1-1-2012

# Evaluation of the Immune Response of Angus Heifers with Different Genetic Markers for Marbling when Challenged with Lipopolysaccharide

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By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in Animal Nutrition
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

December 2012

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Title of Study: Evaluation of the immune response of Angus heifers with different genetic markers for marbling when challenged with lipopolysaccharide

Pages in Study: 57

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Nineteen heifers (274 plus/minus 24 kg) were blocked into two treatment groups based upon DNAm; heifers with no DNAm (noQG), and heifers with one or more DNAm (1+QG). Prior to challenge (24 h), heifers were fitted with indwelling jugular catheters and indwelling vaginal temperature (VT) monitoring devices. Blood samples were collected at 30-min intervals while RT was collected at 1-min intervals from -2 to 8 h relative to a lipopolysaccride (LPS) challenge (0.5 microgram/kg BW) at 0 h. Serum was analyzed for concentrations of cortisol and pro-inflammatory cytokines. All physical, endocrine, and immune measurements increased relative to LPS challenge. No differences observed for IL-6 or TNF-alpha; however, 1+QG heifers had a greater circulating INF-gamma ($P < 0.001$). Furthermore, 1+QG heifers had an elevated VT ($P = 0.04$). This would suggest a different immune system approach to an LPS challenge.

# DEDICATION

I dedicate this thesis to my mother, Larraine Thrash Buntyn for her unconditional love and support in all my life endeavors. My mother has been there for me since day one. She is the reason that I truly love beef cattle and taught me my first life lesson about cattle. Without her, I would never have had the courage to follow my dreams and pursue a career in the beef industry. I would not be where I am today without her.

ACKNOWLEDGEMENTS

No ending is ever complete without a beginning. While this may be the end of my time at Mississippi State, it truly is just the beginning of my career in animal agriculture. I have spent countless hours in the classroom, graduate office, and South Farm, at Mississippi State. I learned the true value of cattle and what cattle mean to me. I am a firm believer you must find your true passion in life. No matter how much sweat, blood, and tears I have lost, at the end of the day, I will always find happiness just looking at cattle.

First and foremost I would like to thank my committee for all of their help, advice, guidance, and the endless hours they have dedicated to me. My committee includes Dr's Ty Schmidt, Daniel Rivera, Jeff Carroll, and Trent Smith. I would like to thank Dr. Ty Schmidt for giving me the opportunity to become his first student worker and taking a chance on a kid that let twelve fat steers take a stroll during the first lab. Without Dr. Schmidt's guidance, I would never have pursued the career path I am on today. I'd like to thank Dr. Carroll for all of his input, direction, and conversation during my program; he has always challenged me to think critically about my project and shown me the science aspect that needed evaluating. I appreciate Dr. Rivera for always helping me with any question I had and always being a phone call away and taking over guidance during a difficult time. I would also like to thank Dr. Smith for showing me there is

always two ways to solve a problem and always patiently waiting until I chose the most appropriate.

I also appreciate the assistance from Dr. Nichole Burdick Sanchez, Dr. Lindsey Hulbert, Jeff Daily, and Jessica Carroll at USDA-ARS Livestock Issues Unit in Lubbock, TX for always being willing to help no matter what I needed. I would also like to thank the faculty and staff of the Animal and Dairy Sciences department for their time, help, and guidance. I would like to thank William White the facilities coordinator for the Levek animal research center, and Tim Armstrong and James Cannon of the Mississippi State Meat Lab for the many laughs we shared at Rand's expense.

I have had the privilege of meeting and working with many outstanding graduate students while pursuing my masters. Most influential was Dr. Shollie Falkenberg who was always willing to help with a problem or just listen to an idea. I would especially like to thank all the graduate students that helped on my projects or provided entertainment in the graduate office. These include: Rand Broadway, Katie Cooley, Joe Binversie, Jessica Graves, Heather Duoss, Lyndi Jury, Thurston Dawson, Katie Pfeiffer, Nick Simmons, Landon Marks, Jonathan Greene, Adam Geiger, and Sam Plank. Lastly, I would like to thank Dr. Whitney Agee for the support and understanding while finishing my thesis.

I greatly appreciate the help from everyone that has been a part of my life during my time at Mississippi State. Finally, I would like to thank God for always being there for me and leading me down the path he has chosen for me.

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# CHAPTER I

# INTRODUCTION

The cattle industry is a very diverse industry with the common goal of producing a product demanded by consumers across the world. The cattle industry can be divided into four distinct sectors. These sectors include: cow/calf, stocker, feedlot, and the abattoir. No matter which sector an animal may be located, at any given time, the genetic potential of this animal is influenced at selection. Natural selection has occurred for thousands of years with evolution following the theory of Darwin's survival of the fittest. With domestication of livestock, the process of selection changed from the need for survival to the need of man. Selection is the process of deciding which animals remain in the breeding population and for how long. Artificial selection, which is used in the livestock industry today, remains under human control. The goal of breeders in today's beef cattle industry has become breeding cattle that are both profitable and provide the end product demanded by the consumer.

With the scientific advances in animal agriculture, selective breeding has become an in-depth process using more tools than ever in the decision process. With the use of expected progeny differences (EPDs), the mapping of the beef genome, and even the use of ultrasound, artificial selection has revolutionized the livestock industry. Expected progeny differences (EPDs) are nothing more than a genetic prediction of an animal's genetic value. This allows animal breeders a chance to select for certain desirable

production traits in a goal of producing desirable offspring (regardless of for the purpose of further generations or for end products). Furthermore, with the mapping of the beef genome, single genes have been discovered that control production traits such as feed efficiency and marbling. The genetic boom of the livestock industry has led to the development of commercially available genetic tests such as GeneSTAR® or the HD 50k (Pfizer Anim. Genetics, Kalamazoo, MI). These genetic tests allow the ability for single trait selection controlled by certain genes, which only selects for one trait of concern.

While single trait selection may have beneficial effects for producers in regard to the specific trait, there is limited data available to describe how single trait selection can impact other important traits such as animal response to immune challenges. Therefore, the objective of this study was to evaluate the immune response of Angus heifers to an endotoxin challenge, when selection was based on genetic markers for intramuscular fat (marbling).

CHAPTER II

Literature Review

## Genetic Selection

The United States beef industry consists of many sectors that have a part in producing and marketing beef to consumers. An important sector is the cow-calf operation. Cow-calf operations are the building block of the entire beef industry with complete control of new genetic input. Therefore, most genetic testing is focused in the cow-calf sector (Garrick and Golden, 2009). Furthermore, this sector can be divided once again into seed stock and commercial cow-calf operations, with only five percent of cow-calf operations being seed stock producers (Garrick and Golden, 2009). Today's current information systems are oriented with breed associations, which posse generations of data collected on numerous cattle (sire, dam, and offspring). The collection of this data by breed associations led to the development of expected progeny differences (EPDs) and economic indexes used to evaluate the potential genetic value of cattle (Garrick and Golden, 2009). Thanks to vast changes in technology, the bovine genome has been mapped, and genetic testing can be utilized to predict cattle production traits based upon specific genes.

Genetic markers used in the cattle industry have three phases that are used to develop these selection tools which include: detection, evaluation, and implementation (Davis and DeNise, 1998). The detection phase is to discover quantitative trait loci

(QTL) on chromosomes that affect production traits. Once discovered, these QTL can be utilized in breeding programs in marker-assisted selection (MAS). First, QTLs are discovered in family lines and evaluated to establish the number of times the alleles repeat on a gene. Markers can be classified as the distance between markers and QTL or direct markers that represent the exact QTL that affects the trait of interest (Davis and Denise, 1998). Linked markers must be evaluated within family lines, whereas direct markers can be used to evaluate entire breeds (Davis and Denise, 1998). Identification of quantitative trait loci being used as a selection aid can greatly benefit cattle traits such as carcass composition and intramuscular fat due to the fact of the difficulty of measuring these traits without harvesting the animal (Casas et al., 2003). Once a QTL has been discovered and classified as either a linked marker or direct marker, markers are evaluated accordingly (Davis and Denise, 1998). Marker-assisted selection programs should take into account the added value of using genetic markers. A producer must consider where the added genetic value will enter production and thus increases product, and who this increase in production value benefits. Most economic value will be noticed in commercial production (Davis and Denise, 1998). In today's cattle industry, purebred operations gain the most from using genetic markers to improve the industry's genetics and as a marketing tool. Furthermore, genetic testing allows the most genetic change due to the fact purebred operations can have an impact on cow-calf sector's output of genetics (Davis and Denise, 1998).

## Genetics

To fully understand the impact of genetic selection, a basic understanding of genetics is needed. The study of heredity and variation within a population or on an

individual basis is called genetics (Klug and Cummings, 2000). Genetic information is housed in the nucleus of eukaryotic cells. Deoxyribonucleic acid (DNA) stores genetic information as a molecule in the nucleoid region of cells (Klug and Cummings, 2000). As a double helix structure, DNA molecules contain heredity units called genes that form chromosomes. A gene is the basic unit of heredity, which is composed of nucleotides and is the basic building blocks of DNA. A gene is the simplest form of genetic storage that can replicate, express, and undergo mutations (Klug and Cummings, 2000). These linear DNA molecules which are associated with proteins form chromosomes. Most eukaryotes have a specific number of chromosomes which are diploid in number. Chromosomes exist in identical pairs and can replicate genetic information by mitosis and meiosis. During mitosis, cell division leads to the production of two cells with identical genetic information (Klug and Cummings, 2000). Meiosis is the production of gametes that contain only the genetic material of the parent cell (Klug and Cummings, 2000). Substitution, duplication, or deletion of nucleotides leads to a different expression of a gene which is called an allele (Klug and Cummings, 2000).

