Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge

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Abstract

Our primary objective of this experiment was to evaluate potential genetic differences between two diverse Bos taurus breeds [Angus (AG) and Romosinuano (RO)] in response to an endotoxin challenge. Eighteen steers (n = 9 steers/breed; 299.4 ± 5.2 kg BW) were acclimated to environmentally controlled chambers maintained at thermoneutrality (19.7 °C) and then fitted with indwelling jugular catheters and rectal temperature (RT) recording devices 1 d before the endotoxin challenge. The next day, blood samples were collected at 30-min intervals from -2 to 8 h, and RT was measured continuously at 1-min intervals throughout the study. At time 0, all steers received an intravenous bolus injection of lipopolysaccharide (LPS; 2.5 µg/kg BW). Serum samples were stored at \(-80°C\) until analyzed for cortisol, proinflammatory cytokines [tumor necrosis factor-alpha (TNF-\(\alpha\)), interleukin-1 beta (IL-1\(\beta\)), IL-6, and interferon gamma (IFN-\(\gamma\))], and acute phase proteins (serum amyloid A, acid soluble protein, ceruloplasmin, and \(\alpha\)-acid glycoprotein). Rectal temperatures increased in both breeds within 1 h after LPS, with RO producing a greater increase in RT than AG steers (\(P < 0.001\)). Serum cortisol and TNF-\(\alpha\) increased (\(P < 0.01\)) in both breeds within 1 h after the LPS challenge. For cortisol, an overall breed effect (\(P < 0.02\)) was detected, such that AG steers had a higher cortisol response than RO steers. A breed \(\times\) time interaction (\(P < 0.01\)) was observed for TNF-\(\alpha\), such that the response was delayed and extended in the RO steers compared...
with the AG steers. At 2 and 2.5 h after LPS, TNF-α concentrations were greater ($P < 0.03$) in RO steers than in AG steers. For IL-1β, a breed × time interaction ($P < 0.04$) was also observed. At 3 h after LPS, IL-1β concentrations were greater ($P < 0.01$) in RO steers than in AG steers. Serum IL-6 and IFN-γ increased ($P < 0.01$) in a similar manner in both groups after the LPS challenge. These data show differences in the innate immune response between two diverse *Bos taurus* breeds which may provide insight about differences observed in productivity, heat tolerance, disease resistance, and longevity among cattle breeds.

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1. **Introduction**

Previous studies have shown the potential for genetic differences associated with immune responses in various breeds of cattle. Blecha et al [1] reported that the immunologic response to shipping stress varied between Angus (AG) and Brahman × AG cross cattle. The researchers noted that the skin-test response to phytohemagglutinin was greater in AG steers than in Brahman × AG cross steers. Muggli et al [2] reported that IgG concentrations were different between AG and Hereford cattle. Another study by Engle et al [3] reported that AG calves had greater total IgG and IgM titers against pig red blood cells and greater lymphocyte proliferation in response to phytohemagglutinin than Simmental calves. The aforementioned studies focus on breed differences in the adaptive immune response. However, there is limited evidence of an effect of breed on the acute innate immune response to a pathogen.

Therefore, the objective of this study was to evaluate potential genetic differences between two diverse *Bos taurus* breeds [AG and Romosinuano (RO)] in response to an endotoxin (lipopolysaccharide; LPS) challenge. The RO breed is a breed native to Colombia and derived its name from its origin in the Sinú river region (sinuano) of northern Colombia and its polled (romo) character [4]. The RO breed is noted for its longevity, docile temperament, and adaptation to tropical stressors. The AG steers used in the present study were from the USDA Agricultural Research Service SubTropical Agricultural Research Station herd that descends from a group of cattle from the University of Florida obtained in the early 1950s. Beginning in 1955, AG bulls from the Wye Plantation (Wye Mills, MD, USA) were used to generate (natural) the SubTropical Agricultural Research Station AG cow herd. In the early 1970s and in the early 1990s, semen from modern Wye bulls was used on some of the cows to introduce different bloodlines.

2. **Materials and methods**

2.1. **Experimental design**

The experiment protocol and procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee before the start of the experiment. Eighteen steers ($n = 9$ AG and 9 RO; $299.4 ± 5.2$ kg BW) were acclimated to environmental chambers maintained at thermoneutrality (19.7°C) in the Brody Environmental Center at the University of Missouri, Columbia. Steers remained in the chambers for 7 d before and during the study. Three environmental chambers were used for the study, and each was divided into 6 stanchions that housed 1 steer/stanchion (3 AG and 3 RO per chamber). Steers were limit-fed a high-concentrate diet as previously described [5] and were provided unlimited access to water. On the day before the challenge, steers were fitted with indwelling jugular vein catheters. Temporary indwelling jugular catheters consisting of approximately 30.5 cm of sterile Tygon tubing (AAQ04133; US Plastics, Lima, OH, USA; 0.05 in i.d. and 0.09 in o.d.) was inserted into the jugular vein with the use of a 14-gauge by 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). The catheter was maintained in place with the use of tag cement and a 5.1-cm wide porous surgical tape around the incision site, and then the entire neck region of the bulls were wrapped with Vet Wrap to ensure stability of the catheterization site. The remaining tubing not inserted into the bull served as the catheter extension for collection of blood samples. In addition, steers were fitted with rectal temperature (RT) recording devices [6,7] that measured RT continuously at 1-min intervals. On the day of the challenge, blood samples were collected at 30-min intervals from −2 to 8 h relative to an intravenous bolus injection of LPS (2.5 μg/kg BW; LPS from *Escherichia coli* 0111:B6; Sigma-Aldrich, St. Louis, MO, USA) at time 0 h. Blood samples were allowed to clot for 30 min at room temperature before centrifugation at 1250g for 20 min at 4°C. Serum was isolated and stored at −80°C until
analyzed for cortisol, tumor necrosis factor α (TNF-α), IL-1β, IL-6, interferon γ (IFN-γ), serum amyloid-A (SAA), ceruloplasmin, α-acid glycoprotein, and serum acid soluble protein (ASP). In addition, respiration rates were determined every hour from −4.5 to 9.5 h relative to the LPS challenge. Respiration rate measurements were made by counting flank movements over a 1-min interval and are presented as breaths per minute.

2.2. Serum analyses

All serum analyses were performed in duplicate or triplicate. Concentration of serum cortisol was determined by RIA (Coat-A-Count; DPC, Los Angeles, CA, USA) in a single assay, according to the manufacturer's directions. The minimum detectable concentration was 2 ng/mL and the intra-assay CV was <5%. Serum concentrations of proinflammatory cytokines TNF-α, IL-1β, IL-6, and IFN-γ were determined with a bovine-specific custom-developed multiplex ELISA that was commercially validated for bovine cytokines (SearchLight; Pierce Biotechnology Inc, Rockford, IL, USA). All results are expressed as milligram per deciliter with intra-assay and interassay CVs of ≤5% and 10%, respectively.

Serum concentrations of acute-phase proteins were analyzed as previously described [8]. Briefly, serum concentration of the acute-phase protein SAA was determined by a commercially available ELISA kit (PHASE; TriDelta, Maynooth, Ireland) with the use of the manufacturer's protocol with an intra-assay variation of ≤5% and an interassay variation of ≤10%. Ceruloplasmin oxidase activity was measured in duplicate with the use of colorimetric procedures described by Demetriou et al [9]. All results are expressed as milligram per deciliter with intra-assay and interassay variation of ≤5% and 10%, respectively. Bovine α-acid glycoprotein concentrations were measured with a commercially available single-radial immunodiffusion kit (Cardiotech, Louisville, KY, USA). A low and high control was provided by the commercial kit, which yielded an interassay variation of ≤5%. For serum ASP concentrations, 0.1 mL of serum sample was mixed with 1.0 mL of 0.6 M perchloric acid and incubated at 20° C to 22°C for 20 min. After centrifugation at 2,000g for 20 min, 0.2 mL of the supernatant fraction was mixed with 1.0 mL of Coomassie Brilliant Blue G-250 solution and incubated for 20 min. The mixture was then read at 590 nm with the use of a GeneSys TM 20 spectrophotometer (ThermoSpectronic, Rochester, NY, USA). A standard curve was generated with a commercial protein standard (BSA; Sigma-Aldrich) with an intra-assay and interassay variation of ≤5% and 10%, respectively.

2.3. Statistical analysis

Before analysis, RT was averaged into 10-min intervals. All data were analyzed by ANOVA specific for repeated-measures with the use of Statview (SAS Institute Inc., Cary, NC, USA) with animal as the experimental unit. Fixed effects in the model included sample time, breed, and their interaction. Specific treatment comparisons were made with a paired t test to compare before challenge and after challenge time points. For comparisons to baseline, specific time points were compared with all data collected from −2 h to 0 h. Values of P < 0.05 were considered significant.

3. Results

3.1. Physiological variables

Although RT was more variable in RO steers than in the AG steers before LPS administration, RT did not differ (P > 0.10) between breeds during this time period. After LPS administration, RT increased within 1 h after LPS (Fig. 1) in both breeds (P < 0.001) with RO steers producing a greater increase in RT than AG steers by 250 min after LPS (P < 0.03). A breed × time interaction (P < 0.001) was observed for RT such that RO steers exhibited a greater peak in RT and a more rapid decline than AG steers. By 440 min after LPS, RT was not different (P > 0.10) from RT at time 0 for RO steers; however, RT in AG steers remained elevated above time 0-h values throughout the entire 8-h sampling period. Before LPS administration AG steers had a greater respiration rate than RO steers (P < 0.001; Fig. 2). In response to LPS administration, respiration rate increased in both breeds within 0.5 h (P < 0.003). Although RO steers produced an overall greater increase from baseline in respiration rate than AG steers in response to LPS challenge (P < 0.05), absolute peak respiration rates were not different (P > 0.10) between the two breeds. By 1.5 h after LPS administration, respiration rates in the AG steers had returned to pre-LPS values (P > 0.10). However, in RO steers respiration rates did not return to pre-LPS values until 6.5 h (P > 0.10) after the LPS challenge.

3.2. Cortisol and acute-phase proteins

Before administration of LPS, serum cortisol concentrations did not differ between AG and RO steers (P > 0.10; Fig. 3). Cortisol concentrations increased
within 1 h of LPS administration ($P < 0.001$) and remained elevated through the remainder of the 8-h sampling period. The highest cortisol concentrations occurred between 2.5 and 4 h for both breed groups. Overall, a breed effect ($P < 0.014$) was associated post-LPS cortisol concentrations such that cortisol concentrations were greater in the AG steers than in the RO steers.

**Fig. 1.** Rectal temperature (RT) at 10-min intervals in Angus (AG; $n = 9$) and Romosinuano (RO; $n = 9$) steers before and after an intravenous bolus injection of lipopolysaccharide (LPS; 2.5 µg/kg BW) administered immediately after collecting a blood sample at time 0. For clarity of presentation, data are presented as means only. Pooled SEM = 0.025. Breed × time interaction: $P < 0.0001$.

**Fig. 2.** Respiration rate at 1-h intervals in Angus (AG; $n = 9$) and Romosinuano (RO; $n = 9$) steers for 4.5 h before and 9.5 h after an intravenous bolus injection of lipopolysaccharide (LPS; 2.5 µg/kg BW) administered at time 0. Respiration rate measurements were made by counting flank movements over a 1-min interval and are presented as breaths per minute. For clarity of presentation, data are presented as means only. Pooled SEM = 0.726. Pre-LPS breed effect: $P < 0.001$; post-LPS change in respiration from baseline breed effect: $P < 0.05$. 

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No effect of breed was observed on SAA concentrations before LPS administration ($P > 0.10$; Fig. 4A). After LPS administration, both AG and RO steers exhibited an increase in SAA concentrations. However, a breed × time effect was observed ($P < 0.013$), such that SAA concentrations increased more rapidly in the AG steers than in the RO steers. Serum concentrations of SAA remained numerically higher in the AG steers than in the RO steers throughout the 8-h sampling period. Although fluctuations in serum concentrations of ASP during the 8-h sampling period resulted in an overall effect of time ($P < 0.001$), there were no effects associated with LPS administration or with breed type ($P > 0.10$; data not shown). For serum concentrations of ceruloplasmin, a breed × time effect ($P < 0.001$) was observed in the pre-LPS period, such that RO steers had greater concentrations of ceruloplasmin than the AG steers (Figure 4A). Although mirrored responses were observed for the two breed types after the LPS challenge, the breed × time effect persisted ($P < 0.001$) throughout the 8-h sampling period (Fig. 4B). In both breeds, a rapid drop in serum concentrations of ceruloplasmin was observed between 1 and 2 h after LPS ($P < 0.01$). However, by 3 h after LPS serum concentrations of ceruloplasmin had rebounded and were not different from time 0 values ($P > 0.10$). As with ASP, serum concentrations of α-acid glycoprotein varied over the 8-h sampling period ($P < 0.04$); however, no effects of LPS administration or breed type were observed ($P > 0.10$; data not shown).

### 3.3. Proinflammatory cytokines

Serum concentrations of TNF-α did not differ between breed types before LPS administration ($P > 0.10$). In response to LPS administration, TNF-α concentration increased in both breed types within 1 h (Fig. 5A; $P < 0.001$). A breed × time interaction was observed for serum concentrations of TNF-α, such that the response was delayed and extended in the RO steers compared with the AG steers ($P < 0.001$). Specifically, peak concentrations of TNF-α for AG steers were observed at 1.5 h, whereas the peak response in RO steers was observed at 2 h. Serum concentrations of TNF-α were greater in RO steers than in AG steers at 2 and 2.5 h after LPS ($P < 0.03$). After peak responses, serum concentrations of TNF-α declined in both groups. Before LPS administration, serum concentrations of IL-1β did not differ (Fig. 5B; $P > 0.10$) between the two breed types. However, during the post-LPS period, there was a breed × time interaction ($P < 0.04$), such that serum concentrations were greater at 3 h after LPS in RO steers than in AG steers. By 6 and 7 h after LPS administration, concentrations of IL-1β were not different between breed types ($P > 0.10$).
administration, serum concentrations of IL-1β had returned to baseline values in the AG and RO steers, respectively \((P > 0.10)\). Breed did not affect serum concentrations of IL-6 (Fig. 5C) or IFN-γ (Fig. 5D) during the periods before or after LPS. Both IL-6 and IFN-γ increased in a similar manner after LPS administration and remained elevated above baseline values for the entire sampling period. Serum concentrations of IL-6 increased above baseline values by 1 h after LPS within both breed types. Serum concentrations of IFN-γ had increased above baseline values for both breed types by 2 h after LPS administration.

4. Discussion

The RO steer is a tropically adapted heat-tolerant breed, which is a trait necessary for beef cattle production in the southeastern United States. However, it is necessary to determine whether the genetic differences
that the RO steer express could have secondary influences, for example, on immune function [5]. This study helped to elucidate differences in innate immune function in AG and RO steers, two different *Bos taurus* cattle breeds, in response to a provocative challenge with a gram-negative endotoxin (ie, LPS). Data from the current study show distinct differences because of breed of cattle in the physiological responses (RT and respiration rate), the acute stress (cortisol and acute-phase proteins) response, and the proinflammatory cytokine response (TNF-α and IL-1β).

The RT response to the LPS challenge is similar to previous observations by others [10–13]. Although a greater increase in RT was observed in RO steers after LPS, RO steers also showed a more rapid return to basal RT than the AG steers. Hammond et al [14] demonstrated that AG cattle had greater RT than Brahman, RO, and Senepol heifers during the summer’s hottest days and during the winter. In addition, others have reported that the thermoregulatory ability of RO cattle is superior to AG cattle [15,16]. Scharf et al [5] suggests that this greater thermoregulatory ability of RO
cattle may be due to an increase in heat loss to the environment, a reduction in heat production, or both. However, in response to the LPS challenge, RT was greater in RO than in AG steers, thus suggesting either an increase in heat production or a reduction in heat loss, or a combination of both, in the RO steers. Therefore, the greater thermoregulatory ability of RO cattle may not necessarily be due to heat production alone, but may also reflect a change in vasoconstriction and the associated heat loss to the environment. The greater increase in RT in response to LPS also suggests potential differences in metabolic rate between AG and RO cattle. Forsberg et al [16] reported that an increase in body temperature of 1°C in response to an immune challenge increases energy usage by 10% to 15%. A greater increase in RT by RO cattle may be indicative of an overall higher energy usage during the immune challenge, which may reflect a redistribution of energy resources away from maintenance and growth and toward support for the immune system. Being more efficient at redistributing energy to combat an immunologic insult could aid in the resiliency of the RO steers to various health challenges compared with the AG steers. Unfortunately though, a redistribution of energy may increase the time necessary for production parameters (ie, ADG, milk production, etc) to recover from an immune challenge. Given that the steers in this study were limit-fed, which may have altered their metabolic rate and that performance after exposure to the endotoxin was not recorded, it is unknown which group of steers actually returned to a
homeostatic condition sooner. Future studies will need to compare the RT response to LPS in ad libitum and limited steers, as well as subsequent growth performance, to clarify any effect of metabolic rate and potential differences in overall recovery.

Parallel to the increase in RT, respiration rate also increased in both AG and RO steers in response to LPS administration, with RO steers producing a greater increase in respiration rate (compared with baseline) than AG steers. Administration of LPS has previously been shown to increase respiration rate in multiparous Holstein cows [12] and in horses [17]. Waldron et al [12] demonstrated an increase in respiration rate in lactating dairy cows in response to three different doses (0.5, 1.0, and 1.5 μg LPS per kg BW), with a more rapid increase in respiration rate occurring as LPS dose increased. The lesser basal respiration rate in the RO steers adds to the possibility of a metabolic difference between the two breed types. If RO steers do indeed have a lesser metabolic rate during homeostatic conditions, then it is feasible to suggest that they have more energy available that could be reapportioned toward the immune system during times of immunologic stress.

Cortisol concentrations increased in both breeds in response to LPS within 30 min and remained elevated through 8 h. The LPS-induced cortisol response is similar to that reported by others [7,10,12,18]. Interestingly, although the RO steers exhibited a greater increase in RT than the AG steers, their stress response, as indicated by serum concentrations of cortisol, was less than that of the AG steers. The lesser concentrations of cortisol may have indicated that the magnitude of stress experienced by the RO steers was less than that of the AG steers. If indeed this was the case, then RO steers may have been more adept at mounting a more robust innate immune response that allowed them to return to homeostasis earlier.

Previous studies have reported increases in circulating concentrations of SAA in calves as a result of exposure to various mild-to-moderate stressors. Arthington et al [19] reported increases in circulating concentrations of SAA in beef calves in response to being commingled. In the same study, these researcher demonstrated that SAA concentrations were elevated even higher in calves that had been transported, thus indicating that, even under conditions of mild stress, differential responses in SAA concentrations can be observed. Likewise, Alsemgeest et al [20] reported increases in circulating concentrations of SAA in calves when maintained on floor types that induced a mild stress response. In more moderate-to-severe stress responses, such as those associated with inflammatory disease, viral exposure, and bacterial challenges, SAA concentrations have been reported to be sensitive indicators of the overall stress response [21,22]. In the present study, SAA concentrations gradually increased in all steers after LPS administration. However, SAA concentrations were elevated to a greater extent in the AG steers than in the RO steers, perhaps indicating that exposure to LPS was more stressful on the AG steers. However, concentrations of SAA may merely be reflective of the cortisol response in these steers because circulating concentrations of cortisol were greater in the AG steers after LPS administration. Interestingly, these data show that a higher RT response in the RO steers than in the AG steers after the LPS challenge is not associated with greater circulating concentrations of SAA and cortisol. Regardless of the interpretation, SAA concentrations reported in previous studies and the current study tend to indicate that SAA may be an accurate and sensitive indicator of the level of stress an animal is experiencing.

The distinct drop in serum concentrations of ceruloplasmin between 1 and 2 h after LPS administration is similar to that previously reported in Brahman × AG calves [23]. This consistently observed decline in serum concentrations of ceruloplasmin in previous reports has been speculated to be associated with the redistribution of iron in the host after exposure to bacteria [23]. Because iron is a limiting nutrient for bacterial growth, mammals undergo a process of iron redistribution as an adaptive protective response to limit bacterial growth and to ensure survival of the host [24].

Although both groups of steers in the current study displayed this precipitous drop in ceruloplasmin after the LPS challenge, AG steers maintained lesser serum concentrations of ceruloplasmin throughout the study. In a study that used 1,038 calves from the same population as the current study, Qui et al [25] reported greater concentrations of ceruloplasmin in calves after weaning. Specifically, Brahman-AG and RO-AG F₁ calves had greater ceruloplasmin concentrations than purebred Brahman and AG calves after weaning. In addition, Qui et al [25] reported that calves born to AG sires had lesser ceruloplasmin responses to weaning and transportation stressors than did RO and Brahman breeds, suggesting differences in the manner at which the different breeds respond to stress. This data support the ceruloplasmin concentrations reported in the current study. Differences in the secretion of acute-phase proteins may be explained by the secretion of cytokines, specifically IL-1 and TNF-α, which stimulate the release of acute-phase proteins [26]. Whether the differ-
ences in circulating concentrations of ceruloplasmin observed for AG and RO steers would affect the resistance to or recovery from a live bacterial challenge warrants further investigation.

Evidence in the literature suggests differences in innate immune function between *Bos indicus* and *Bos taurus* cattle breeds. A study investigating skin gene expression in the skin of Holstein-Friesian and Brahman tick-infested cattle found differences in the expression of innate immune genes at the site of tick infestation [27]. Differences in the manner by which the innate immune system responds to pathogens in different breeds can potentially be manipulated to enhance the immune responses of cattle breeds that produce a lesser or nondirected response. In the current study, concentrations of TNF-α and IL-1β did not differ between breeds before LPS administration. However, differences were observed in the cytokine response after administration of LPS. Specifically, the duration in which the concentrations of TNF-α and IL-1β were elevated in RO steers was greater than in AG steers. This is suggestive of an increase in the duration of the proinflammatory response or a more robust proinflammatory response. Although an enhanced proinflammatory response can benefit the host by augmenting the clearance of pathogens, an over-exuberant proinflammatory response can result in damage to healthy tissue [28]. Cortisol is a potent anti-inflammatory hormone produced by the adrenal cortex and is essential in the regulation of the inflammatory response. Because AG steers produced a greater cortisol response to LPS, AG cattle may be better at controlling the inflammatory response than RO cattle. Thus, on the basis of the proinflammatory cytokine responses, AG steers may be more likely to return to homeostasis quicker from the inflammatory insult than RO steers. Note that no differences in the IL-6 and IFN-γ responses to LPS were observed between AG and RO steers.

In conclusion, these data demonstrate differences in the innate immune response between two diverse *Bos taurus* breeds which may provide insight about differences observed in productivity, heat tolerance, disease resistance, and longevity among cattle breeds.

References


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