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Effect of winter season and feeding techniques on the growth performance and blood expression profile of the stress-, inflammation-, and cellular integrity-associated genes in *Bos taurus* and *Bos indicus* breeds

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We determined the effects of the winter season (cold stress), from October to March 2023, on the growth performance and expression profile of the blood chemo-cytokines and tight junction proteins in two cattle breeds [*Bos indicus* (Brahman) *vs. Bos taurus* (Angus)] reared under two feeding techniques or treatments: traditional with growth-promoting technology (GPT) (TRT treatment) *vs.* natural without GPT (NAT treatment) (n = 50 steers/breed per treatment) in Colorado State, USA. The body weight (BW), the average daily BW gain (ADG), the dry matter (DM) feed intake (DMI), and the feed efficiency (FE; kilograms gain/kilogram DMI) were monitored. Total RNA was extracted from blood at the end of each month and subjected to reverse transcription and real-time quantitative PCR to measure the relative expression of target genes using the $2^{-\Delta\Delta Ct}$ method. There were no significant period-by-breed-by-feeding technique (P × B × T) interactions for BW, ADG, and FE. When the factors were analyzed separately, the data showed a significant increase of BW for both breeds during the study period. However, Angus cattle had higher BW, ADG, and FE compared with their Brahman counterparts. These differences were associated with higher feed intake (p < 0.0001) in Angus *versus* Brahman. The blood expression levels of heat shock protein 90 (*Hsp90*), interleukin 6 (*IL6*), chemokines (*Ccl4*, *Cxcl12*, and *Xcl1*), and chemokine receptor (*Cxcr2*) were significantly upregulated in November, which coincided with the lower environmental temperature. The abundance of occludin (*Ocln*) mRNAs peaked in January after an initial increase from October, followed by a downward trend through March. Brahman cattle exhibited a significant higher expression of *Hsp90*, *Ccl4*, and *Xcl1* compared with their Angus counterparts. The TRT treatment significantly downregulated the expression of the *Cxcr2* gene compared with the NAT feeding technique. Together, to the best of our knowledge, this is the first study showing a differential expression of *Hsp90* and chemokines between a cold-resilient (Angus) and a cold-sensitive (Brahman) cattle. This work provides novel opportunities and offers new avenues for the identification of molecular signatures for marker-assisted selection.

KEYWORDS

cattle breed, chemokines, cold stress, cytokines, HSPs, production system, tight junction proteins

1 Introduction

The evidence for rapid climate change is compelling and beyond doubt (Oerlemans, 2005; Tollefson, 2025a, b). Although it seems counterintuitive, global warming is plummeting, with an Arctic oscillation shift bringing unexpected global extreme cold events (Briffa and Osborn, 2002; Cohen et al., 2021; Blackport and Fyfe, 2024; Wu et al., 2025). For instance, and to mention a few, the severe cold surges of February 2021 that struck the Great Plains caused the air temperature to drop by 20°C within a single day (Doss-Gollin et al., 2021). A similar scenario happened in China in February 2024 with record-breaking blizzards, 14°C temperature decrease, and freezing rain (Ting et al., 2025). These extreme cold surge events, influenced by sun radiation, wind speed, and relative humidity, can have profound deleterious effects on livestock health, welfare, and production sustainability (Young, 1981; Debnath et al., 2024).

When the environmental temperature falls below the lower critical temperature (LCT), the heat dissipation is greater than the heat production, which in turn results in cattle thermal balance disruption and cold stress (Roland et al., 2016; Wang et al., 2023). It is worth mentioning that the LCT for beef cattle varies depending on their body condition and hair coat, ranging from approximately −7.7°C (18°F) or lower for a dry heavy winter coat to 15°C (59°F) for a wet summer coat (Young, 1981). Additional factors affecting the LCT in cattle are the wind velocity and direction, as well as the pen or pasture conditions (dry *vs.* muddy).