## GeneSTAR® Technology

Within the beef industry, carcass quality is an important trait as it can have an effect on the price/cut received for cattle. Typically, carcass quality refers to the USDA Quality grade, which is influenced by the amount of intramuscular fat located in the longissimus muscle at the $12^{th}$ and $13^{th}$ rib interface of a beef carcass (USDA, 1997). United States Department of Agriculture Quality grades greater than Low Choice, can provide producers' premiums for cattle that fit into certain markets. Quantitative trait loci have been identified that are directly related to the amount of intramuscular fat that is

deposited within the muscle. Identification of these QTL's has allowed cattle producers to utilize this information when selecting cattle for breeding (Casas et al., 2003). With the discovery of QTLs, companies have taken this information and developed marketable gene tests such as GeneSTAR® MARB™ or GeneSTAR® Quality Grade marker (Pfizer Anim. Genetics, Kalamazoo, MI) but is now part of the Pfizer 50k chip and referred to as the Quality panel. GeneSTAR® MARB™ marker was the first commercially genetic test available to cattle producers the opportunity to use direct gene marker evaluation. GeneSTAR® MARB™ evaluates QTL polymorphisms on the 5' thyroglobulin gene (TG5) that have been reported to have a direct effect on intramuscular fat deposition. The use of GeneSTAR® MARB™ can improve carcass traits in two ways: discovering cattle within breeding herds that have exceptional carcass traits and in the marketing of cattle with genetic potential for improved carcass traits (Rincker et al., 2006).

However, research is limited on the actual carcass benefits of breeding selections based solely on the use of GeneSTAR® MARB™ (Rincker et al., 2006). The National Beef Cattle Evaluation Consortium was put in charge of the validation process of commercial genetic test. The purpose of this checkpoint was/is to validate genotype and phenotype differences of cattle that exhibit these markers which are marketed by commercial testing companies. Focusing on the GeneSTAR® Quality Grade marker also known as GeneSTAR® MARB™, the marker is evaluated by C/T single nucleotide polymorphism (SNP) located 537 base pair upstream of thyroglobulin on RNA polymerase III (Van Eenennaam et al., 2007). The previously discussed marker is commercially marketed as the TG5 or QG1 marker. Also, the GeneSTAR® MARB™

panel consists of a second marker known as QG2 SNP; however, the location of QG2 has not been released or published (Van Eenennaam et al., 2007).

Van Eenennaam et al., (2007) evaluated the effects of the TG5 and QG2 markers in 409 offspring (Charolais x Angus) in which carcass quality was reported as the percentage of carcasses receiving a grade of USDA Select, Choice, or Prime, based on GeneSTAR® MARB™ markers rather than an actual increase in marbling score. Based upon this classification, there was an 8.6% increase of carcasses receiving the USDA Grade Choice or Prime that possessed one copy of the TG5 allele. For the QG2 allele, a 2.9% increase was observed in carcasses grading Choice or Prime for each copy of the allele. Overall, a 6.2% increase of carcasses grading Choice or Prime was attributed to one GeneSTAR® Quality Grade marker. Rinker et al., (2006) evaluated 192 Simmental steer's final quality grade using GeneSTAR® MARB™. Cattle were placed into populations based on the number of STAR markers observed from genetic test of each steer. The results indicated that steers possessing 0, 1, or 2 STARs, where STARs represent the number of the TG5 genes expressed; did not result in difference in final quality grades of steers (Rinker et al., 2006).

Single nucleotide polymorphisms, such as the one used to develop GeneSTAR® MARB™ panel, allow the evaluation of certain alleles over several generations due to the lack of change to these alleles (Stone et al., 2005). Smith et al, (2009) evaluated the frequency of the TG5 allele in Brahman cattle originating in Louisiana. The result indicated that 99% of the Brahman steers exhibited the unfavorable CC base pair combination of this allele. Furthermore, 0.8% of the steers possessed the favorable heterozygous T genotype, and no homozygous TT genotypes were observed

(Smith et al., 2009). Van Eenennaam et al., (2007) reported that Wagyu cattle had the greatest amount of heterozygous and homozygous alleles at the TG5 marker for the favorable T allele with 50 and 38%, respectively of all cattle tested Van Eenennaam et al., (2007). Furthermore, the Angus cattle tested exhibited 39% heterozygous and 11% homozygous for the favorable allele at the TG5 marker. Other breeds of cattle exhibit fewer favorable alleles at the TG5 marker with *Bos indicus* cattle exhibiting the fewest of all cattle (Van Eenennaam et al., 2007). Overall, the GeneSTAR® MARB™ marker can provide producers the genetic potential of cattle; however, the GeneSTAR® MARB™ marker does not take into account any environmental factors that can also affect ending USDA quality grades (Van Eenennaam et al., 2007).

## Impact of Genetic Selection

Genetic selection is usually based upon producing a superior animal in some aspect of production. Animals selected for specific production traits may tend to posse less than favorable behavioral, physiological, and immunological issues (Rauw et al., 1998). In the dairy industry, genetic merit has been measured by milk production. However, it has been observed that dairy cattle with greater genetic merit for milk production have decreased fertility (Veerkamp et al., 2007). Selection for greater genetic merit for milk production is believed to alter energy partitioning in lactating dairy cattle diverting energy from reproductive tissue, resulting in less fertility efficiency. The genetic change is hypothesized to affect the GH/IGF-I axis resulting in a negative energy balance due to the genetic alteration (Veerkamp et al., 2007). In an attempt to lessen the effects of elevated milk production in dairy cows, emphasis has been placed on female fertility. This genetic selection is based on cow performance and daughter performance

in an attempt to select dairy cows that not only excel at milk production but also remain in a breeding population (Weigel, 2006). The poultry industry has also placed great importance on single trait selection for muscling; however, the immune competence of poultry has been reported to decrease (Siegel and Dunnington, 1997).

The use of genetic markers for disease resistance is still a developing technology. The three arguments of genetic selection for disease resistance are sustainability, feasibility, and desirability for these tools (Stear et al., 2001). While there are commercially available tests for milk production and marbling, there are no genetic evaluation tools available today that impact the health aspects of animal production. With an increase in animal welfare concerns, selection tools for animal health maybe seen in the future of livestock industries (Weigel, 2006). In sheep, selection for the ARR allele for scrapie resistance has been evaluated. No significant differences were observed of production traits of sheep selected for the ARR allele; however, when homozygous ARR alleles were selected, genetic variation of the flock decreased (Alfonso, 2006).

## Innate Immune System

The immune system of mammals is composed of three primary segments: physical, innate, and acquired. The first segment is the physical barriers, such as skin, hair within the nasal cavity, mucosal linings of the respiratory tract and the gastrointestinal tract. The body has three different physical protection mechanisms that include mechanical, chemical, and microbiological. The skin serves as a mechanical barrier for the body preventing pathogenic agents from entering the body. Furthermore, the flow of fluids throughout the body serves as a mechanical barrier to pathogens by

always moving these agents away from a potential infection site. Chemical barriers include acid concentrations, such as the acidity of the gastrointestinal tract and lysozyme in tears. However, these barriers cannot completely eliminate the access of the body to pathogenic agents. The second segment (line of defense) in response to a pathogen is the innate immune system. The innate immune system is comprised of the compliment system, macrophages, neutrophils, and natural killer cells. Innate immunity does not adapt to a specific pathogen as it serves as a broad spectrum defense mechanism (Parham, 2009). The final segment is acquired immunity, which encompasses two types of lymphocytes. B and T lymphocytes recognize a specific pathogen antigen which leads to the destruction of the pathogen and aids in the development of immunological memory (Parham, 2009).

When a pathogen penetrates the physical barriers, the innate immune system becomes the first internal line of defense responsible for stopping any pathogenic agents. The innate immune system is inherited by offspring from both parents (Parham, 2009). At this point of infection, the innate immune system mobilizes a defense strategy against invaders. First, the body must recognize that a pathogen has entered the body. Soluble proteins and cell-surface receptors are responsible for locating pathogens and relaying this message to effector mechanisms. Effector cells supply these effector mechanisms that are responsible for destroying pathogen cells to prevent the spreading of infection. Furthermore, serum proteins are in search of foreign cells in an attempt to flag and attack pathogens to elicit the help of effector cells. This is known as complement within the innate immune response (Parham, 2009).

Infections caused by pathogens can be classified as either extracellular infections or intracellular infections (Parham, 2009). Depending on the type of infection, the innate immune system will respond accordingly. First, complement is activated causing a release of proteases that target the infectious pathogen. Complement is comprised of more than 30 proteins; however, it has been reported that complement component 3 (C3) serves as one of the major proteins in eliciting an immune response (Parham, 2009). Complement activation has three different pathways of activation: alternative pathway, lectin pathway, and classical pathway. The alternative pathway activates complement simply when a pathogen cell surface allows for activation (Parham, 2009). The lectin pathway requires the binding of mannose-binding lectin to the surface of a pathogen; whereas, the classical pathway requires a c-reactive protein or antibody binding to a specific antigen on a pathogen (Parham, 2009). No matter which pathway is triggered, complement becomes activated. Once activated, C3 is cleaved into a small C3a particle and a larger C3b particle. The C3b particle binds to the pathogen's cell wall and flags the pathogen for destruction (Parham, 2009). Once flagged, pathogens are attacked by cells of the innate immune system which include: macrophages, polymorphonuclear neutrophils (PMN), and natural killer (NK) cells (Marsh and Kendall, 2006). Furthermore, cells that release inflammatory mediators such as basophils, mast cells, and eosinophils, are observed in an innate immune response (Carroll, 2008). The innate immune response serves as the internal first line of defense for the body and leads into an inflammatory response.

## Inflammatory response

At the moment of injury, damaged cells begin to release histamine to serve as a signal that begins the cascade of defense mechanisms (Tizard, 2009). These signals can be classified as either exogenous or endogenous. Exogenous signals are produced by the occurrence of microorganisms; whereas, endogenous signals are produced by damaged or dead cells (Tizard, 2009). Due to the vast number of microorganisms and the fluid dynamics of the microbiom, the innate immune system is not designed to detect every microorganism. Pathogen-associated molecular patterns (PAMPs) are responsible for recognizing an array of molecules that are similar to entire classes of pathogens (Tizard, 2009). Examples of PAMPs include the lipopolysaccharide (LPS) of gram-negative bacteria cell wall components. Once PAMPs recognize a foreign molecule, pattern recognition receptors (PRRs) located on sentinel cells bind PAMPs initiating an innate immune response (Tizard, 2009). The major PRRs are classified as toll-like receptors (TLR), which can be located intracellular or extracellular depending on the pathogen for which the toll-like receptors are responsible for detecting (Tizard, 2009). The binding of PAMPs to PRRs initiated the sentinel cells to release cytokines, chemokines, and enzymes (Tizard, 2009). Sentinel cells include macrophages, dendritic cells, and mast cells; with a common goal of locating and destroying foreign invaders. Furthermore, sentinel cells are responsible for inflammation at the site of infection and the release of mediating molecules that signal the receptor sentinel cells to release cytokines (Tizard, 2009). Cytokines are proteins produced in response to a foreign molecule with the purpose of binding to a cells specific receptor to elicit a response from a particular cell (Parham, 2009). These inflammatory mediators produce or initiate the five cardinal signs

of acute inflammation that includes: heat, redness, swelling, pain, and loss of function (Tizard, 2009). At the site of infection or injury, blood flow decreases allowing leukocytes the opportunity to bind to blood vessel walls. Then, blood flow increases at the local site of infection or injury and blood vessels allow passage of fluid into the infection site (Tizard, 2009). Physical/visible symptoms of inflammation are related to increase of blood flow and the movement of fluids from blood vessels to the site of the immune insult (Parham, 2009). Acute inflammation serves as a beneficial tool in the presence of an immune insult by mounting a defensive strike against invaders. Pro-inflammatory cytokines serve a major role during inflammation.

## Pro-inflammatory cytokines

Cytokines are protein molecules produced by cells (macrophages, dendritic cells, and mast cells) of the immune system in response to an immune insult (Tizard, 2009). Cytokines can function via three modes of action; autocrine, paracrine, or endocrine (Webster Marketon and Glaser, 2008). With the start of an innate immune response, helper T cell formation is stimulated by Interleukin-12 (IL-12) and Interferon-γ (INF-γ) (Parham, 2009). Helper T cells (h-T) are derived from T lymphocytes and process unique receptors for binding bacteria cell components with one of the most notable being lipopolysaccride deriving from gram-negative bacteria cell wall destruction (Parham, 2009). When bacterial cell components bind to the receptors on the h-T cell, binding of the receptor initiates a signal cascade for the production of cytokines (Parham, 2009). Macrophages contain the Toll-like receptor 4 (TLR4) which recognizes the bacterial LPS and initiates the signal for an innate immune response due to the presence of a gram-negative bacterium (Parham, 2009). The initial step for the signal is activation of the cell

nucleus that activates the transcription of genes for inflammatory cytokines. These inflammatory cytokines are produced in the cytoplasm then shuttled to the extracellular fluid (Parham, 2009). There are two types of h-T cells which produce helper T 1 (h-T 1) and helper T 2 (h-T 2) cytokines (Webster Marketon and Glaser, 2008). Helper T 1 cells are responsible for producing tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interferon-γ (IFN-γ). Tumor necrosis factor-α serves many roles within the immune response including; activating cells (mast cells, macrophages, lymphocytes, and neutrophils), killing tumor cells, and eliciting an inflammation response (Tizard, 2009). Interleukin-6 is a systemic cytokine that acts on the hypothalamus, muscle, and adipose cells in an attempt to elevate body temperature (Parham, 2009). Interferon-γ is also produced by h-T 1 cells has a primary function of activating macrophages (Parham, 2009). Helper T 2 cells produce numerous cytokines that enhance B cell production (Tizard, 2009). These B cells or B lymphocytes are responsible for producing immunoglobulins and antibodies seen during adaptive immunity (Parham, 2009).

## Tumor necrosis factor-α

Tumor necrosis factor-α is produced and released by macrophages and T lymphocytes of the h-T 1 cells in response to an immune insult. After invasion of a pathogen, TNF-α is one of the first cytokines released into circulation during the immune response (Marsh and Kendall, 1996). Tumor necrosis factor-α is produced when PAMP binding takes place to receptors on macrophages. Tumor necrosis factor-α recruits macrophages to remove or kill invading pathogens (Marsh and Kendall, 1996). As TNF-α production increases, TNF-α takes on an autocrine role that assist in the production of interleukin-1 (IL-1) and an indirect role of controlling the production of interleukin-6.

Once bound to polymorphonuclear neutrophils (PMN) cells, TNF-α regulates the function of neutrophils during an immune insult (Marsh and Kendall, 1996). Polymorphonuclear neutrophils (PMNs) or neutrophils are short lived phagocytic leukocytes that respond early on during immune insult (Marsh and Kendall, 1996).

## Interleukin-6

Interleukin-6 is a glycoprotein produced by macrophages, T cells, and mast cells in response to the presence of bacterial endotoxins, IL-1, and TNF-α (Tizard, 2009). Interleukin-6 functions in both the acute-phase response and the autoimmune response. Furthermore, IL-6 is pivotal for an inflammation and fever response associated with an infection. Interleukin-6 has also been documented to function during an anti-inflammatory response by the down regulating TNF-α and IL-1 (Tizard, 2009). During an immune insult, IL-6 has been reported to direct the immune response from a neutrophil attack to a more macrophage approach (Tizard, 2009). Neutrophils and macrophages have similarities and difference but are both phagocytic (ingesting) cells. Neutrophils are short lived cells that circulate in the bloodstream until called to sites of infection by macrophages with the one goal of killing invaders. Macrophages are long lived cells located in tissues that begin killing invaders at the start of a challenge; however, macrophages are not just phagocytic cells because of their alarming and signaling capabilities (Parham, 2009).

## Interferon-gamma

Interferon-γ, like IL-6 is also a glycoprotein that has been primarily thought of as only an antiviral cytokine; however, IFN-γ serves to regulate h-T 1 cells as well as

antiviral properties (Tizard, 2009). Helper T 1 cells, cytotoxic T cells, and natural killer (NK) cells produce IFN-γ (Tizard, 2009). Interferon-γ regulates macrophage activation by directly activating macrophages or preparing macrophages to be able to detect limited concentrations of LPS (Marsh and Kendall, 1996). Although h-T1 cells and cytotoxic T cells produce IFN-γ, NK cells produce the majority of IFN-γ in the presence of mitogens or microbial products (Marsh and Kendall, 1996). Interferon-γ production is controlled by the interaction of many cytokines that in return are regulated by IFN-γ that leads to the production of IFN-γ (Marsh and Kendall, 1996).

## Lipopolysaccharide model

Gram-negative bacteria contain a cell wall composed of peptidoglycans surrounded by a layer of lipopolysaccharide (LPS); (Tizard, 2009). When a pathogen is recognized within the body, complement of the innate immune system is activated. Complement is composed of plasma proteins that mark bacteria and extracellular virus particles by attaching these plasma proteins to the cell walls of invaders (Parham, 2009). This protein coating allows for the phagocytosis of invading cells to take place due to the thick cell walls of some bacteria (Parham, 2009). Complement receptors CR3 and CR4 located on the surface of macrophages are responsible for recognizing LPS and microbial ligands (Parham, 2009). Receptor-mediated endocytosis takes place when receptors located on macrophages bind microbial ligands leading to the destruction of the pathogen cell. This cascade of events also leads to the release of cytokines causing an innate immune and a pro-inflammatory response (Parham, 2009).

Lipopolysaccharides have been utilized by research in human and animal health to study the pro-inflammatory response. The use of LPS to study the pro-inflammatory

response allows for researchers to evaluate the immune response without using live pathogens, as LPS will induce a pro-inflammatory response similar to that of an actual pathogen (Carroll et al., 2008). A gram-negative bacterium causes illness when LPS is released from the degradation of the cell wall (Steiger et al., 1999).

The LPS model is often used to gain a better understanding of the role of the innate immune response. Steiger et al., (1999) reported that a 2 μg/kg of body weight infusion of LPS over a 100 min period could reduce feed intake when administered to Holstein x Jersey heifers with a mean body weight of 311 kg. After 160 min specific animal intake was reduced and intake remained decreased over the following 24 h period. Furthermore, concentrations of serum TNF-α increased during the LPS challenge and remained elevated for 10 h post LPS challenge (Steiger et al., 1999). Waggoner et al., (2009) reported that LPS infusion to steers resulted in an increase demand for amino acids by the immune system in response to the immune challenge. Carroll et al., (2008) used a 2.5 μg/kg of body weight to evaluate the effects of LPS on the cattle immune system. The results suggested that TNF-α and IL-6 serum concentrations rapidly increased following LPS infusion ($P = 0.0001$). Furthermore, INF-γ concentrations increased but at a slower rate (Carroll et al., 2008) when compared to IL-6 and TNF- α. The results of these trials clearly indicated that the LPS challenge model can be successfully utilized to incite a pro-inflammatory response of cattle that is typically caused by gram-negative bacteria. The elevation of body temperature and the production of pro-inflammatory cytokines in response to a LPS challenge is an assurance an immune response was elicited (Burdick et al., 2010). Due to this, the LPS model has become widely accepted to elicit an immune response.

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# CHAPTER III

# PRELIMINARY TRIAL - EVALUATION OF THE IMMUNE RESPONSE OF ANGUS HEIFERS WITH DIFFERENT GENETIC MARKERS


## Abstract

Twenty-three heifers (223 ± 44 kg) were blocked into two treatment groups based upon DNAm; heifers with no DNAm (noQG), and heifers with one or more DNAm (1+QG). Prior to challenge (24 hrs), heifers were fitted with indwelling jugular catheters and indwelling rectal temperature (RT) monitoring devices. Blood samples were collected at 30 min intervals while RT were collected at 1 min intervals from -2 to 8 h relative to a lipopolysaccride (LPS) challenge (0.25 μg/kg BW) at 0 h. Serum was analyzed for concentrations of cortisol and pro-inflammatory cytokines. There was a different (P = 0.02) at 2 h post LPS with 1+QG displaying a greater RT. However, noQG heifers had greater circulating concentrations of interferon-gamma (IFN-γ), interleukin-6 (IL-6), and Tumor necrosis factor-alpha (TNF-α). These results suggest that while there was an innate immune response to the LPS challenge, there was a limited response, and this LPS dose of 0.25 μg/kg BW was easily handled by the heifers, and thus should allow for an increase to a typical dose of 0.5 μg/kg BW to fully challenge the innate immune response of these unique heifers.

## Introduction

Bovine respiratory disease (BRD) continues to be a problem in the cattle industry. It is reported that the cattle industry loses over $500 million to BRD annually (Miles, 2009). With the increase of genetic technology, there still remains no genetic evaluation of a cattle's immune system. Our hypothesis was to evaluate the immune system of Angus heifers based on the occurrence of the GeneSTAR® MARB™ markers for intramuscular fat deposition. It is widely anticipated that adipose depots are used as energy storage sites in animals (Pond, 1978). The purpose of this preliminary study was to evaluate the immune response of Angus heifers selected for genetic markers could handle a LPS challenge dosage of 0.25 μg/kg BW, which is typically half the dose was administered to the heifers in this study. This 0.25 μg/kg BW is one-half the typical dose, and thus this decreased dosage while less than usual, may provide some insight of these genetically selected heifers ability to respond to an endotoxin challenge.

## Materials and Methods

Use of animals and the procedures utilized in this study were approved by the Mississippi State University Animal Care and Use Committee prior to initiation of the experiments (#10-034).

A preliminary trial was conducted at the Levek animal research center ruminant metabolism facility located at Mississippi State University to evaluate the innate immune response of heifers with genetic differences in intramuscular adipose development. Heifers utilized for this trial were fall-born heifers from the Angus GeneSTAR (GSm) herd; a pure-bred herd located at Mississippi State University. The objective of this herd

is producing Angus cattle with genetic variation in the occurrence of QG 1 and QG 2; offspring posses either zero markers (noQG) or one or more markers (1+QG).

To determine the presence or absence of QG 1 and QG 2, hair samples were collected from the tail of the heifers during processing at pre-weaning (202 d of age). Hair samples were shipped to Pfizer Animal genetics for analysis. After collection of hair, heifers were returned to the herd paddocks and maintained there until weaning. Fourteen d after weaning heifers were separated from the resident genetic herd and placed into a 1.62 hectare paddock and allowed 21 d to acclimate to environment, diet, and human contact. The diet for study consisted of: 26.2494% soybean hull pellets, 22.0341% corn gluten feed, 27.6565% of Bermudagrass (*Cynodon dactylon*) hay, 23.5592% cracked corn, and 0.5008% mineral premix (on a DM basis).

Twenty-three heifers (223 ± 44 kg) were blocked into two treatment groups based upon DNAm; heifers with no DNAm (noQG), and heifers with one or more DNAm (1+QG). The percentages of 1+QG heifers are shown in Table 3.1. Indwelling jugular vein catheters for serial blood collection were inserted - 1 d prior to the start of the trial (d -1). Catheters consisted of approximately 150 mm of polytetrafluoroethylene tubing (6417-41 18TW, Cole-Palmer; o.d. = 1.66 mm) that was inserted into a jugular vein using a 14-gauge × 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). The catheter was maintained in place using tag cement and a 5.1-cm-wide porous surgical tape. An extension consisting of sterile plastic tubing (Tygon S-50 HL; VWR Scientific, West Chester, PA; i.d. = 1.59 mm; o.d. = 3.18 mm) was attached to the catheter for collection of blood samples with minimal disturbance of the heifers. Between blood samples, all catheters were flushed with 5 mL of saline (0.9% wt/vol NaCl) followed by 3

mL of heparinized saline (1 ml of heparin 10,000 IU/mL in 500 mL of saline) to replace fluid volume and to maintain catheter patency.

On d -1, heifers were also fitted with indwelling rectal temperature (RT) monitoring devices that recorded RT every min (Reuter et al., 2010). After insertion of catheters and RT devices, heifers were placed into individual tie stalls. On d 0, blood samples were obtained from each heifer in 30 min intervals from -2 to 0 h to serve as a control. At 0 h, heifers received an i.v. bolus dose of lipopolysaccharide (LPS; 0.25 μg/kg of BW) via the jugular catheter. After the bolus of LPS, nine ml of blood were obtained every half hour staring at 0.5 h and ending at 8 h relative to challenge. Blood samples were allowed to clot for 30 min at 21ºC and then centrifuged at 2,000x *g* for 30 min (4ºC) and serum was separated. Serum was collected and transferred into 15 mL microcentrifuge tubes for storage and frozen (-80ºC) for later analysis.

**Serum analysis**

Serum cortisol concentration was determined by radioimmunoassay (Coat-ACount; DPC, Los Angeles, CA) per manufactures directions in a single assay with a detection limit of 2-ng/mL and less than 5% intra-assay coefficient of variation. Serum concentrations of the pro-inflammatory cytokines, interferon-gamma (IFN-γ), interleukin-6 (IL-6), and Tumor necrosis factor-alpha (TNF-α) were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines (SearchLight, Pierce Biotechnology Inc., Rockford, IL). For all cytokines, the intra-assay variation was less than 5 %, and the inter-assay variation was less than 20 %.

## Statistical analyses

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS as a block design; and the model included sampling time, treatment, and sample time x treatment. Sample treatment x time was used as the error term to test whole plot effects. Rectal temperature was initially recorded at 1 min intervals, but subsequently averaged over 30 min intervals to facilitate comparisons to other immune and physiological parameters. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pair wise differences among least squares means at various sample times were evaluated with the PDIFF option of SAS. Results from the area under the curve calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

## Results

All physiological, endocrine, and immune measures changed with time in response to the LPS challenge ($P < 0.001$). There was a treatment x time interaction ($P < 0.001$) for rectal temperature (RT; Figure 1). No differences ($P = 0.78$) in initial RT (-8 to 0 h) were observed between the two treatment groups. Difference in RT between noQG and 1+QG heifers appeared at 2 h post-challenge. However, by h 3, RT was not different ($P > 0.06$) between treatments and had returned to baseline measurements by 18 h post-challenge.

No treatment x time interaction ($P = 0.26$) was detected for cortisol (Figure 2), however there were various times points that met discussion. While there were no

differences in overall cortisol concentrations, 1+QG heifers had greater ($P = 0.05$) circulating cortisol concentrations at 2.5 and 3 h post-challenge. At 3.5 h post-challenge, noQG heifers had a greater ($P = 0.03$) circulating concentration of cortisol. By 24 h, cortisol concentrations had returned to concentrations similar to those observed during the pre-challenge time points.

There was a treatment x time interaction ($P < 0.001$) for IFN-γ. Heifers in the noQG group had increased ($P < 0.05$) circulating concentration of IFN-γ at 4 and 4.5 h post-challenge (Figure 3). By 12 h post-challenge both 1+QG and noQG heifers had IFN-γ concentrations similar to concentrations observed prior to the LPS challenge.

There was a treatment by time interaction ($P < 0.001$) observed for TNF-α with two time points being significantly different between 1+QG and noQG heifers (Figure 4). During the trial, overall TNF-α concentrations were not different ($P > 0.14$) between 1+QG and noQG. At 1 and 1.5 h post-challenge, noQG heifers had a greater ($P < 0.001$) circulating concentration of TNF-α when compared to 1+QG. Two h post-challenge, circulating concentrations of TNF-α were not different ($P > 0.07$) with concentrations steadily decreasing and reaching baseline concentrations by 24 h post-challenge.

A treatment x time interaction ($P < 0.001$) was observed in IL-6 circulating concentrations (Figure 5). There were no differences in initial IL-6 concentrations between 1+QG and noQG heifers prior to the LPS challenge. Differences ($P < 0.01$) observed in IL-6 concentrations were observed at 4, 4.5, 5, 5.5, 6.5, 7.5, and 8 h post-challenge, noQG heifers had greater concentrations of IL-6 at these time points when compared to 1+QG heifers. While noQG heifers had increased concentrations of IL-6 at these time points, both treatment groups possessed a similar pattern in circulating IL-6

response patterns. Starting at 1 h post-challenge, both noQG and 1+QG heifers had an increase in circulating concentrations of IL-6. The greatest ($P < 0.001$) concentration of IL-6 for noQG heifers was observed at 8 h post-challenge. Twenty-four h post-challenge, IL-6 concentrations had decreased and returned to concentrations similar to pre-challenge concentrations for noQG and 1+QG heifers.

## Discussion

An increase in body temperature is one parameter that has been used to evaluate the effectiveness of an LPS challenge (Burdick et al., 2010). In this study, RT increased approximately at 0.5 h post-challenge and spiked at 4 h post-challenge. This follows the same pattern as observed by Carroll et al., 2008; Burdick et al., 2010; and Reuter et al, 2010 relative to a LPS challenge. Furthermore, cytokine profiles followed the same patterns as seen in these studies.

## Conclusion

The purpose of this genetic herd is the development of a resident Angus herd with variations in the presence of the DNAm for intramuscular fat. Thus, the offspring of this herd are vital to the research of the bovine genetic group here at MSU. With the use of LPS at the 0.5 μg/kg BW, while a safe an effective model for evaluating the innate immune, there is the chance of adverse responses that can lead to the lose of some animals. Therefore, before we proceeded with an LPS dose of 0.5 μg/kg BW that is typically utilized, we wanted to ensure these genetically selected heifers could safely handle the typical LSP dosage. By conducting this preliminary study, we were able to

elicit an immune response in Angus heifers with different genetic markers without adverse effects. However, the dose used did not elicit a robust response resulting in lower cytokine production. Due to this, we hypothesized that by increasing the dose of LPS and conducting another study we could achieve the goal of eliciting a more robust and typical innate immune response.

Table 3.1 Percentile breakdown of 1+QG heifers being either homozygous T or heterozygous T (One or Two stars, respectively) for Quality Grade markers (QG) utilized to evaluate the innate immune response of heifers with varying genetic markers for intramuscular fat

| | Heterozygous T One Star | Homozygous T Two Star |
|---|---|---|
| QG1 | 84.6% | 0% |
| QG2 | 61.5% | 30.7% |

[a] QG1 is 537 base pairs upstream from the first exon of the thyroglobulin gene
[b] QG2 is an anonymous SNIP identified by Pfizer Animal Genetics

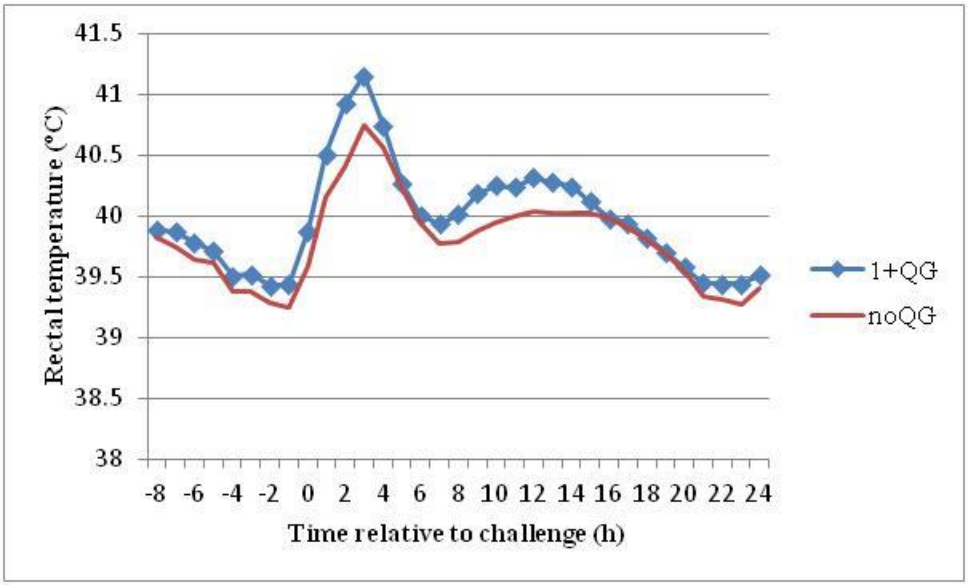


Figure 3.1 Mean rectal temperature (RT) for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.25 µg/kg of BW)

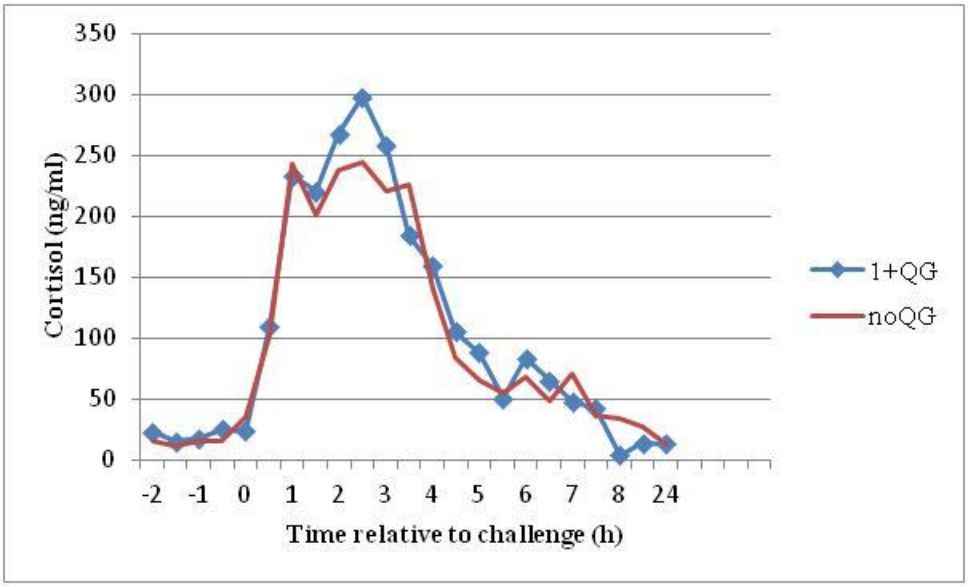


Figure 3.2 Mean serum concentrations of cortisol for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.25 µg/kg of BW)

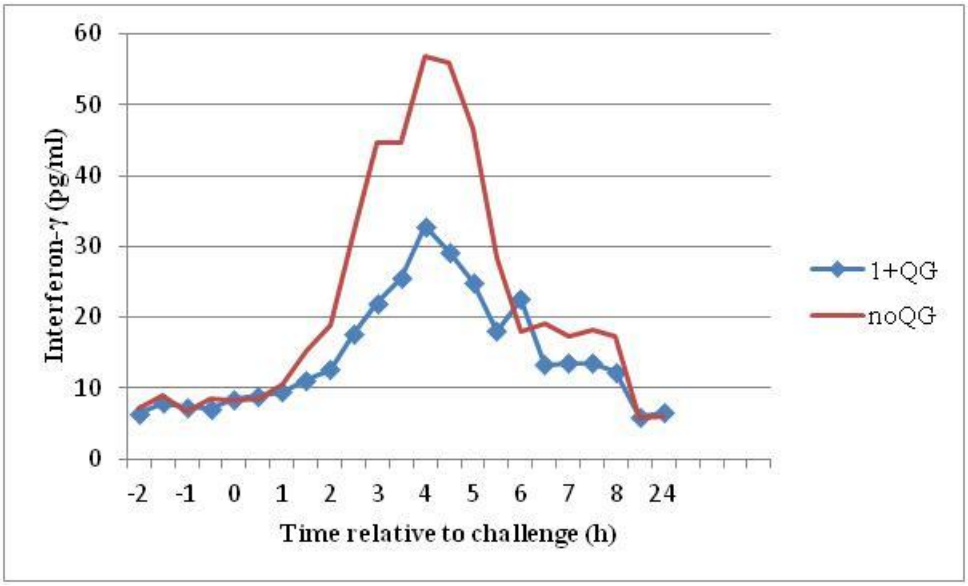


Figure 3.3 Mean serum concentrations of IFN-γ for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.25 μg/kg of BW)

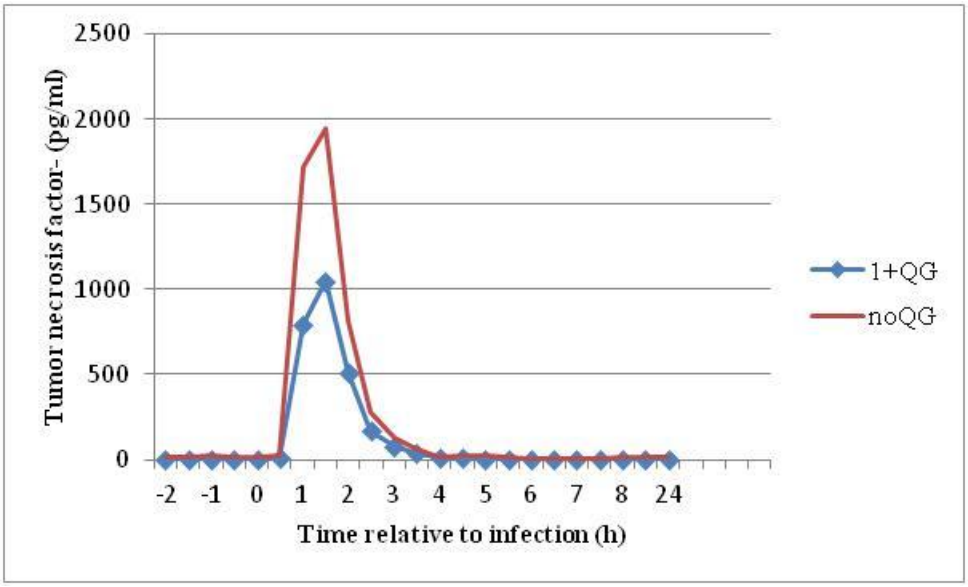


Figure 3.4 Mean serum concentrations of TNF-α for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.25 μg/kg of BW)

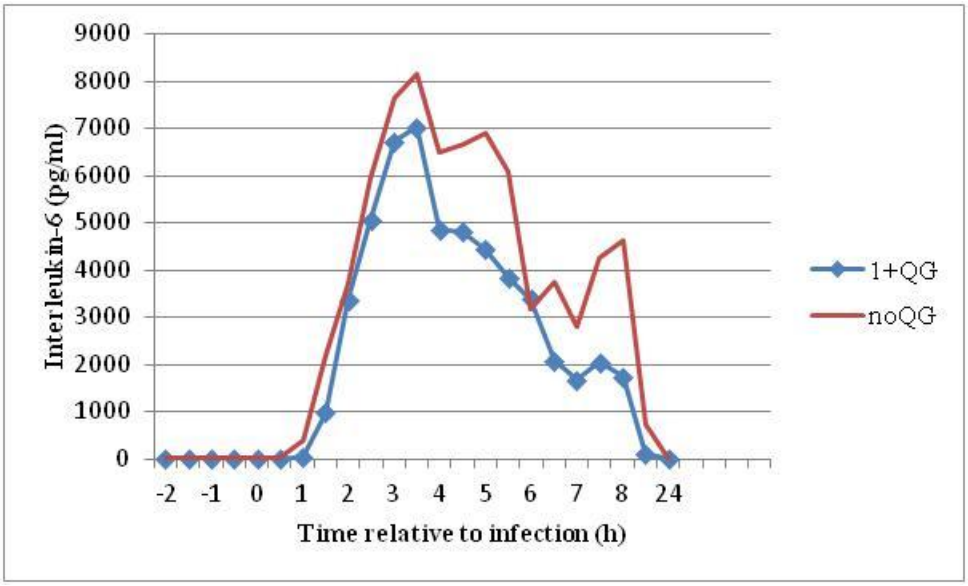


Figure 3.5 Mean serum concentrations of IL-6 for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.25 µg/kg of BW)

CHAPTER IV

# EVALUATION OF THE IMMUNE RESPONSE OF ANGUS HEIFERS WITH DIFFERENT GeneSTAR™ MARKERS FOR MARBLING BY USING AN LIPOPOLYSACCHARIDE (LPS) CHALLENGE


## Abstract

Nineteen heifers (274 ± 24 kg) were blocked into two treatment groups based upon DNAm; heifers with no DNAm (noQG), and heifers with one or more DNAm (1+QG). Prior to challenge (24 hrs), heifers were fitted with indwelling jugular catheters and indwelling vaginal temperature (VT) monitoring devices. Blood samples were collected at 30-min intervals while RT were collected at 1-min intervals from -2 to 8 h relative to a lipopolysaccride (LPS) challenge (0.5 μg/kg BW) at 0 h. Serum was analyzed for concentrations of cortisol and pro-inflammatory cytokines. All physical, endocrine, and immune measurements increased relative to LPS challenge. No differences observed for IL-6 or TNF-α; however, 1+QG heifers had a greater circulating INF-γ ($P < 0.001$). Furthermore, 1+QG heifers had an elevated VT ($P = 0.04$). This would suggest a different immune system approach to an LPS challenge.


## Introduction

During an immune insult, metabolic priorities change in the host to support the immune response and repair damaged tissues (Lochmiller and Deerenberg, 2000).

Because immune insults suppress the intake of food, the body relies on stored protein and energy reserves to supply the nutrients during this time (Lochmiller and Deerenberg, 2000). Furthermore, the immune system is controlled by the availability of nutrients to mount an immune response (Carroll and Forsberg, 2007). A febrile and immune response is an energy demanding response. The febrile response alone has been estimated to increase metabolism rates by 10% to 13% for every degree Celsius increase in animal temperature (Kluger and Rothenburg, 1979). Also, the production of pro-inflammatory cytokines requires energy (Carroll and Forsberg, 2007). During an immune response, animal behavior changes resulting in more time of rest, less time eating, and decreased sexual behavior. During this time of behavioral change, metabolism also is increased in support of an immune response (Carroll and Forsberg, 2007). Without an increase in body temperature and the production of pro-inflammatory cytokines, animal survival and production traits such as weight gain and milk production would decrease at a greater rate when compared to the amount of energy required to support an immune response (Carroll and Forsberg, 2007).

For the past three plus decades, the ability to predict carcass merit of cattle has increased as grid-based marketing has evolved. This increased importance is related to premiums applied to carcasses that achieve a USDA Quality Grade of Low Choice or better. Thus, marbling is the second most important factor affecting the final carcass value of beef cattle (carcass weight is the primary factor). With marbling serving as a key profitability factor, numerous tools are available to producers to improve/predict carcass quality grades. These tools range from live animal evaluation, real-time ultrasounds, EPD's, and marker assisted selection.

With the sequencing of the bovine genome, several genes affecting marbling have been identified (no omnipotent genes); thus commercially available DNA marker assisted tests for marbling are available for cattle producers. A primary DNA marker assisted test available is the GeneSTAR Molecular Value Predictions (GS-MVP) which is a 56-marker panel utilized to evaluate three core management traits of beef cattle, which are feed efficiency, marbling, and tenderness. With this technology, producers can make selection based upon the genetic make-up of a sire and dam to improve carcass merit.

A portion of the GS-MVP is the GeneSTAR Quality Grade that evaluates a single nucleotide polymorphism (SNP) in the 5` leader sequence of the thyroglobulin gene (TG) which is referred to as QG1 and three anonymous SNP's referred to as QG2, QG3, and QG4. While three of the SNP's are anonymous, the TG is known to produce thyroglobulin that is a precursor to thyroid hormone that has a role in adipose metabolism (Barendse, 1999). This role in adipose metabolism while possibly playing an important role in development of marbling may also play an important role in animal health. Newly received feed-lot cattle are subject to two forms of stress which includes weaning/relocating stress, and exposure to new infectious agents that weaken the immune system (Galyean et al., 1999). Thus selection of sire and dam combinations for marbling based upon TG may also provide a benefit to cattle during an immune challenge. Therefore, the objective of this study was to evaluate the innate immune response of cattle genetically selected to posses either one or more copies of the favorable alleles for TG5 or zero copies of the favorable alleles for TG5.

## Materials and Methods

Use of animals and the procedures utilized in this study were approved by the Mississippi State University Animal Care and Use Committee prior to initiation of the experiments (#10-034).

A study was conducted in June 2010 at the Levek animal research center ruminant metabolism facility located at Mississippi State University, Starkville. Heifers utilized for the study were fall-born heifers from the Angus GeneSTAR (GSm) herd; a pure-bred herd located at Mississippi State University. The objective of this herd is producing Angus cattle with genetic variation in the occurrence of QG 1 and QG 2; offspring posses either zero markers (noQG) or one or more markers (1+QG).

To determine the presence or absence of QG 1 and QG 2, hair samples were collected from the tail of the heifers during processing at pre-weaning. Hair samples were shipped to Pfizer Animal genetics for analysis. After collection of hair, heifers were returned to the herd paddocks and maintained there until weaning. For the study, 14 d after weaning heifers were separated from the resident genetic herd and placed into a 1.62 hectare paddock and allowed 21 d to acclimate to environment, diet, and human contact. The diet for study consisted of: 26.2494% soybean hull pellets, 22.0341% corn gluten feed, 27.6565% of Bermudagrass (*Cynodon dactylon*) hay, 23.5592% cracked corn, and 0.5008% mineral premix (DM basis).

For the study, 19 heifers (274 ± 24 kg; 10 noQG heifers and 9 1+QG heifers, percentages of 1+QG heifers are shown in Table 4.1) were fitted with indwelling jugular vein catheters for serial blood collection were inserted - 1 d prior to the start of the trial. Catheters consisted of approximately 150 mm of polytetrafluoroethylene tubing (6417-41

18TW, Cole-Palmer; o.d. = 1.66 mm) that was inserted into a jugular vein using a 14-gauge × 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). The catheter was maintained in place using tag cement and a 5.1-cm-wide porous surgical tape. An extension consisting of sterile plastic tubing (Tygon S-50 HL; VWR Scientific, West Chester, PA; i.d. = 1.59 mm; o.d. = 3.18 mm) was attached to the catheter for collection of blood samples with minimal disturbance of the heifers. Between blood samples, all catheters were flushed with 5 mL of saline (0.9% wt/vol NaCl) followed by 3 mL of heparinized saline (1 ml of heparin 10,000 IU/mL in 500 mL of saline) to replace fluid volume and to maintain catheter patency.

Additionally, on d -1, heifers were also fitted with indwelling vaginal temperature (VT) monitoring devices that recorded VT every min (Burdick et al. 2012). After insertion of catheters and VT devices, heifers were placed into individual tie stalls. On d 0, blood samples were obtained from each heifer in 30 min intervals from -2 to 0 h to serve as a control. At 0 h, heifers received an i.v. bolus dose of lipopolysaccharide (LPS; 0.5 μg/kg of BW) via the jugular catheter. Following LPS challenge, blood samples were collected at 30 min intervals from 0.5 to 8 h post LPS and at 12, 16, 20, and 24 h. After the bolus of LPS, nine ml of blood were obtained every half hour staring at 0.5 h and ending at 8 h relative to challenge. Blood samples were allowed to clot for 30 min at 21ºC and then centrifuged at 2,000 x *g* for 30 min (4ºC) and serum was separated. Serum was collected and transferred into 15 mL microcentrifuge tubes for storage and frozen (-80ºC) for later analysis.

**Serum analysis**

Serum cortisol concentration was determined by radioimmunoassay (Coat-ACount; DPC, Los Angeles, CA) per manufactures directions in a single assay with a detection limit of 2-ng/mL and less than 5% intra-assay coefficient of variation. Serum concentrations of the pro-inflammatory cytokines, interferon-gamma (IFN-γ), interleukin-6 (IL-6), and Tumor necrosis factor-alpha (TNF-α) were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines (SearchLight, Pierce Biotechnology Inc., Rockford, IL). For all cytokines, the intra-assay variation was less than 5 %, and the inter-assay variation was less than 20 %.

**Statistical analyses**

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS as a block design; and the model included sampling time, treatment, and sample time x treatment. Sample treatment x time was used as the error term to test whole plot effects. Rectal (T-1) and vaginal (T-2) temperature was initially recorded at 1 min intervals, but subsequently averaged over 30 min intervals to facilitate comparisons to other immune and physiological parameters. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pair wise differences among least squares means at various sample times were evaluated with the PDIFF option of SAS. Results from the area under the curve calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

## Results

For the study, all physiological, endocrine, and immune measures changed ($P \leq 0.05$) with time in response to the LPS challenge. There was a treatment x time interaction ($P < 0.001$) observed for vaginal temperature (VT, Figure 4.1). No differences ($P > 0.05$) in initial VT (-8 to 0 h) were observed between the two treatment groups. Difference in VT between 1+QG and noQG heifers was observed starting at 6 – 9 and 13 – 21 h post-challenge. Heifers within the 1+QG group had a greater ($P < 0.04$) VT during these two timeframe when compared to noQG heifers. Twenty-four h post-challenge, VT in both 1+QG and noQG heifer's had returned to temperatures observed pre-challenge.

There was no treatment x time interaction ($P > 0.17$) for cortisol (Figure 4.2). There were no differences in initial cortisol concentrations between 1+QG and noQG heifers pre-challenge. Post-challenge, circulating cortisol concentrations began to rise at 0.5 h and peaking between 4.5 and 5 h. Twenty-four h post-challenge, cortisol concentrations were similar to those observed in both treatments pre-challenge.

A treatment x time interaction ($P < 0.001$) was observed for IFN-γ (Figure 4.3). There were no differences observed in IFN-γ concentrations observed during the pre-challenge period, however, 1+QG heifers had greater (P – value) IFN-γ concentrations from 3.5 - 7 h post-challenge. The greatest ($P < 0.001$) concentration of IFN-γ for 1+QG heifers was observed at 4 and 4.5 h post-challenge 362.09 pg/ml and 363.9 pg/ml respectively. Sixteen h post-challenge, IFN-γ concentrations had returned to baseline concentrations for both 1+QG and noQG heifers.

No treatment x time interaction was detected for TNF-α ($P > 0.05$; Figure 4.4). Circulating concentrations of TNF-α followed the same response pattern for both treatment groups. Twenty-four h post-challenge, both 1+QG and noQG concentrations of TNF-α had returned to baseline concentrations observed during the pre-challenge period.

There was no treatment x time interaction detected for IL-6 ($P > 0.17$; Figure 4.5). Both 1+QG and noQG heifers had similar circulating concentrations of IL-6 in response to the LPS challenge. Twenty-four h post challenge, IL-6 concentrations had decreased but were still detectable whereas IL-6 concentrations were not detectable pre-challenge.

## Discussion

During the release of cytokines due to a pro-inflammatory response, metabolism changes to a catabolic state rather than an anabolic state (Elsasser et al., 2008). During this time, storage depots are used to supply energy for an immune response with adipose lipid storage being the first reserves used (Elsasser et al., 2008). Although no fat measurements were obtained from these Angus heifers, treatment groups were based on GeneSTAR Quality Grade markers. Van Eenennaam et al. (2007) observed that cattle, possessing the TG5 favorable allele that is represented by the one GeneSTAR quality Grade star, had an increase of 6.2 % of cattle grading Prime or Choice for each GeneSTAR Quality Grade star (Van Eenennaam et al., 2007). With this increase in quality grade, it appears that cattle with the favorable TG5 allele have the genetic ability for increased intramuscular marbling resulting in a greater propensity for the cattle to grade Prime or Choice (Van Enennaam et al., 2007).

In this study, an increase in vaginal temperature (VT) was observed which is indicative of the typical febrile response elicited by the LPS challenge. This increase in

core body temperature in response to an LPS challenge is stimulated by the increase of pro-inflammatory cytokines (Burdick et al., 2010). Peak VT was observed at 5h post-LPS challenge. Carroll et al. (2009) and Burdick et al. (2010) have reported the febrile response to an LPS challenge to be dose-, breed- and animal-dependent. All immune measurements changed with time relative to the LPS challenge. The increase in circulating concentrations of cortisol and pro-inflammatory cytokines were similar to responses reported by Carroll et al. (2009) and Elsasser et al. (2008). This response was similar to the TNF-α response observed by Carroll et al., (2009) in cattle receiving a 1.0 μg/kg BW LPS challenge. Cortisol concentrations were not different but followed a similar pattern to that observed by Burdick et al. (2010). When IFN-γ was analyzed, IFN-γ was different at time points 3.5-7 h post-LPS challenge with 1+QG heifers having greater circulating concentrations of IFN-γ. However, the peak IFN-γ concentrations during (362.09 pg/ml) in 1+QG heifers at 4 h post-LPS challenge were greater than concentrations observed by Carroll et al. (2009), but the IFN-γ profile did follow a similar time pattern observed by Carroll et al. (2009). The overall IL-6 profile followed a similar pattern reported by Carroll et al. (2009). Furthermore, the immune system is controlled by the availability of nutrients to mount an immune response (Carroll and Forsberg, 2007). A febrile and immune response is an energy demanding response. The febrile response alone has been estimated to increase metabolism rates by 10% to 13% for every degree Celsius increase in animal temperature (Kluger and Rothenburg, 1979). Also, the production of pro-inflammatory cytokines requires energy (Carroll and Forsberg, 2007). The elevated VT and increased IFN-γ concentrations observed in 1+QG heifers displays an increased use of nutrients during an immune insult. The half degree

Celsius increase alone would calculate into 1+QG heifers having an increased metabolism of 5% to 7% based on the febrile response observed in this study. It is still unclear at this time which body reserves were being used at the time of challenge. During an immune response, animal behavior changes resulting in more time of rest, less time eating, and decreased sexual behavior. During this time of behavioral change, metabolism also is increased in support of an immune response (Carroll and Forsberg, 2007). Without an increase in body temperature and the production of pro-inflammatory cytokines, animal survival and production traits such as weight gain and milk production would decrease at a greater rate when compared to the amount of energy required to support an immune response (Carroll and Forsberg, 2007). The overall change in metabolism due to an immune insult is energy demanding (Carroll and Forsberg, 2007).

Previous research has evaluated the use of adipose tissue during an immune insult. Adipocytes have been observed secreting or binding cytokines such as TNF-α and interleukins during an immune insult (Pond, 2005). It has been shown that fatty tissue can account for more than half of an animal's body weight to almost undetectable amounts (Pond, 2012). However, it has become widely accepted these fat depots for the most part are energy reserves to be used during times of energy needs (Pond, 2012). For this study, non esterified fatty acids (NEFA) and blood urea nitrogen (BUN) concentrations should be measured. By measuring these blood parameters, the observation could be analyzed looking to see if 1+QG heifers were metabolizing fat depots that they would be expected to contain based on the genetic markers for intramuscular fat deposition.

