, Il7 and IL21).
NM_173920	IL3	Interleukin 3	-	Granulocyte/macrophage colony-stimulating factor which acts in hematopoiesis by controlling the production, differentiation and function of granulocytes and monocyte-macrophages.
NM_001075297	IL33	Interleukin 33	MGC142386	IL-1 family that drives production of TH2 associated cytokines.
NM_173921	IL4	Interleukin 4	BSF-1, IL-4	Functions in B-cell activation. Co-stimulator of DNA-synthesis. Induces the expression of class II MHC molecules on resting B-cells. Enhances secretion and cell surface expression of IgE and IgG1.
NM_173922	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	IL-5, TRF	Factor that induces terminal differentiation of late-developing B-cells to immunoglobulin secreting cells. Growth and differentiation of eosinophils.
NM_001110785	IL6R	Interleukin 6 receptor	-	Part of the receptor for IL6. Binds to IL6 with low affinity; however no signal is transduced without IL6ST association.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
XM_002696322	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	IL6RB CD130	Part of the IL-6 receptor complex which acts as a signal transducer. Must be involved for signal transduction for IL-6 signaling.
NM_173924	IL7	Interleukin 7	MGC152606	Hematopoietic growth factor capable of stimulating lymphoid progenitor proliferation.
NM_173925	IL8	Interleukin 8	IL-8	Chemotactic factor which attracts neutrophils, basophils and T-cells. Involved in neutrophil activation.
XM_002689203	IL9	Interleukin 9	-	Supports IL2 and IL4 independent growth of T-helper cells.
XM_002683814	IL9R	Interleukin 9 receptor	-	Receptor for IL9; requires IL2RG to form a complex.
NM_001013401	LTA	Lymphotoxin alpha (TNF superfamily, member 1)	-	Cytokine that binds to TNFRSF1A, TNFRSF1B and TNFRSF14. In its heterotrimeric form with LTB binds to TNFRSF3.
XM_002697371	LTB	Lymphotoxin beta (TNF superfamily, member 3)	-	Plays a specific role in immune response regulation. Provides the membrane anchor for the attachment of the heterotrimeric complex to the cell surface.
NM_001033608	MIF	Macrophage migration inhibitory factor)	MGC127044	Pro-inflammatory cytokine involved in the innate response to bacterial pathogens.
XM_002686767	NAMPT	Nicotinamide phosphoribosyltransferase	PBEF1	Catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, an intermediate in the biosynthesis of NAD. Secreted form behaves and a cytokine and an adipokine.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_175713	OSM	Oncostatin M	-	Encodes a growth regulator with inhibits the proliferation of a number of tumor cell lines.
NM_001101062	PF4	Platelet factor 4	-	Released during platelet aggregation. Neutralizes the anticoagulant effect of heparin. Chemotactic for neutrophils and monocytes. Inhibits endothelial cell proliferation.
NM_174187	SPP1	Secreted phosphoprotein 1	-	Acts as a cytokine involved in enhancing production of IFN-g and IL12 and reducing the production of IL10.
NM_173966	TNF	Tumor necrosis factor	TNFa	Cytokine which binds to TNFRSF1A. Secreted by macrophages, induces cell death.
NM_001098056	TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b	OPG OCIF	Neutralizes function in osteoclastogenesis. Plays role in preventing arterial calcification.
XM_002684917	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	APO2L TRAIL	Binds to a number of TNFRSF members, induces apoptosis. Activity may be modulated by binding to decoy receptors.
NM_001205770	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11	OPGL TRNACE ODF RANKL	Osteoclast differentiation and activation factor. Augments the ability of dendritic cells to stimulate naïve T-cell proliferation. May be an important regulator of T-cell interactions with dendritic cells.
NM_001034647	TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13	APRIL, MGC128565	Cytokine which binds to TNFRSF13B and TNFRSF17, plays a role in the regulation of tumor cell growth. May be involved in monocyte/macrophage mediated immunological processes.

Reference Sequence	Symbol	Full Name	Alternative Names	Gene Function
NM_001114506	TNFSF13 B	Tumor necrosis factor (ligand) superfamily, member 13b	-	Binds to TNFRSF13B and BCMA. Forms 2 ligands-2receptors pathway involved in the stimulation of B and T cell function and the regulation of humoral immunity. Inhibits isoform 1 secretion and bioactivity.
NM_001101855	TNFSF14	Tumor necrosis factor (ligand) superfamily, member 14	MGC157058	Activates NFkappaβ, stimulates the proliferation of T-cells.
NM_001205715	TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4	GP34 OX40L	Co-stimulates T-cell proliferation and cytokine production.
NM_174216	VEGFA	Vascular endothelial growth factor A	VEGF	Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels.