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# Influence of environmental temperature on the physiological, endocrine, and immune responses in livestock exposed to a provocative immune challenge

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## Abstract

Although livestock experience many stressors throughout their life, one of the most commonly experienced, and most difficult to control, is stress caused by fluctuations in environmental temperatures that extend beyond the thermoneutral (TN) zone for an animal. In swine, cold stress has long been recognized as a main cause of neonatal morbidity and mortality. A possible explanation for this increased morbidity and mortality may be related to their inability to generate a febrile response. Previously, we reported that the acute phase immune response, including the generation of fever, after exposure to lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA) is substantially altered in neonatal pigs maintained in a cold environment (ie, 18°C). Neonatal pigs that were maintained in a cold environment and administered LPS experienced a period of hypothermia coupled with altered endocrine and proinflammatory cytokine responses that could prove detrimental. In cattle, we previously reported differences in the acute phase immune response of two diverse breeds of *Bos taurus* cattle (Angus and Romosinuano) when maintained under TN conditions and exposed to LPS. More recently we have reported that differences in the stress and immune responses of Angus and Romosinuano heifers varies, depending on whether the cattle were housed at either TN or heat stress air temperatures. Our data clearly show that even intermittent periods of heat stress similar to that experienced in production environments can have significant effects on the stress and innate immune responses of cattle. Understanding the effect of thermal stress on livestock is critical to developing and implementing alternative management practices to improve their overall health and well-being.

*Keywords:* Cattle; Cold stress; Heat stress; Immunity; Swine

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## 1. Introduction

Although livestock experience many stressors throughout the production cycle, one of the most commonly experienced, and most difficult to control, is stress caused by fluctuations in environmental temperatures that extend beyond thermoneutrality for an animal. Cold stress and infectious disease are suspected to contribute to the 13% to 15% mortality rate reported for piglets between farrowing and weaning [1]. In addition, losses because of extreme changes in environmental temperature [heat stress (HS)] result in significant losses throughout the livestock industry every year. Specifically, mortality losses because of HS resulted in an estimated US$728 million dollars in five states (California, Nebraska, North Carolina, Oklahoma, and Texas) [2]. Death losses because of HS have been highlighted during heat waves, such as those that occurred across midcentral United States in the summer of 1995.

This brief review focuses on two types of thermal stress that have been found to be significant factors contributing to the overall health and well-being of livestock, 1) cold stress in neonatal pigs and 2) HS in beef cattle, and briefly highlights research performed by the authors.

## 2. Cold stress in neonatal pigs

In pigs, the neonatal period is a critical time of development, and subsequent health and performance can be manipulated through changes in the neonatal thermal environment [3]. This is mainly because the neonatal pig's thermoregulatory system at birth is not fully mature. Simmons [4] reported that the thermoneutral (TN) zone for neonatal pigs ranges from 30°C to 34°C yet shifts to 25°C to 30°C by the time pigs are 3 to 4 wk of age (weaning). However, Herpin et al [5] reported that the lower crucial temperature (LCT) is 34.6°C at 2 h of age for neonatal pigs, which has become an industry standard, and highlights the importance of providing an adequate set of environmental conditions. Because of the difference in environmental temperature between the womb and the outside environment, producers often offer supplemental heat in the form of heat pads and heat lamps to maintain environmental temperatures above the LCT for neonatal pigs. Providing this supplemental heat not only improves piglet performance but may also be essential for proper function of the immune system and survival.

Cold stress has been long recognized as a main cause of neonatal morbidity and mortality in swine and is considered a main contributing factor associated with high percentages of neonatal losses [6–8]. Cold exposure begins at birth with a rapid 15°C to 20°C transition from the uterine environment to ambient temperature conditions. Although HS is rarely a problem for the newborn pig, the industry standard of an LCT of 34.6°C at 2 h of age [5] highlights the concerns of producers to take effective and proactive measures to mitigate the negative effect associated with the transition of the newborn piglet into an environment conducive to rapid heat loss. The LCT of a neonatal pig is much higher compared with other species, such as the calf (13°C) or lamb (29°C) [9], and as such the neonatal pig is more susceptible to the detrimental effects of cold stress. Herpin et al [5] stated that reduced pig vigor can result when neonatal pigs are exposed to cold stress at birth, which can affect the aggressiveness of nursing and therefore the amount of colostrum and nutrients the pig receives to use for thermogenesis and immune protection. Unlike calves and lambs, pigs lack sufficient amounts of subcutaneous fat and have a sparse hair coat, which prevent the pig from conserving heat [9]. Previous studies have reported that maintaining neonatal pigs in cold environmental temperatures for the first 15 d of life results in decreased growth and increased number of deaths [10]. Blecha and Kelley [11] reported that pigs maintained at 10°C for 2.5 h after birth and before colostrum intake had decreased concetrations of serum gamma globulin for up to 14.5 h after the return of pigs to their dams, and serum gamma globulin tended to remain lower in cold-stressed pigs for an additional 48 h. In addition, pigs that died within the first 21 d after birth had lower total concentrations of serum gamma globulin than pigs that lived when serum was collected on the first day of life [11]. Furthermore, it has been suggested that cold environmental temperatures may interfere with the neonatal pig's ability to clear pulmonary bacterial infections [12].

The effects of cold thermal environments on the physiological and endocrinologic characteristics in neonatal pigs have been studied for more than four decades. However, there is limited data delineating the effects of cold stress on the immune system of young pigs. Nienaber et al [13] reported an increased feed intake, reduced gain, and an increase of feed needed to add a unit of gain in growing pigs housed at 5°C compared with 20°C. In addition, finishing pigs housed at 5°C had greater plasma concentrations of cortisol and greater adrenal weights compared with pigs housed at 20°C. A trend was observed for a greater neutrophil-to-lymphocyte ratio after 2 wk of treatment; however, no effect of temperature on skin thickness was observed in response to phytohemagglutinin or the antibody response to sheep red blood cells after 4 wk of treatment [13]. In contrast, Minton et al [14] found limited effects of fluctuating ambient temperature (in which temperatures fluctuated from 35°C for 12 h to 15°C for 12 h) on immune parameters in weaned pigs. However, all the aforementioned studies were performed on growing and finishing pigs, not on neonatal pigs that are at a heightened risk of becoming hypothermic/cold stressed because of their immature thermoregulatory system. In fact, a possible explanation for the increased morbidity and mortality associated with cold stress in neonatal pigs may be related to the inability of the neonatal pig to generate a febrile response.

The induction of a febrile response to the invasion of pathogens is necessary to initiate countermeasures to control and eliminate invading pathogen and to prevent overstimulation of the immune system [15,16]. Environmental temperature plays a main role in the maintenance of core body temperature in the neonatal pig, and it may also play a significant role in the febrile response to an infectious agent. Previously, we found that the acute phase immune response, including the generation of fever, after exposure to a provocative immune challenge is substantially altered in neonatal pigs maintained in a cold environment (18°C) compared with pigs maintained in a TN environment (34°C) [1]. Specifically, male pigs were taken from their dams at 24 h of age. Body weights and rectal temperatures were recorded before the pigs were removed from the sow and transferred to environmentally controlled chambers that were maintained at 50% relative humidity and either at 18°C or 34°C (n = 14 pigs/environmental temperature). Immediately after being placed into their respective chambers, pigs received an intraperitoneal injection of either saline (Control; n = 7 pigs/environmental temperature) or lipopolysaccharide (LPS derived from *Escherichia coli* O111:B4; Sigma-Aldrich, St Louis, MO, USA; 150 $\mu$g/kg of BW; n = 7 pigs/environmental temperature). Rectal temperatures were recorded with a handheld thermometer every 15 min for a 3-h period after which time all pigs were humanely sacrificed for blood and tissue collection. Pigs that were maintained in a cold environment and exposed to LPS experienced a period of hypothermia, apparent within 1.25 h after challenge, which lasted until 2.75 h after challenge (Fig. 1). Becoming hypothermic in a production environment could prove detrimental in and of itself because it would alter the behavior of the pigs, causing them to seek warmth which could increase their risk of being crushed by the sow. In contrast, pigs housed at 34°C did not produce a rectal temperature response to LPS challenge. In addition, pigs exposed to an environmental temperature of 18°C and administered LPS lost more weight (Fig. 2) and had greater concentrations of serum cortisol (Fig. 3A) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$; Fig. 3B) than any other treatments. Exposure to an environmental temperature of 18°C alone caused an increase in serum cortisol concentrations in control piglets compared with control pigs maintained at 34°C. The exaggerated TNF-$\alpha$ response observed in pigs exposed to an environmental temperature of 18°C and administered LPS can cause additional complications, because TNF-$\alpha$ can increase lethargy and sickness behavior [17], thus delaying recovery. Pigs maintained in a warm (34°C) environment exhibited no visual signs of illness and only minimal activation of the endocrine and immune systems associated with the LPS challenge.

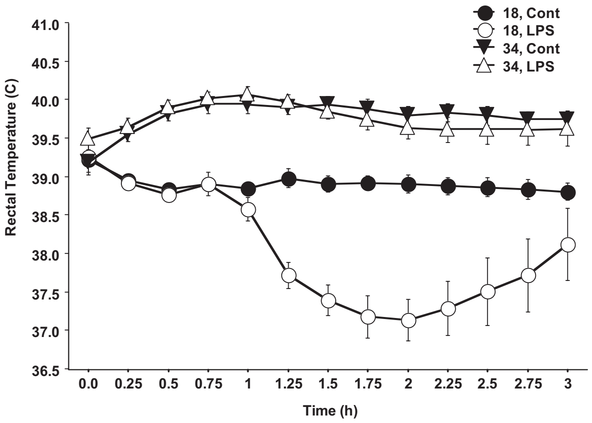

Fig. 1. Effect of environmental temperature (ET; 18°C or 34°C) and lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA) treatment on rectal temperature (RT) in neonatal pigs during a 3-h period. Rectal temperatures were recorded at 15-min intervals for a 3-h period. Before removing the pigs from their sows, RTs were recorded (crate temperature = 0 h). Pigs were then transferred to environmentally controlled chambers maintained at 18°C or 34°C with 50% relative humidity. On entering their respective chambers, pigs received intraperitoneal injections of either saline (Control; n = 7 pigs/ET) or LPS (n = 7 pigs/ET) at 150 $\mu$g/kg. Beginning at 0.25 h and lasting throughout the 3-h period, RTs were lower ($P < 0.05$) for Control pigs maintained at 18°C than for Control pigs maintained at 34°C. In pigs maintained at 34°C, RTs were not affected by LPS challenge. The LPS-treated pigs maintained at 18°C had lower RT ($P < 0.05$) than Control pigs maintained at 18°C between 1.25 and 2.75 h after LPS challenge. Recreated from Carroll et al [1].

Given that the primary function of a febrile response is to assist in the removal of invading pathogens, these data show a strong linkage between environmental tem-

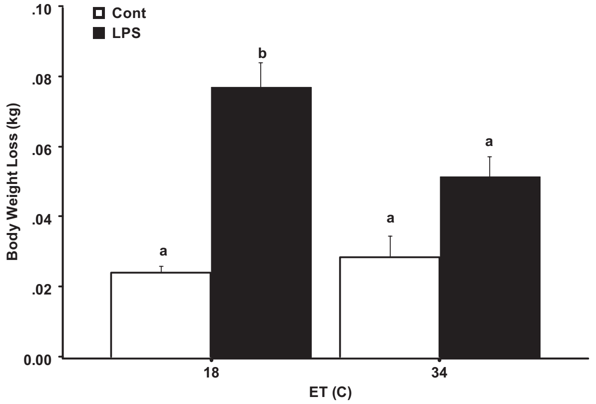

Fig. 2. Effect of environmental temperature (ET; 18°C vs 34°C) on mean (±SEM) BW loss in neonatal pigs after a 3-h period after having received intraperitoneal injections of either saline (Control; n = 7 pigs/ET) or lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA; 150 μg/kg; n = 7 pigs/ET). $P < 0.001$ for a vs b. Recreated from Carroll et al [1].

perature and the ability of the neonatal pig to not only mount an adequate immunologic response but to survive as well. These results also indicate that, when combined, cold stress and exposure to endotoxin induce a rapid and potentially dangerous loss of body heat in the neonatal pig. Although routine management practices include supplying supplemental heat sources (eg, heat lamps and/or heat pads) to newborn pigs, the overall advantage of this practice may not be fully appreciated. Perhaps providing the additional heat source may reduce the potential for, the severity of, and the duration of illness.

## 3. Heat stress in cattle

The pathologic manifestations of HS occur because of an inability to maintain homeothermy due primarily to environmental factors, such as ambient temperature, humidity level, radiant heat load, and wind speed. Exposure to environmental temperature above thermoneutrality and elevated humidity can reduce reproductive performance, weight gain, milk production, and feed intake in cattle [18]. In addition to production losses, HS can increase the incidence of mortality [18,19]. St-Pierre et al [2] estimated beef cattle losses because of HS at US $87 million in the breeding herd and greater than US $282 million in finishing cattle. Therefore, HS has serious economic implications in beef cattle, with locations in the south-central United States (Kansas, Nebraska, Oklahoma, and Texas) experiencing the greatest losses.

Several factors can influence the thermoregulatory ability of cattle, including sex and breed [18]. Our laboratories have previously found that there are significant differences between two diverse heat-sensitive (Angus) and heat-tolerant (Romosinuano) *Bos taurus* breeds for their rectal temperature and innate immune response after an LPS challenge [20]. The Romosinuano is a breed native to Colombia, South America, and derived its name from its origin in the Sinú river region (sinuano) of northern Colombia and its polled (romo) character [21]. Romosinuanos are noted for their longevity, docile temperament, and adaptation to tropical stressors, such as HS.

In our previous study, Romosinuano steers exhibited a greater rectal temperature response to the LPS challenge and a more rapid return to basal rectal temperature compared with Angus steers (Fig. 4). Other researchers have also reported differences in rectal

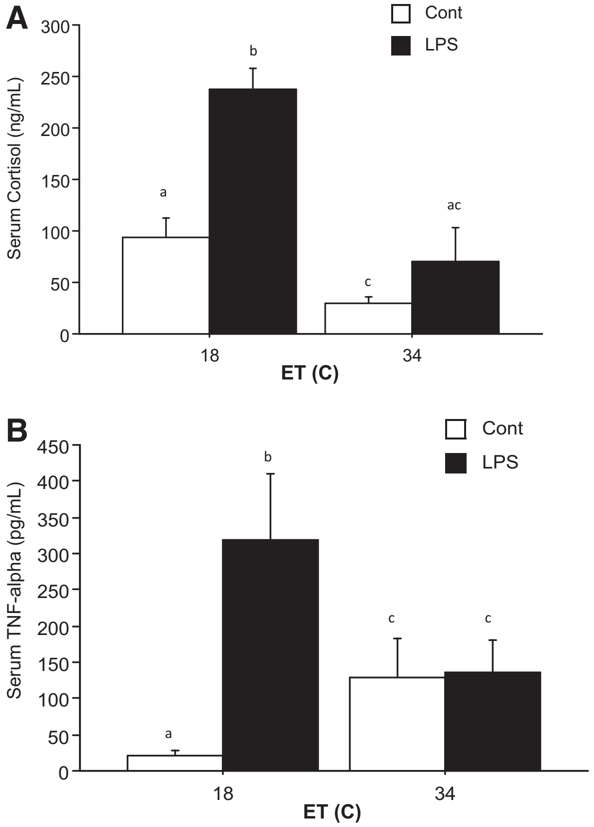

Fig. 3. Effect of environmental temperature (ET; 18°C vs 34°C) on mean (±SEM) serum concentrations of cortisol (A) and tumor necrosis factor-α (TNF-α; B) in neonatal pigs after a 3-h period after having received intraperitoneal injections of either saline (Control; n = 7 pigs/ET) or lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA; 150 μg/kg; n = 7 pigs/ET). $P < 0.001$ for a vs b, and b vs c; $P < 0.05$ for a vs c (A). $P < 0.01$ for a vs b; $P < 0.05$ for b vs c (B). Recreated from Carroll et al [1].

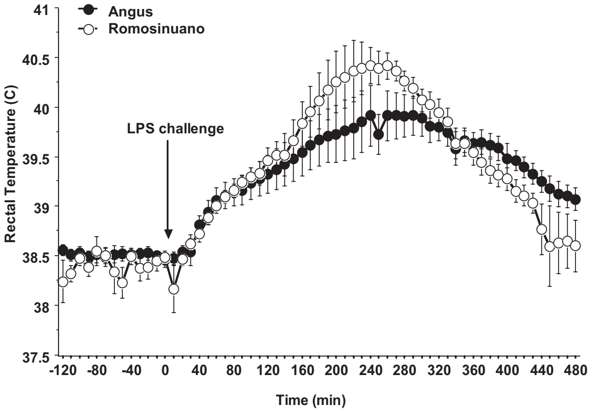

Fig. 4. Rectal temperature (RT) at 10-min intervals in Angus (n = 9) and Romosinuano (n = 9) steers before and after an intravenous bolus injection of lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA; 2.5 $\mu$g/kg of BW) administered immediately after the collection of a blood sample at time 0. Rectal temperatures were collected at 1-min intervals and then averaged into 10-min intervals before analysis. A breed × time interaction ($P < 0.0001$) was observed for RT such that Romosinuano steers exhibited a greater peak RT and a more rapid decline compared with Angus steers. Recreated from Carroll et al [1].

temperature responses between Romosinuano and Angus cattle when exposed to adverse environmental conditions and have suggested that the thermoregulatory ability of the Romosinuano is superior to that of the Angus [22,23]. A greater thermoregulatory ability of Romosinuano cattle could partially be explained by reduced heat production, an ability to increase heat loss to the environment, or a combination of both of these physiological processes [24]. An ability to increase heat loss to the environment could partially be explained by differences we have previously observed in respiration rates in which Romosinuano steers produced a greater increase in respiration rate compared with Angus steers during an LPS challenge [20]. An increased respiration rate after the LPS challenge could possibly provide a means by which the Romosinuano could dissipate more heat. Whether differences in the thermoregulatory ability or potential differences in metabolic rate are responsible for the differences in the LPS-induced febrile responses observed in our previous study remains ambiguous at this time.

Angus steers had greater cortisol concentrations in response to the LPS challenge (Fig. 5), whereas the serum TNF-$\alpha$ response to LPS was delayed and extended in Romosinuano compared with Angus steers. A breed × time interaction was also observed for serum concentrations of interleukin-1$\beta$ (IL-1$\beta$) in response to LPS, such that 3 h after challenge, concentrations of IL-1$\beta$ were greater in Romosinuano steers than in Angus steers. However, no effect of breed was observed on the responses of interferon-$\gamma$ and IL-6 to LPS administration. Changes in the production of acute-phase proteins were also observed. Although a greater response of serum amyloid A was observed in Angus steers, Romosinuano steers had greater ceruloplasmin concentrations throughout the period before and after challenge. However, $\alpha$-acid glycoprotein and acid-soluble protein were not affected by breed. Collectively, the data support the conclusion that differences in the physiological, endocrine, and immune responses between these two diverse cattle breeds exist in how each breed handles the complicated effect of environmental (heat) in combination with immune (LPS) stressors. On the basis of our interpretation of the data, Romosinuano cattle are considered more robust than Angus cattle in their ability to manage heat and immune stressors.

In a more recent study, our laboratories conducted a subsequent study to elucidate potential genetic differences in the stress and innate immune responses after an LPS challenge in these same two breeds of cattle while maintained under either TN or HS conditions (J.A. Carroll et al, 2010, unpublished data). In this study, Angus heifers displayed greater rectal tempera-

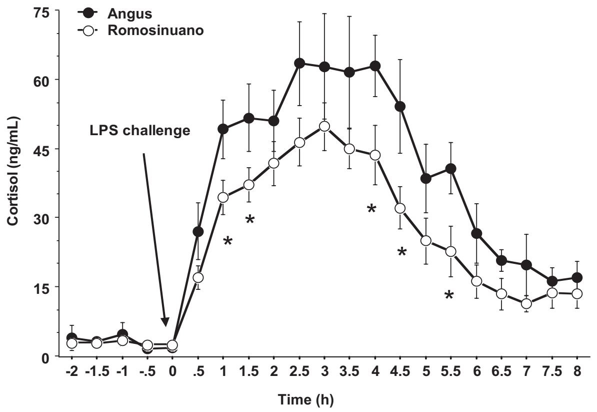

Fig. 5. Serum concentrations of cortisol at 30-min intervals in Angus (n = 9) and Romosinuano (n = 9) steers before and after an intravenous bolus injection of lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA; 2.5 $\mu$g/kg of BW) administered immediately after the collection of a blood sample at time 0. Basal serum cortisol concentrations did not differ between Angus and Romosinuano steers ($P > 0.10$). Serum cortisol concentrations increased within 1 h of LPS administration ($P < 0.001$) and remained elevated through the remainder of the 8-h sampling period. Peak cortisol concentrations occurred at 2.5 and 3 h for Angus and Romosinuano steers, respectively. *$P < 0.10$ for Angus vs Romosinuano as specific time points); time effect after LPS, $P < 0.001$; breed effect after LPS, $P < 0.014$. Recreated from Carroll et al [1].

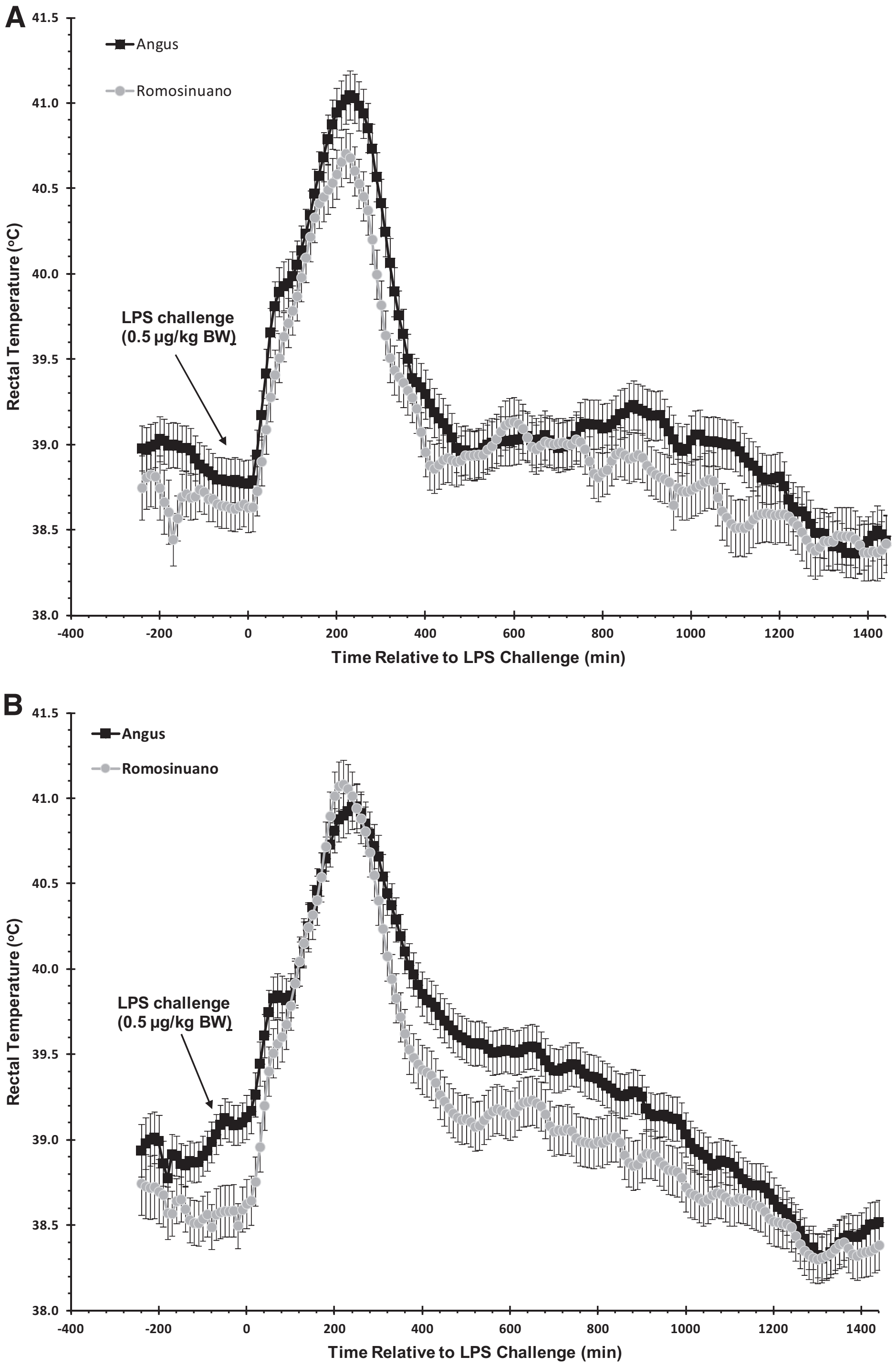

Fig. 6. Effect of environmental temperature and lipopolysaccharide (LPS; *Escherichia coli* O111: B4; Sigma-Aldrich, St Louis, MO, USA; 0.5 µg/kg of BW) challenge on rectal temperature (RT) responses of Angus (n = 11; 306 ± 26 kg of BW) and Romosinuano (n = 10; 313 ± 32 kg of BW) heifers. Heifers were housed in individual stanchions in four temperature-controlled environmental chambers. Ambient temperature was within cycling thermoneutrality (18.5°C to 23.5°C; A) for a 1-wk adjustment period, followed by an increase in two chambers to cycling heat stress (24°C to 38°C; B) for 2 wk. Rectal temperatures were collected at 10-min intervals from −4 to 24 h relative to the LPS challenge at 0 h. Angus heifers displayed greater basal RT than Romosinuano heifers when housed at either thermoneutrality or heat stress temperatures ($P < 0.01$) and produced an overall greater febrile response after the LPS challenge (ie, had greater average rectal temperature after LPS; $P < 0.01$) (J.A. Carroll et al, 2010, unpublished data).

ture than Romosinuano heifers when housed at either TN (cycling from 18.5°C to 23.5°C; Fig. 6A) or HS (cycling from 24°C to 38°C; Fig. 6B) temperatures and produced a greater overall febrile response to LPS administration (ie, had greater average rectal temperature after LPS administration). Sickness behavior scores (eg, based on a 1 to 5 scale with 1 indicating the animal displayed the least amount of sickness behavior, such as head distension, increased respiration, and labored breathing, and 5 indicating the animal displayed the greatest amount of sickness behavior) assigned by a trained observer in response to LPS administration were similar between breeds when housed at TN. However, when housed in an HS environment, Romosinuano heifers displayed greater scores of sickness behavior compared with Angus heifers after LPS administration. Environmental temperature also played a significant role in the release of proinflammatory cytokines after LPS administration. For example, heifers housed in an HS environment produced greater concentrations of IL-6 after LPS administration than heifers housed at TN. These data show that even intermittent periods of HS similar to that experienced in production environments can have significant effects on the stress and innate immune responses of cattle. Other factors that may influence the cytokine response to LPS during HS include feed intake, as studies have found that as body temperature increases, feed intake decreases [2,18,25]. Therefore, the available nutrients, and nutrient partitioning (to immunity vs to homeostatic processes), may differ between cattle housed in TN vs HS, contributing to differences observed in hormone and cytokine concentrations. Understanding the effect of thermal stress on the immunologic responses of livestock is critical to developing and implementing alternative management practices that would improve the overall health and well-being of animals in production systems.

This study also highlighted the use of different methods for measuring body temperature in heat-stressed cattle. During this study, in addition to measurement of rectal temperature, body temperature was also determined by measuring vaginal temperature, skin temperature (rump and ear), and ruminal temperature [26]. Differences in skin temperature were observed before the challenge, with HS heifers having greater rump and ear temperature than heifers housed in TN. In response to LPS, a decrease in ear temperature was observed in heifers housed at TN, yet there was no significant change in ear temperature in HS heifers. In contrast, rump temperature increased in HS heifers but decreased in heifers housed in TN. Increases in skin temperature occurred before an increase in core body temperature and was more visible in heifers housed in TN compared with an HS environment. Rectal and vaginal temperature responses were similar, with Romonsinuano heifers producing a greater temperature response than Angus heifers. In addition, the increase in rectal and vaginal temperatures in response to LPS administration was >1°C and surpassed the ruminal temperature for both breeds and treatments. In contrast to rectal, vaginal, and skin temperatures, a clear ruminal temperature response to LPS was not observed. Therefore, a more accurate core body temperature response to LPS administration was observed by measuring vaginal and rectal temperatures. Ambient temperature had little effect on the core body temperature response of Angus heifers to LPS administration. However, the heat-tolerant Romosinuano breed appears to exhibit a slightly greater core temperature response to LPS administration when exposed to an HS environment.

These data show differences in the stress and innate immune responses between two heat-sensitive and heat-tolerant *Bos taurus* breeds under both thermal neutral and HS conditions which may aid in our ability to elucidate other physiological mechanisms that contribute to differences in productivity, disease resistance, and longevity among cattle breeds. In addition, the data from this research highlight the importance associated with the effect that environmental temperature can have on the stress and innate immune responses of livestock.

## 4. Conclusion

Regulation and balance of stress and immunity among livestock is important for health and, ultimately, for production. Further elucidation and a greater understanding of the factors that influence the stress and innate immune responses in livestock, including the influence of environmental conditions, will undoubtedly increase our capability of developing various management practices that enhance production efficiency and overall health in livestock.

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