**Evaluation of the innate immune response of Angus heifers with genetic marker variation
for intramuscular fat deposition following a lipopolysaccharide challenge^{1,2}**

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Abstract: This study evaluated the effect of genetic selection for markers related to marbling deposition in Angus heifers on the immune response following a lipopolysaccharide (LPS) challenge. Fall-born heifers ($n = 19$; ~7 months of age, 274 ± 24 kg) with genetic variation for marbling were utilized including 10 heifers with no genetic markers (No-MVP) and 9 heifers with ≥ 1 markers (1+MVP). Genotypic variation for quantitative trait loci QG1 and QG2 heifers was determined. Twenty-four hour (h) prior to the LPS challenge, heifers were fitted with jugular catheters and vaginal temperature (VT) monitoring devices. Temperature monitoring devices were programmed to record VT in 1 min intervals. On day (d) 0, 2 h prior to LPS challenge and 8 h post challenge, serum was collected in 0.5 h intervals (0800 – 1800 h). Serum was also collected at 12, 16, 20, and 24 h post LPS challenge. The LPS challenge was administered to the heifers at 1000 h via intravenous bolus ($0.5 \mu\text{g/kg BW}$). Serum was analyzed for cortisol, $\text{TNF-}\alpha$, IL-6, $\text{IFN-}\gamma$, and haptoglobin (HAPT). A treatment x time interaction existed ($P \leq 0.001$) for VT; prior the LPS challenge, VT was similar. Vaginal temperature was elevated from 6 to 21 h post-LPS in 1+MVP compared to No-MVP. A treatment x time interaction ($P = 0.02$) was observed for $\text{IFN-}\gamma$. Prior to the LPS challenge, $\text{IFN-}\gamma$ concentrations were similar between the two groups. However, 3.5 to 7 h post-LPS concentrations of $\text{IFN-}\gamma$ were greater ($P \leq 0.05$) for 1+MVP compared to No-MVP. There was no treatment effect ($P \geq 0.17$) for cortisol, $\text{TNF-}\alpha$ or IL-6. A treatment effect ($P = 0.02$) was observed for HAPT; 1+MVP had decreased HAPT compared to No-MVP. Overall, results suggest that heifers selected based upon genetic markers for marbling may have an altered immune in response when challenged with LPS.

Keywords: Acute phase response, Cattle, Cytokines, Marbling, Quantitative trait loci

1. Introduction

With the mapping of the bovine genome, numerous quantitative trait loci for important production traits of cattle have been identified and as such, commercially available DNA marker assisted tests are available for cattle producers. A primary DNA marker assisted test available is the GeneSTAR[®] Molecular Value Predictions (GS-MVP; Zoetis, NJ) which is a 56-marker panel utilized to evaluate three core management traits of beef cattle; feed efficiency, marbling, and tenderness. For marbling, two markers have been identified to estimate marbling: QG1, which is *TG5*, a single-nucleotide polymorphism upstream from the start of the first exon of thyroglobulin, and QG2, which is an anonymous SNP (VanEenennaam et al., 2007). The *TG5* favorable allele T has been associated with increases in marbling scores and accounts for 6.5% of the residual variance for the marbling phenotype (Barendse, 1999).

Intramuscular adipose tissue is directly related to beef quality (USDA quality grades), enhanced flavor, and an overall improvement in consumer satisfaction of beef. However, in the living system, intramuscular adipose tissue provides an important source of triacylglycerols during periods of increased energy demand (Shaw et al., 2010). Human kinesiology researchers have reported that a 2 h cycling event at 60% maximum oxygen uptake can result in a 60% reduction in intramuscular adipose tissue content (Shaw et al., 2010). While cattle may never experience maximum oxygen uptake, there are times when cattle have a need for increased energy such as during an immune insult. When challenged with an immune insult, metabolic priorities change in the host to support the activation and response of the immune system (Lochmiller and Deerenberg, 2000). Furthermore, as a result of the immune challenge, there may be suppression of feed intake, thus the body must rely on energy reserves to supply energy to support the immune response (Lochmiller and Deerenberg, 2000). Additionally, the febrile

response during an immune insult has been estimated to increase metabolism rates by 10% to 13% for every degree Celsius increase in body temperature (Kluger and Rothenburg, 1979). While the exact physiological function of intramuscular adipose in beef cattle has not been fully elucidated, it is a source of stored energy, and as an energy source may provide additional energy in times of need.

Due to the important role of intramuscular adipose tissue, both within the living animal and the final product, the selection of sire and dam combinations for marbling QTL's may provide a dual benefit. The benefits marbling in the final product are well documented (Casas et al., 2007; Rinker et al., 2006; VanEenennaam et al., 2007). However, to date, there has been no evaluation of the possible benefits for selection of marbling QTL's on the immune response of cattle. As an energy storage depot, intramuscular adipose tissue could potentially be an energy source when energy demands increase in order to initiate an immune response. Therefore, the objective of this study was to evaluate the innate immune response of cattle genetically selected to possess either one or more copies of the two QTL's associated with marbling deposition (QG1 and/or QG2).

2. Materials and methods

2.1: Experimental design

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 (approval #10-034). Fall-born heifers (n = 19; BW 274±24 kg) were selected from the Angus GeneSTAR herd were utilized for this trial. The Angus GeneStar[®] herd is a pure-bred herd maintained for producing Angus cattle with genetic variation in the occurrence of QG 1 and QG 2.

Determination of the presence or absence of QG 1 and QG 2 was determined via hair samples analyzed by Zoetis animal genetics (Florham Park, NJ). Based upon QTL determination, 10 heifers with no QTL markers (No-MVP) for marbling and 9 heifers with one or more QTL makers (1+MVP) for marbling were identified for inclusion into the study.

Fourteen day (d) post-weaning, identified 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 the duration of the trial consisted of: 26.25% soybean hull pellets, 22.03% corn gluten feed, 27.66% of Bermudagrass (*Cynodon dactylon*) hay, 23.56% cracked corn, and 0.50% mineral premix (DM basis).

Twenty-four hour (h) prior to the immune challenge (d -1), heifers were fitted with indwelling jugular vein catheters for serial blood collection as well as indwelling vaginal temperature (VT) recording devices, programmed to record VT at 1min. intervals for the duration of the trial (Burdick et al., 2012). 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.

After insertion of catheters and VT devices, heifers were placed into individual tie stalls and allowed ad libitum access to feed and water. On d 0 (day of the immune challenge), starting at 0800 h and continuing until 1600 h, blood samples were collect every 30min. (8 h time period) and again at 12, 16, 20, and 24 h post-lipopolysaccharide (LPS) challenge (2100, 0100, 0500, and 1000 h). Following the collection of blood at 1000 h, heifers received an i.v. bolus dose of LPS (0.5 µg/kg of BW LPS from *E. coli* O111:B4; Sigma Aldrich, St. Louis, MO USA) via the jugular catheter. Whole blood was collected into 9 mL monovette tubes with no additive

(Sarstedt Inc., Newton, NC, USA). After collection, blood samples were allowed to clot for 30 min at 21°C, centrifuged at 2,000 x g for 30 min. (4°C). Serum was collected and transferred into 1.5 mL microcentrifuge tubes and stored at -80°C prior to analysis

2.2 Cortisol, Tumor Necrosis Factor – α , Interferon – γ , Interleukin – 6, and Haptoglobin

All serum samples were analyzed in duplicate. Serum cortisol concentrations were determined using a commercially available enzyme immunoassay kit according to the manufacturer's directions (Arbor Assays, Ann Arbor, MI) by comparison of unknowns to standard curves generated with known concentrations of cortisol. The minimum detectable cortisol concentration was 45.4 pg/mL, and the intra- and inter-assay coefficients of variation were 5% and 20%, respectively. Data are presented as ng/mL.

Serum cytokine concentrations (TNF- α , IFN- γ , and IL-6) were determined by a custom bovine 3-plex sandwich-based chemiluminescence ELISA kit (Searchlight-Aushon BioSystems, Inc., Billerica, MA). The minimum detectable concentrations were 0.5, 0.1, and 3.3 pg/mL for TNF- α , IFN- γ , and IL-6, respectively. All intra-assay coefficients of variation were less than 5% and all inter-assay coefficients of variation were less than 20% for all assays. Data are presented as pg/mL. Serum haptoglobin concentrations were determined by measuring haptoglobin/hemoglobin complex by the estimation of difference in peroxidase activity (Hulbert et al., 2011). Results were expressed in arbitrary units resulting from the absorption reading at 450 nm x 100.

2.3 Statistical analysis

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed as repeated

measures within the MIXED procedure of SAS as a block design (No-MVP vs. 1+MVP) with individual animal serving as the experimental unit; 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. Vaginal temperature was initially recorded at 1min. intervals, but subsequently averaged over 30min. 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.