Table 4.1 Percentile breakdown of 1+QG heifers being either homozygous T or heterozygous T (One or Two stars, respectively) for Quality Grade markers (QG) utilized to evaluate the innate immune response of heifers with varying genetic markers for intramuscular fat

| | Heterozygous T One Star | Homozygous T Two Star |
|---|---|---|
| QG1[a] | 45.5% | 55.5% |
| QG2[b] | 33.3% | 22.2% |

[a] QG1 is 537 base pairs upstream from the first exon of the thyroglobulin gene
[b] QG2 is an anonymous SNIP identified by Pfizer Animal Genetics

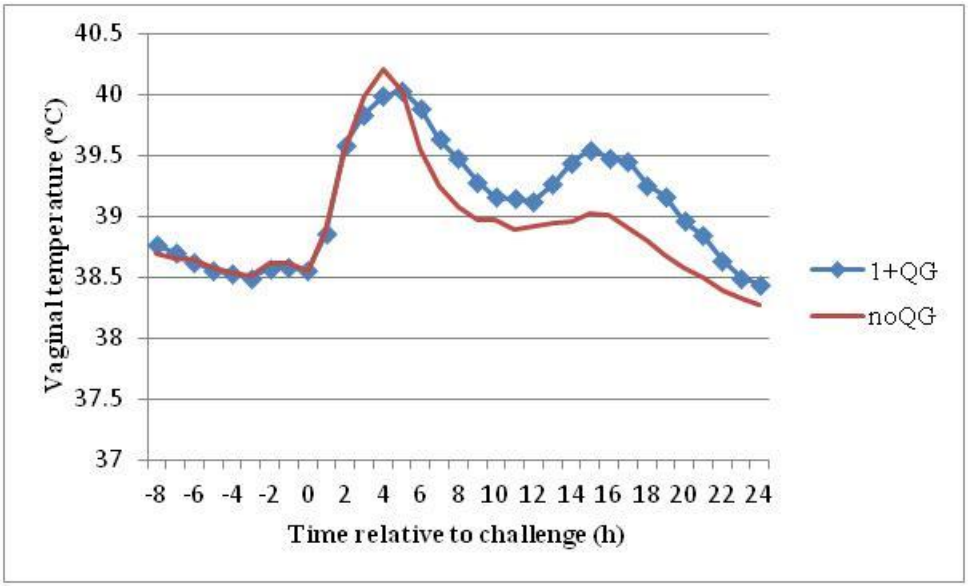


Figure 4.1 Mean vaginal temperature (VT) for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.5 µg/kg of BW)

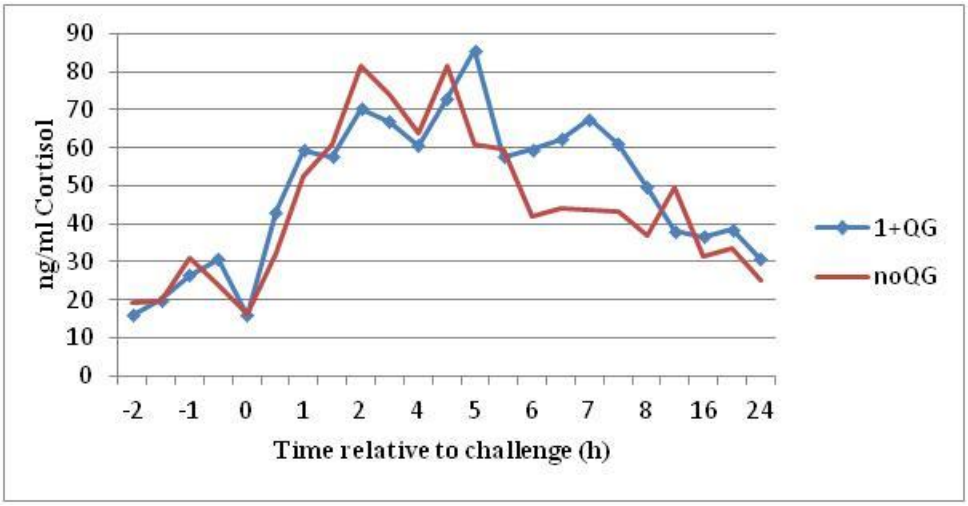


Figure 4.2 Mean serum concentrations of cortisol for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.5 μg/kg of BW)

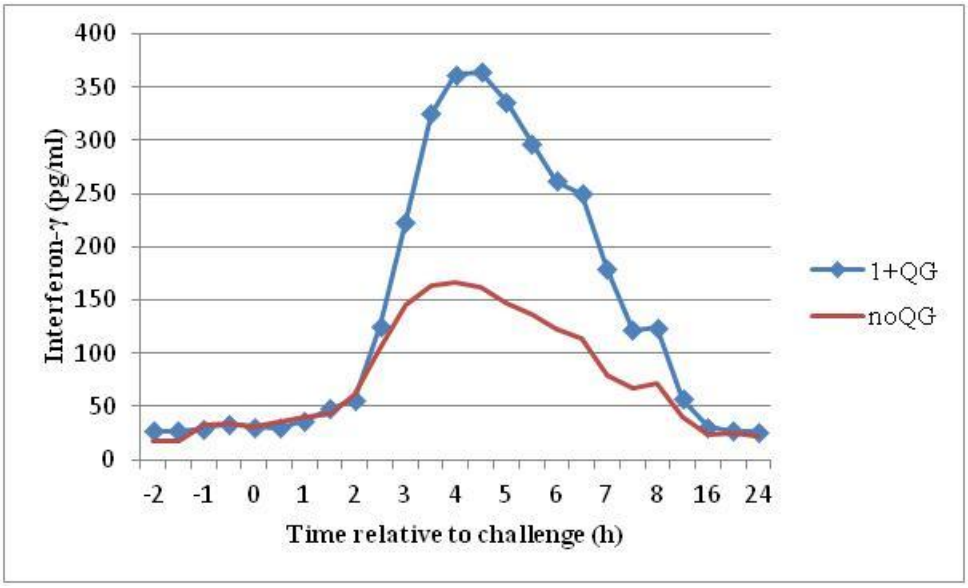


Figure 4.3 Mean serum concentrations of IFN-γ for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.5 μg/kg of BW)

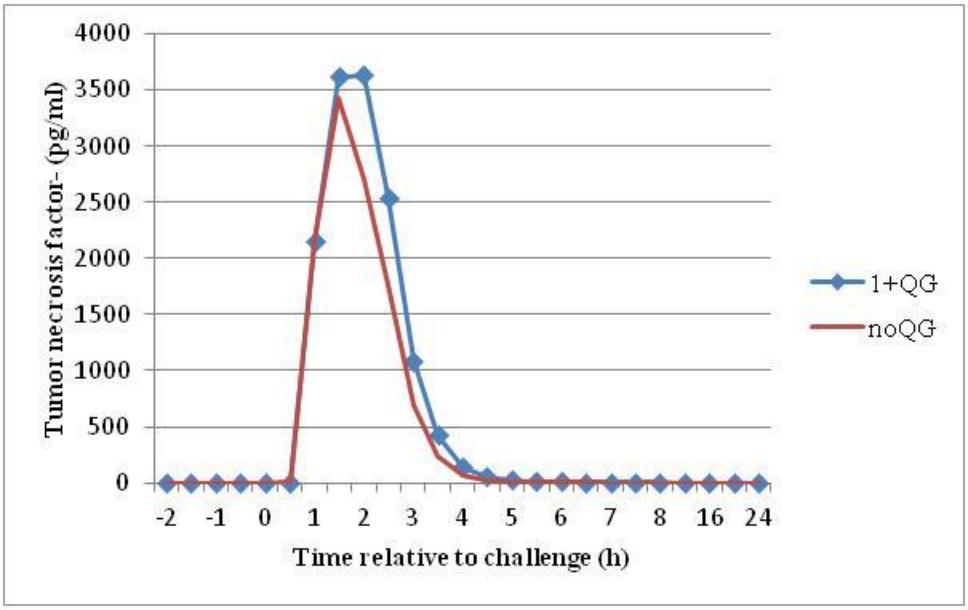


Figure 4.4 Mean serum concentrations of TNF-α for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.5 µg/kg of BW)

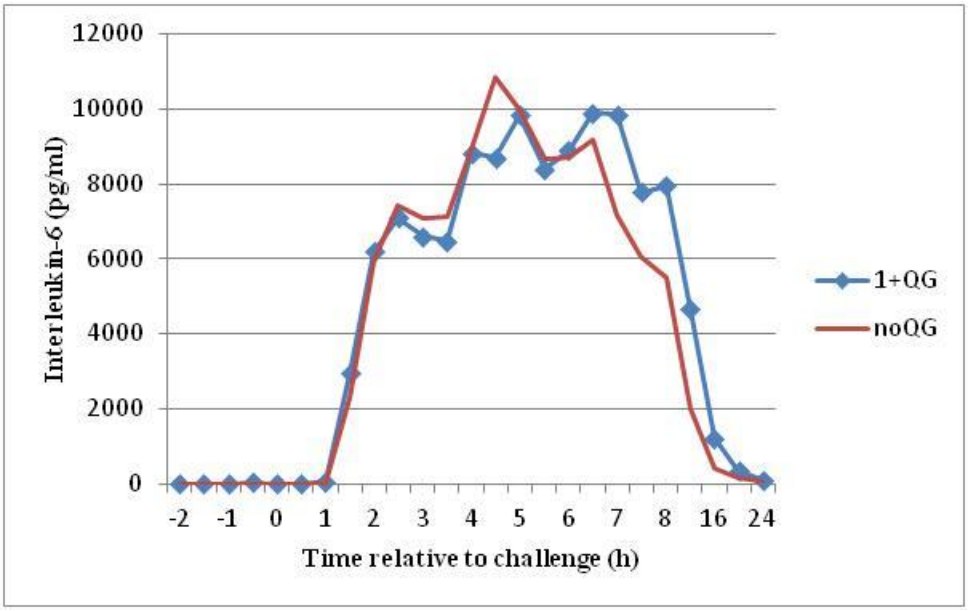


Figure 4.5 Mean serum concentrations of IL-6 for 1+QG and noQG heifers following an i.v. bolus dose of lipopolysaccaride (LPS) challenge (0.5 µg/kg of BW)

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