When it occurs during the adverse winter months, cold stress increases the feed intake and thermogenesis, lowers the passage rate and digestibility by 0.2% for each degree decrease below 20°C (68°F) (Kennedy et al., 1986), and slows down the body weight gain and growth (Kang et al., 2016; Wang et al., 2023). At the physiological level, cold stress can modulate the circulating hemato-biochemical profile (Berian et al., 2019), disrupt the electrolyte balance (Prasanna et al., 2022), suppress the immune function (Kim et al., 2023), and induce oxidative stress (Li et al., 2021), leading to tissue injuries such as frostbite. In extreme conditions, hypothermia advances and cold stress can lead to bradypnea and bradycardia, which may result in sentience loss and collapse (Roland et al., 2016). Altogether, these adverse effects lead to substantial and hefty financial losses to livestock worldwide, particularly in the long and cold Northern Hemisphere.

At the cellular and molecular levels, cold stress initiates a neuroendocrine response via activation of the corticotropin-releasing hormone and the adrenocorticotropic hormone, which in turn stimulate cortisol secretion that triggers target metabolic tissues (Carlin et al., 2006). Target tissues and cells respond to cold stress through a series of complex mechanisms, including stress (heat shock proteins, HSPs), inflammation (chemo-cytokines), and cellular integrity (tight junctions) pathways, aiming at protecting and maintaining cell homeostasis and survival or inducing cell death, dependent on the duration and the severity of cold stress on one hand and the cattle breed, body condition, and hair coat on the other hand. Interestingly, the breed Angus (*Bos taurus*) originated in cold climate (Scotland) and has developed a thick and dry winter coat with a low LCT. However, its counterpart Brahman (*Bos indicus*) emerged from a hotter climate (India) and has a short, glossy, and wet summer coat with a higher LCT. Together, this indicates that these breeds have developed different physiological adaptive pathways to cope with different environmental conditions, where Brahman cattle are thermotolerant (Hernandez-Ceron et al., 2004) and the Angus breed is cold-resistant (Smakuyev et al., 2021). As a follow-on to our previous investigation where we reported the effect of the summer season (Branine et al., 2025), this study was undertaken to determine the effect of the winter season and the feeding technique (with or without growth-promoting technology, GPT) on the blood expression of the stress (HSP)-, immunity and inflammation (cyto-chemokine)-, and cellular integrity (tight junction protein)-associated genes in the Brahman and Angus breeds.

2 Materials and methods

2.1 Environmental conditions monitoring

The daily ambient temperature (*T*), relative humidity (RH), clear-sky solar radiation (RSO), and wind speed (WS) were recorded by and acquired from an official automated meteorological station at Colorado State University's Agricultural Research, Development and Education Center (ARDEC) Beef Cattle Research Farm. The temperature humidity index (THI) was calculated using a previously published equation (NOAA, 1976).

2.2 Ethics statement

The experiment was conducted at Colorado State University's ARDEC research feedlot in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee at the Colorado State University (protocol no. 3712-13).

2.3 Experimental design, animal husbandry, and feeding systems

In October 2023, Brahman (*B. indicus*) and Angus (*B. taurus*) steers (n = 100 steers/breed) were shipped from ranches in Texas and Montana, respectively, to ARDEC in Fort Collins, CO. Upon arrival, all cattle were allotted an acclimation period of 2 weeks and provided with roughage-based ration and free access to water. On October 19 and 20, 2023 (day −1 and day 0), steers were individually weighed and the initial body weight (iBW) recorded. Based on the iBW, the steers were randomized into blocks consisting of four pens/block comprising two feeding systems within each breed. All steers were housed by block (five blocks/ feeding system) in 10-head research feedlot pens (four pens/block) for the first 84 days of the study. The feeding systems evaluated (traditional with GPT, TRT) are similar to those described by Branine et al. (2025) and consisted of administering to both Angus and Brahman steers GPTs commonly used in commercial beef feedlots. Specifically, these included providing anabolic in-ear implants on day 0 (100 mg trenbolone acetate/14 mg estradiol benzoate) (Synovex Choice®, Zoetis, Parsippany, NJ, USA) and day 84 (200 mg trenbolone acetate/28 mg estradiol benzoate) (Synovex Plus®, Zoetis, Parsippany, NJ, USA). The terminal implant was administered approximately 96 days prior to slaughter. In the growing and finishing diets, TRT cattle were fed 35 g monensin/ ton dry matter (DM) (Rumensin®, Elanco, Greenfield, IN, USA) and 7 g tylosin/ton DM basis (Tylan®, Elanco, Greenfield, IN, USA). Approximately 42 days prior to slaughter, 27 g ractopamine hydrochloride (RAC)/ton DM basis (Actogain®; Zoetis Animal Health, Parsippany, NJ, USA), allowing for a 2-day withdrawal period prior to harvest. The second feeding system consisted of providing Angus and Brahman steers with no GPT and was designated as a non-technology or natural (NAT) feeding program. At the initial processing, all cattle were vaccinated for respiratory (Bovi-Shield GOLD® 5; Zoetis, Kalamazoo, MI, USA) and clostridial diseases (Ultrachoice® 8, Zoetis, Kalamazoo, MI, USA). For internal and external parasites, all steers were administered subcutaneously with avermectin (Dectomax®, Zoetis, Kalamazoo, MI, USA) and orally drenched with albendazole (Valbazen®, Zoetis, Kalamazoo, MI, USA). Both a visual and a radiofrequency identification tag were administered to provide a means for individual animal identification. On day 180 (March 2024), all cattle were weighed and shipped to a commercial abattoir in Fort Morgan, CO, where individual carcass data were collected.

2.4 Body weight recording and blood sampling

Individual body weights (BWs) were recorded at the beginning of the trial and on a monthly basis, with approximately 28-day intervals throughout the study, with the final BWs taken prior to processing. At each weighing, three steers/pen (n = 15 steers/breed per production system) were randomly selected for blood collection via jugular venipuncture throughout the trial. Individual blood samples were quickly drawn (less than 2 min) into heparinized tubes (BD Vacutainer™ Plastic Blood Collection Tubes with Sodium Heparin; Hemogard™, Fisher Scientific, Waltham, MA, USA) and kept on ice until they were aliquoted with TriZol LS (Life Technologies, Carlsbad, CA, USA) for total RNA isolation and then stored at −80°C until further analysis. From the 60 animals from which whole blood was collected, 12 steers were randomly selected per breed × production system (24 total) for further gene expression analysis.

2.5 Gene expression analysis

Total RNAs were extracted from blood (n = 12 randomly selected steers/group) using TriZol LS reagent (Life Technologies, Carlsbad, CA, USA) in accordance with the manufacturer's standard protocol and recommendations. The RNA integrity and quality were assessed with 1% agarose gel electrophoresis, which showed good structure, sharp and distinct bands of ribosomal 28S, 18S, and 5S RNA. The total RNA concentrations and purities were determined for each sample with Take 3 Micro-Volume Plates using a Synergy HT multi-mode microplate reader (BioTek, Winooski, VT, USA). Samples with $OD_{260}/_{280}$ ratios between 1.8 and 2 were used for cDNA synthesis. DNase-treated RNAs (1 µg) were reverse-transcribed using the qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD, USA) in a 20-µl total reaction and incubated at 42°C for 30 min followed by 85°C for 5 min. The cDNA samples were diluted 10× and subjected (2.5 µl) to real-time quantitative PCR (qPCR) (Applied Biosystems 7500 Real-Time PCR System, Carlsbad, CA, USA) in the presence of 0.5 µM of each forward and reverse primer and SYBR Green Master Mix (ThermoFisher Scientific, Rockford, IL, USA) in a 12.5-µl total reaction. The thermal cycling parameters and the amplification conditions were as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 58°C for 1 min. A dissociation protocol (Sequence Detection System, Thermo Fisher Scientific) was used at the end of the amplification, and a melting curve analysis was applied to exclude contamination with unspecific PCR products. An agarose gel electrophoresis (2%) was also used to confirm the presence of only one definite band of the predicted size, followed by DNA sequencing (Branine et al., 2025). When cDNAs were omitted (negative control), no bands were detected by the agarose gel electrophoresis. The relative expression of the target genes was determined using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) with normalization to ribosomal 18S RNA as the housekeeping gene. The breed Brahman, the traditional treatment (TRT), and the month October were used as calibrators. Oligonucleotide primer sequences specific for the cattle HSPs (*Hsp60*, *HspA1A*, and *Hsp90*), cytokines [interleukin 6 (*IL6*), *IL18*, and *IL-1β*; tumor necrosis factor alpha (*Tnfα*); and C-reactive protein (*Crp*)], chemokines [C–C motif chemokine ligands *Ccl2*, *Ccl5*, and *Ccl20*; C–C motif chemokine receptor type 2 (*Ccr2*); C–X–C motif chemokine ligands *Cxcl12* and *Cxcl14*; C–X–C motif chemokine receptors *Cxcr1* and *Cxcr2*; and X–C motif chemokine ligand 1 (*Xcl1*)], and tight junction proteins [claudin 1 (*Cldn1*) and occludin (*Ocln*)] have been previously reported (Branine et al., 2025).

2.6 Statistical analysis

Data on the growth performance and relative gene expression were analyzed using three-way repeated measures ANOVA with breed (Brahman *vs.* Angus), feeding system or treatment (TRT *vs.* NAT), period (month effect), and their interactions as the main factors. If ANOVA showed significant effects, the means were compared with Tukey's honestly significant difference (HSD) multiple comparison test using GraphPad Prism version 10.00 for Windows (GraphPad Software, La Jolla, CA, USA). If the interactions were not significant, the main factors (i.e., breed, feeding technique, and/or period) were analyzed separately using two-way ANOVA, one-way ANOVA, or Student's "*t*," as appropriate. The random effect of the animal ID was not significant, as demonstrated by the linear mixed model analysis. Data are presented as the mean ± SEM (n = 12 steers/breed per treatment), and the difference was considered statistically significant at $p < 0.05$.

3 Results and discussion

Cold stress, driven and intensified by climate change (Briffa and Osborn, 2002), is a real threat to agriculture in general and to livestock production sustainability in particular. While the global and US annual cost and loss estimates are not readily available in the literature and search results, cold stress does cause heavy economic downturn and damage to the industry due to the increased feed costs, the reduced feed efficiency and performance, and the higher illness rates and potential fatalities (Cox et al., 2016; Wang et al., 2023; Debnath et al., 2024). As the effect of cold stress is dependent on its severity, duration, and intensity on one hand and on the body condition and cattle breed on the other hand, the present study aimed to determine the effect of the winter season (from October 2023 to March 2024) on cold-sensitive Brahman and cold-resistant Angus beef cattle reared in Fort Collins, Colorado (40°33′33″ N, 105°4′41″ W) (Figure 1A).

During the study period, the weather in Fort Collins, CO, was characterized by a varying average air temperature from −7.21°F to 64°F (from −21.78°C to 17.77°C) and RH from 27% to 96%, which resulted in a deviated THI from 1.81 to 61 (from −16.77 to 16.11) (Figures 1B–D). The average WS changed from 4.72 to 35 km/h, while the average RSO fluctuated from 11.62 to 303 W/m^2 (Figures 1E, F). These temperatures were lower compared with the lowest average habitual temperature (20°F), indicating that the 2023 winter season was indeed cold.

As shown in Tables 1, 2, and 3, there were no significant period-by-breed-by-feeding technique (P × B × T) interactions for BW, average daily gain (ADG), and feed efficiency (FE). When the main factors were analyzed separately, one-way ANOVA revealed a period effect ($p < 0.0001$) (Table 1), with the average BW significantly increased from 343 ± 3.84 kg in October 2023 to reach 544 ± 6.71 kg in March 2024. Within the study period, Student's *t*-test revealed significant differences between breeds, with higher BW in Brahman in October and November 2023 ($p < 0.0001$) (Table 1) and an elevated BW in Angus in January, February, and March 2024 ($p < 0.05$) (Table 1). However, no differences were observed in December 2023. The ADG and FE were significantly higher in Angus compared with Brahman cattle during the study period, except during February–March (Tables 2, 3). These differences may be associated with the maturation stage and growth rate, as Angus are an early- while Brahman a later-maturing breed (Chenoweth et al., 1996; Martins et al., 2024). It is also plausible that these differences are related to inherent and genetically based variations in the breed's inherent physical, metabolic functions, and physiological traits that enable them to adapt to specific environmental conditions. Here, originated from rugged and cold climate (Scotland), the Angus breed is characterized by its insulating thick and dense coat, hardiness, and thicker backfat, allowing them to be more resilient and to better withstand cold stress, thereby partitioning a greater proportion of the energy intake toward growth instead of thermogenesis and survival (Smakuyev et al., 2021; Farias et al., 2024). Furthermore, a previous study has shown differences in the metabolic hormones between these breeds, with Angus cattle exhibiting lower serum glucose, insulin, and leptin levels (Obeidat et al., 2002). According to the glucostatic or the glucodynamic theory, falling levels of glucose trigger hunger and stimulate feed intake (Cha et al., 2008). Both insulin and leptin are anorexigenic hormones, signaling the hypothalamus to suppress appetite and reduce feed intake (Woods et al., 1979; Gardner et al., 1998; Foote et al., 2015; Tan et al., 2024). In the experimental condition here, despite the significant P × B × T interaction, the feed intake was significantly higher in Angus compared with Brahman cattle (Table 4), which is in agreement with the abovementioned changes explaining the higher BW and ADG. In addition, the lower glucose, insulin, and leptin levels suggest a lower resting energy expenditure in Angus, which might also explain their higher BW and growth rate compared with their Brahman counterparts.

Cold stress acts as a potent modulator of critical physiological pathways within an organism, resulting in multifaceted responses of the intricate crosstalk between the systems responsible for the maintenance of cellular homeostasis. Specifically, cold stress triggers the activation of the stress response pathways, including the upregulation of HSPs. Concurrently, the immune functions are affected, as cold stress can lead to immunosuppression or, conversely, the exacerbation of the inflammatory responses through the release of (chemo)cytokines. Ultimately, these

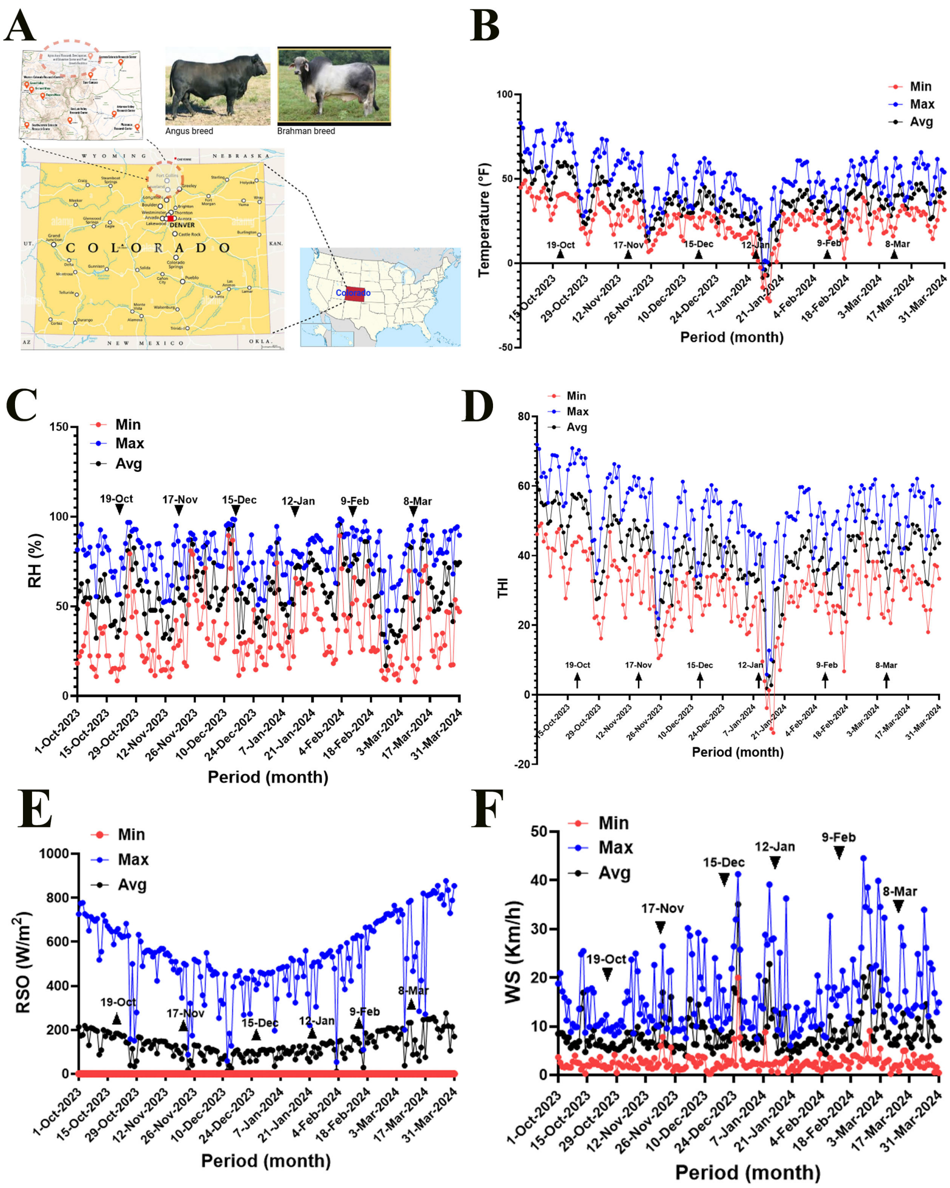

FIGURE 1
Winter study location **(A)** and daily environmental conditions. The daily minimum, maximum, and mean temperatures **(B)**, relative humidity (RH0 **(C)**, temperature humidity index (THI) **(D)**, clear-sky solar radiation (RSO) **(E)**, and wind speed (WS) **(F)** were recorded by and acquired from an official automated meteorological station at Colorado State University's Agricultural Research, Development and Education Center (ARDEC) beef cattle research farm. *Arrows* denote days when the samples were collected.

modulated responses affect the cell integrity and function, potentially leading to cellular survival (physiological adaptability) or damage and homeostasis disruption. The role of HSPs, cytokines, and chemokines in thermotolerance has been previously reported (Li et al., 1995; Hershko et al., 2003; Evgen'ev et al., 2023; Vasek et al., 2024). Furthermore, HSPs bind to and protect tight junctions and activate protective pathways to strengthen the cellular barrier integrity (Wang et al., 2018). This interconnected biological network underscores the potential profound impact of environmental factors on cattle health and productivity. To deepen our understanding and in attempt to delineate the molecular mechanisms associated with cold stress response in our experimental conditions, we next determined the expression of the genes associated with stress, inflammation, and cellular integrity. There was a significant P × B × T interaction for blood *Hsp60* and *HspA1A* expression, with higher mRNA abundance in Brahman in November 2023 (Figures 2A, B), which coincided with the low air temperature (−3.72°C), THI (−3.16), and RH (0.421%). The expression of blood *Hsp90* was significantly affected by period and breed, with higher levels in November compared with other months (Figures 2C, D) and in Brahman as opposed to Angus cattle (Figure 2E). The induction of these HSPs by low temperature was not surprising as cold stress has been shown to upregulate the expression of HSPs in various tissues in numerous species,

TABLE 1 Effects of winter season, breed, and production system on beef cattle body weight.

Breed[a]	Brahman (*Bos indicus*)		Angus (*Bos taurus*)					
Period (P)[b]/System (PS)[c]	TRT	NAT	TRT	NAT	Three-way ANOVA			
					S. of variation	**MS**	***F* (DFn, DFd)**	***p*-value**
October	367 ± 5.2	362 ± 6.4	321 ± 4.7	323 ± 4.3	P	418,658	545.3	<0.0001
November	382 ± 6.3	379 ± 5.5	353 ± 4.0	348 ± 4.7	B	28,291	36.85	<0.0001
December	422 ± 6.8	421 ± 7.6	436 ± 3.6	417 ± 4.8	PS	13,443	17.51	<0.0001
January	452 ± 7.1	452 ± 8.8	500 ± 6.4	478 ± 5.7	P × B	36,046	46.95	<0.0001
February	488 ± 7.6	482 ± 10.2	564 ± 8.0	533 ± 6.1	P × PS	1,335	1.739	0.1248
March	519 ± 9.0	499 ± 11.3	595 ± 8.2	563 ± 7.4	B × PS	3,456	4.502	0.0345
					P × B × PS	612.8	0.7982	0.5515

Period effect

October	November	December	January	February	March	One-way ANOVA (*p*-value)
343 ± 3.84a	366 ± 3.29b	424 ± 3.14c	471 ± 4.46d	517 ± 6.00e	544 ± 6.71f	<0.0001

Breed effect

Period	Brahman (*B. indicus*)	Angus (*B. taurus*)	Student's *t*-test (*p*-value)
October	364 ± 4.13	322 ± 5.65	<0.0001
November	381 ± 4.15	351 ± 3.10	<0.0001
December	421 ± 5.04	427 ± 3.44	0.3296
January	452 ± 5.60	489 ± 4.71	<0.0001
February	485 ± 6.32	548 ± 5.71	<0.0001
March	509 ± 7.38	579 ± 6.18	<0.0001
Average	435 ± 4.40	453 ± 7.14	0.0361

Production system effect

Period	TRT	NAT	Student's *t*-test (*p*-value)
October	344 ± 5.25	343 ± 4.96	0.8904
November	368 ± 4.33	364 ± 4.41	0.5200
December	429 ± 3.88	419 ± 4.30	0.0896
January	476 ± 6.16	465 ± 5.53	0.1891
February	526 ± 8.42	507 ± 7.18	0.0913
March	557 ± 8.76	531 ± 8.51	0.0375
Average	450 ± 6.25	438 ± 5.63	0.1591

[a]Data are the mean ± SEM (n = 50/group).
[b]*B*, breed; *P*, period; *PS*, production system.
[c]*NAT*, natural; *TRT*, traditional with growth-promoting technology.
Different lowercases letters indicate significant differences at P<0.05

including cattle (Mayengbam et al., 2016; Xu et al., 2017). As key processors for protein quality control and stress response, the functions of HSPs include the folding and assembly of new synthesized polypeptides, the refolding of stress-denatured and misfolded proteins, and the regulation of signaling pathways to maintain cellular homeostasis (Theodoraki and Caplan, 2012; Song et al., 2025). The upregulation of *Hsp90* in Brahman confirms that this breed is more sensitive to cold than its Angus counterpart. It is known that Brahman has a short, shiny, and glossy coat that helps in heat tolerance adaptation, but is not sufficient in an extreme cold environment. Although it is still unknown in mammals, *Hsp90* has recently been found to be a nutrient-responsive molecular chaperone that regulates feed intake in *Drosophila* (Ohhara et al., 2021). Thus, its upregulation in Brahman cattle might further explain the differential growth and feed intake observed between the two studied breeds. In an *in vitro* study using neuronal SH-SY5Y cells, Hosoi and co-workers showed that Hsp90 regulates the leptin–STAT3 pathway, which suggests a potential key role of Hsp90 in feed intake regulation (Hosoi et al., 2016).

Cold stress has been reported to suppress the host immune defense by modulating the expression of (chemo)cytokines (Girotti et al., 2011). Here, there was a significant P × B × T interaction for *IL10*, *IL-1β*, *Tnfα*, and *Crp*, but not for *IL6* expression, with increased mRNA levels in Brahman for *IL10*, *IL6*, and *Crp* in November, as well as *IL-1β* and *Tnfα* in November and January (Figures 3A–F), which again coincided with the low air temperature and THI, slow weight changes, and the drop in DMI and FE (Tables 1, 4, 3). There was a significant effect of period on *IL6* expression, with higher mRNA abundance in November and March (Figure 3C). Before discussing the broader impact, two key points could be highlighted. Firstly, the expression of the pro-inflammatory cytokines did not follow the same trend during the winter season, and this was probably associated with the cytokine individual endogenous circadian rhythms (Rahman et al., 2015), the time lag sensitivity and response to cortisol (DeRijk et al., 1997), specific triggers and functions (Cui et al., 2024), and/or the complex interactions between the cytokines themselves and between cytokines and other factors (Turrin and Plata-Salaman, 2000). Secondly, there was a simultaneous increase in both anti-inflammatory (*IL10*) and pro-inflammatory (*IL6* and *Crp*) cytokines during the month of November, and this is foreseeable due to a negative feedback loop to control the magnitude and

TABLE 2 Effects of winter season, breed, and production systems on the average daily gain (ADG, in kilograms) in beef cattle.