3. Results

3.1 Rectal temperature:

There was a treatment x time interaction ($P < 0.001$) for vaginal temperature (Figure 1). Prior to the LPS challenge (-8 to 0 h), VT between the two groups was similar ($P > 0.60$). Within 1 h of administering the LPS challenge, VT increased for both groups. Maximum VT was achieved at 4 h post-LPS challenge for the No-MVP heifers ($40.2^{\circ}\text{C} \pm 0.08$) and maximum VT for 1+MVP heifers was achieved at 5 h post-LPS challenge ($40.0^{\circ}\text{C} \pm 0.08$). After achieving maximum VT, temperature in both groups began to decrease. During these time periods (6 to 9 h post-LPS challenge) 1+MVP heifers had a greater ($P \leq 0.04$) VT, when compared to No-MVP heifers. Nine h post-LPS challenge, VT was $39.3^{\circ}\text{C} \pm 0.06$ and $39.0^{\circ}\text{C} \pm 0.11$ for 1+MVP and No-MVP, respectively. For a 3 h period (10 – 12 h post-LPS challenge), there was no difference ($P \geq 0.08$) in VT between the two treatment groups. Starting at h 13, a biphasic response was observed within both treatment groups. For a two-h period (13 – 15 h), VT increased in both treatment groups. However, this increase in VT for 1+MVP was greater ($P < 0.03$) than that of

the No-MVP heifers. This increase in VT resulted in the 1+MVP heifers having greater ($P < 0.03$) VT for the next 6 h (16 – 21 h post-LPS challenge), when compared to No-MVP heifers. There was no difference ($P \geq 0.10$) in VT between the two groups for the remainder of the trial (22 – 24 h).

3.2 Cortisol

There was no treatment effect ($P = 0.17$) or treatment x time interaction ($P = 0.15$) for cortisol concentrations. Cortisol concentrations were similar between the two treatment groups for the duration of the LPS challenge (prior and post-LPS challenge; Table 1). There was a time effect ($P < 0.001$) for cortisol concentrations (Figure 2). Prior to the LPS challenge (- 2 to 0 h), cortisol concentrations were on average, $21.8 (\pm 6.8)$ ng/mL for 1+MVP heifers and $21.9 (\pm 6.8)$ ng/mL for No-MVP heifers. Cortisol concentrations in both groups of heifers increased ($P = 0.003$) 30 min after the LPS challenge, and continued to increase until maximum cortisol concentrations were achieved at 4.5 h for No-MVP heifers ($81.7 \text{ ng/mL} \pm 9.8$) and 5 h for 1+MVP heifers (85.6 ± 9.8 ng/mL). Following 4.5 h (No-MVP) and 5 h (1+MVP) time period, cortisol concentrations steadily declined. Cortisol concentrations at 24 h post-LPS challenge were not different from baseline concentrations ($P > 0.08$).

3.3 Tumor Necrosis Factor - α

For the pro-inflammatory cytokine TNF- α , no treatment ($P > 0.52$) or treatment x time interaction ($P = 0.98$) was observed (Table 1). There was a time effect ($P < 0.001$) for both treatment groups TNF- α concentrations followed a similar pattern. Concentrations for TNF- α were not detectable prior to challenge and a large spike ($> 3,500$ pg/mL) in TNF- α

concentrations occurred at 1 h post-LPS. This spike in TNF- α was sustained for a 3 h period, and returned to baseline concentrations at 4 h post-LPS challenge.

3.4 Interleukin - 6

No treatment ($P = 0.70$) or treatment x time interaction ($P = 0.17$) was observed for the pro-inflammatory cytokine IL-6 (Table 1) however, there was a time effect ($P < 0.001$). Prior to the LPS challenge (-2 – 0 h), concentrations of IL-6 were minute (< 25 pg/mL). One h post LPS challenge, regardless of treatment, concentrations of IL-6 began to increase and remain elevated ($> 5,000$ pg/mL) until 16 h post-LPS challenge.

3.5 Interferon - γ

A treatment x time interaction ($P < 0.001$) was observed for IFN- γ . Prior to the LPS challenge, there was no difference between the two treatment groups (Figure 5). Concentrations of IFN- γ for both groups were < 30 pg/mL, and remained < 60 pg/mL until 2 h post-LPS challenge. At 3.5 h post-LPS challenge, concentrations of IFN- γ increased in both treatment groups ($P < 0.001$). This difference ($P \leq 0.007$) in IFN- γ concentrations between the 1+MVP heifers and the No-MVP heifers remained until 7.5 h post-LPS challenge. Concentrations of IFN- γ in both treatment groups did not return to baseline (< 30 pg/mL) until 16 h post-LPS challenge.

3.6 Haptoglobin

A treatment ($P = 0.02$) and time ($P < 0.001$) effect were observed for the acute phase protein haptoglobin; however, no interaction ($P = 0.20$) was observed. Overall, haptoglobin concentrations were greater in No-MVP heifers when compared to 1+MVP heifers (3.68 ± 0.12

pg/mL vs. $3.28 \text{ pg/mL} \pm 0.13$; Figure 6). Prior to the LPS challenge, regardless of the treatment, concentrations of haptoglobin were negligible ($< 2.0 \text{ pg/mL}$). Following the LPS challenge, haptoglobin concentrations within both treatments became elevated between 2.0 pg/mL and 4.0 pg/mL , and fluxuated within this range until 8 h post-LPS challenge (Figure 7). Starting 12 h post-LPS challenge, concentrations of haptoglobin increased to $8 \text{ pg/mL} - 12 \text{ pg/mL}$; and remained at these concentrations until the conclusion of the trial (24 h post-LPS challenge).

4. Discussion

Intramuscular adipose tissue (IMAT) is of significance importance to beef cattle; it is the major determination factor for USDA Beef Quality Grades and is vital for the flavor and palatability of beef. Aside from the value of IMAT to meat quality, and storage form of triacylglycerol's, very little is known in regards IMAT. Researchers are still trying to unlock the mechanism of IMAT development, however it is estimated that this development occurs mainly during the late fetal-neonatal stage to about 250 d of age in beef cattle. Furthermore, there is limited data available in regards to the biological function of IMAT during homeostasis and during times of increased energy demands.

In terms of animal health, available energy is one of the major limiting factors in an animal's ability to mount an immune response. During an immune insult there is a major metabolic shift from an anabolic state to a catabolic state (Elsasser et al., 2008). This change in metabolism is designed to provide energy to the immune system. As discussed previously, the febrile response alone during an immune insult is estimated to increase metabolic energy demands by 10 – 13% for every degree Celsius increase in body temperature (Kluger and Rothenburg, 1979). During this catabolic shift, adipose tissue serves as the first energy reserve

utilized (Elsasser et al., 2008). Therefore, the hypothesis of the present study was that cattle selected for possession of GeneStar[®] Molecular Value Predictions QTL's for marbling, (greater amount of triacylglycerol's stored in intramuscular adipose tissue), would have a different immune response to an LPS challenge when compared to cattle lacking QTL's for marbling.

Alteration in body temperature was observed between the No-MVP heifers and 1+MVP heifers. Prior to the LPS challenge, there was no difference in VT between the two groups of heifers. This lack of a difference in VT was maintained until after 6 h post-LPS challenge. Excluding a 3-h period (10 – 12 h post-LPS challenge), VT in the 1+MVP heifers was greater than No-MVP heifers from 6 h to 22 h post-LPS challenge. While it has been typically thought that an elevation in body temperature is a negative response to an immune insult, this is not the case. Researchers in both humans and animals have reported benefits of increased body temperature following an immune challenge (Hasday et al., 2000; Kluger et al., 1998). Mild systemic heat treatment (raising core body temperature to 39.5°C) to mice challenged with LPS was reported to enhance the production of TNF- α and IL-6 within serum (Ostberg et al., 2000). It has also been reported that heat treatment to mice challenged with LPS enhanced LPS-induced TNF- α production (Lee et al., 2012). Furthermore, within the same study, heat treatment increased the number of TNF- α producing macrophages and enhanced TNF- α transcription through increasing LPS-induced nuclear factor kappa of activated B cells and heat shock protein 70 secretion (Lee et al., 2012). While the driving factor for this increase in VT of the 1+MVP heifers cannot be fully elucidated from this study, a possible explanation for this difference could be based upon selection for IMAT. Heifers from the 1+MVP group may have greater energy reserves from which to pull from to sustain the increased body temperature.

Activation of the hypothalamic-pituitary-adrenal axis is a key component to an animal's immune response to viral and bacterial pathogens. During an immune response, pro-inflammatory cytokines are released from various tissues; nearly all nucleated cells are capable of synthesizing cytokines (Dinarello, 2000). Release of cortisol serves to counter balance the production of pro-inflammatory cytokines to prevent a hyper-inflammatory state within the body. However, evidence suggests that cortisol may play multiple roles in the immune response, depending upon the magnitude duration of the stress response. Research has reported that peripheral and central nervous system concentrations of pro-inflammatory cytokines were increased in rats following an acute stressor (O'Connor et al., 2003). Acute stress has also been reported to enhance peripheral inflammation due to glucocorticoid enhanced leukocyte migration to areas of localized inflammation. The increase in cortisol concentrations rapidly following the LPS challenge in the present study is very similar to changes reported by other researchers (Burdick et al., 2010; Carroll et al., 2009). Increases in cortisol concentrations in response to an LPS challenge were reported to have occurred before observed increases in cytokines (Carroll et al., 2009). In a trial utilizing Brahman bulls, a rather rapid increase in cortisol concentrations (~30 min post-LPS challenge) occurred when bulls were challenged with LPS (Burdick et al., 2010). The increase in cortisol concentrations following the LPS challenge may also be linked to the need for the amino acid glutamine by leukocytes. Leukocytes have a particularly large requirement for the glutamine; glutamine consumption exceeds even the need for energy (Pond and Newsholmes, 1999). Glucocorticoids stimulate the up regulation of glutamine synthetase, which results in glutamine being extracted from muscle (S. R. Max; Mills J. Mearow K, 1988). During stress, glutamine and alanine account for $\frac{1}{2}$ to $\frac{2}{3}^{\text{rd}}$ of the amino acid nitrogen released

from muscle and transported to other tissues (Brooks et al., 1986; Loderet al., 1990; Fong et al., 1990).

A major action of the innate immune response to an immunological insult is the release of pro-inflammatory cytokines (IL-6, TNF- α , and IFN- γ). These pro-inflammatory cytokines have numerous effects throughout the body. Functions of TNF- α include: 1) initiation of a cascade activation of other cytokines and growth factors for the inflammatory response, widely considered the prototypic pro-inflammatory cytokine (Sommer and Kress, 2004); 2) growth and differentiation of B cells (Akira et al., 1990); and 3) induction of endothelial adhesion molecules (in conjunction with IL-1 (Dinarello, 2000). Functions of IL-6 include: 1) acting as an important trigger for the production of acute phase proteins (Cray et al., 2009); T cell activation and differentiation (Akira et al., 1990); and 3) macrophage differentiation (Akira et al., 1990). Interferon- γ , classically considered an antiviral cytokine also plays an important role in the innate immune response regardless of the type of pathogen (Dinarello, 2000). Functions of IFN- γ include: 1) activator of pathway that leads to cytotoxic T cells (Dinarello, 2000); 2) induces nitric oxide production (Dinarello, 2000); 3) augmentation of TNF- α activity (Dinarello, 2000); 4) enhancement of natural killer cell activity (Schroder et al., 2004); and 5) increased activation of macrophages (Schroder et al., 2004). During this study, the LPS challenge was able to stimulate the production of TNF- α , IL-6, and IFN- γ , regardless of treatment groups. The magnitude and timing of cytokine production for this study was very similar to reports by others (Burdick et al., 2010; Carroll et al., 2013; Carroll et al., 2009; Waggoner et al., 2009). For TNF- α and IL-6, there were no difference between the No-MVP heifers and the 1+MVP heifers; yet, there was a difference in the production of IFN- γ . Following the LPS challenge, both treatment groups respond to the challenge within 2.5 h. However, 3.5 h after the LPS challenge, IFN- γ

concentrations in the 1+MVP heifers were greater than the No-MVP heifers and remain greater until 7 h post-LPS challenge. During this 4 h period (3.5 to 7 h), the concentrations of IFN- γ for the 1+MVP heifers were typically 1-fold greater than IFN- γ concentrations of No-MVP heifers. This 1-fold increase in IFN- γ in the 1+MVP heifers represents a rather intriguing response. One of the most important cell products that directly stimulate macrophages during an immune response is IFN- γ (Schroder et al., 2004). Macrophages possess microbicidal properties such as induction of NADPH-dependent phagocyte oxidase system, priming for nitric oxide production, tryptophan deletion, and up regulation of liposomal enzymes capable of destroying microbes (Decker et al., 2002). This greater production of IFN- γ by the 1+MVP heifers likely required more energy than that of the No-MVP heifers for IFN- γ production. Thus, it is hypothesized that the heifers selected based upon the possession of one or more QTL's for marbling may have a greater propensity for storage of excess energy (greater energy reserve), thus allowing for more energy to meet the demand associated with increased production of IFN- γ .

The acute phase proteins play major roles in the body's systemic response to inflammation (Ceciliani et al., 2012). Haptoglobin, which is produced by hepatocytes, is considered to be the most prominent acute phase protein in cattle (Cooray et al., 2007). The primary activity of haptoglobin is the formation of the haptoglobin-hemoglobin complex (Hp-Hb complex) (Ceciliani et al., 2012). By binding with hemoglobin, haptoglobin ensures that free hemoglobin (from damaged erythrocytes) is rapidly cleared from circulation (Shih. et al., 2014), thus preventing oxidative damage to hemoglobin (Buehler et al., 2009), albumin (Bunn and Jandl, 1968), lipids (Melamed-Frank et al., 2001), and the tissue of the kidneys (Ceciliani et al., 2012). The formation of the Hp-Hb complex also has an anti-inflammatory effect. The Hp-Hb complex can bind to CD163 of monocytes/macrophages, which

signals the up-regulation of anti-inflammatory cell products such as inducible heme oxygenase – 1 and IL-10 (Philippidis et al., 2004; Schaer et al., 2006). Furthermore, the formation of the Hp-Hb complex also has a bacteriostatic effect by preventing bacteria from utilizing the iron from hemoglobin. Heifers within the No-MVP group had greater overall haptoglobin concentrations when compared to the 1+MVP heifers; there was no treatment x time interaction. The greater haptoglobin concentrations in the No-MVP heifers may indicate a greater need to either sequester free hemoglobin due to damage of erythrocytes or to reduce inflammation related to the LPS challenge.

5. Conclusion

Overall, the results of this study suggest some variation associated with the immune response of heifers possessing one or more markers for intramuscular fat when compared to the heifers in the No-MVP group. Heifers possessing one or more GeneStar[®] Molecular Value Prediction QTL's, when challenged with LPS had an increase in vaginal temperature, increased IFN- γ concentrations, and decreased haptoglobin production compared to the No-MVP heifers. These distinct differences in the immune response may be related to an increase in available energy.

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Figure 1

Figure 1. Vaginal temperature of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n = 10) challenged with 0.5 µg/kg BW of lipopolysaccharide

^{a, b} Lsmeans at specific time points without a common subscript are different between treatment groups if ($P \leq 0.05$)

Figure 2

Figure 2. Serum cortisol concentrations of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n = 10) challenged with 0.5 μ g/kg BW of lipopolysaccharide

Figure 3

Figure 3. Serum tumor necrosis - α concentrations of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n=10) challenged with 0.5 μ g/kg BW of lipopolysaccharide

Figure 4

Figure 4. Serum interleukin - 6 concentrations of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n =9) or no QTL's for marbling (No-MVP; n =10) challenged with 0.5 µg/kg BW of lipopolysaccharide

Figure 5

Figure 5. Serum interferon – γ concentrations of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n = 10) challenged with 0.5 μ g/kg BW of lipopolysaccharide

^{a, b} Lsmeans at specific time points without a common subscript are different between treatment groups if ($P \leq 0.05$)

Figure 6

Figure 6. Serum haptoglobin concentrations of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n = 10) challenged with 0.5 µg/kg BW of lipopolysaccharide

Table 1. Endocrine and immune variable means of Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9) or no QTL's for marbling (No-MVP; n = 10) challenged with 0.5 µg/kg BW of lipopolysaccharide.

Variables	1+MVP ²	No-MVP ³	SEM	P - value		
				Treatment	Time	Treatment x Time
Cortisol	49.0	44.6	2.2	0.17	<0.001	0.15
TNF – α ⁴	548.7	443.9	378.2	0.52	<0.001	0.98
IL – 6 ⁵	4631.5	4303.4	1243.7	0.70	<0.001	0.17
IFN – γ ⁶	137.1	76.2	50.1	0.09	<0.001	<0.001
Haptoglobin	3.3 ^a	3.7 ^b	0.9	0.02	<0.001	0.20

¹ Serum concentrations of all variables are presented

² Angus heifers possessing one or more GeneSTAR[®] Molecular Value Predictions QTL's for marbling (1+MVP; n = 9)

³ Angus heifers possessing no GeneSTAR[®] Molecular Value Predictions QTL's for marbling (No-MVP; n = 10)

⁴ Tumore necrosis – α

⁵ Interleukin – 6

⁶ Interferon – γ

^{a, b} Lsmeans within a column, within a treatment, without a common subscript are different between treatment groups if ($P \leq 0.05$)