Breed[a]	Brahman (*Bos indicus*)		Angus (*Bos taurus*)					
Period (P)[b]/system (PS)[c]	TRT	NAT	TRT	NAT	Three-way ANOVA			
					S. of variation	**MS**	***F* (DFn, DFd)**	***p*-value**
					P	16.98	79.51	<0.0001
October–November	0.53 ± 0.05	0.63 ± 0.13	1.10 ± 0.11	0.94 ± 0.07	B	47.88	224.2	<0.0001
November–December	1.43 ± 0.07	1.46 ± 0.12	2.92 ± 0.07	2.47 ± 0.06	PS	2.142	10.03	0.0017
December–January	1.09 ± 0.06	1.10 ± 0.07	2.34 ± 0.15	2.17 ± 0.09	P × B	3.105	14.54	<0.0001
January–February	1.29 ± 0.10	1.08 ± 0.16	2.29 ± 0.14	1.95 ± 0.18	P × PS	0.1752	0.8202	0.5132
February–March	1.11 ± 0.11	0.61 ± 0.16	1.07 ± 0.21	1.07 ± 0.11	B × PS	0.2269	1.062	0.3036
					P × B × PS	0.503	2.356	0.0540

Period effect					
Oct–Nov	**Nov–Dec**	**Dec–Jan**	**Jan–Feb**	**Feb–Mar**	**One-way ANOVA (*p*-value)**
0.79 ± 0.05a	2.07 ± 0.09b	1.66 ± 0.09c	1.64 ± 0.09c	0.96 ± 0.08a	<0.0001

Breed effect			
Period	**Brahman (*B. indicus*)**	**Angus (*B. taurus*)**	**Student's *t*-test (*p*-value)**
October–November	0.58 ± 0.08	1.01 ± 0.06*	<0.0001
November–December	1.45 ± 0.07	2.69 ± 0.06*	<0.0001
December–January	1.10 ± 0.04	2.25 ± 0.09*	<0.0001
January–February	1.18 ± 0.09	2.12 ± 0.11*	<0.0001
February–March	0.86 ± 0.10	1.07 ± 0.11	0.1631
Overall	1.03 ± 0.09	1.82 ± 0.15	<0.0001

Production system effect			
Period	**TRT**	**NAT**	**Student's *t*-test (*p*-value)**
October–November	0.78 ± 0.09	0.80 ± 0.07	0.8614
November–December	2.15 ± 0.14	1.98 ± 0.11	0.3436
December–January	1.69 ± 0.14	1.64 ± 0.11	0.7798
January–February	1.77 ± 0.12	1.52 ± 0.14	0.1804
February–March	1.09 ± 0.11	0.84 ± 0.10	0.0980
Overall	1.51 ± 0.15	1.34 ± 0.14	0.4108

[a]Data are the mean ± SEM (n = 50/group).
[b]*B*, breed; *P*, period; *PS*, production system.
[c]*NAT*, natural; *TRT*, traditional with growth-promoting technology. * and different lowercase letters indicate significant differences at P<0.05

duration of the inflammation-associated with cold stress and to maintain the cellular homeostasis (Ostrowski et al., 1999; Tamayo et al., 2011; Cicchese et al., 2018). These changes in cytokine expression could also explain the differential feed intake and growth observed between the two breeds. Although they use a different transducing system, *IL-1β*, *IL6*, and *Tnfα* have all been reported to suppress feed intake, which is a shared phenomenon (Plata-Salaman, 1998; Buchanan and Johnson, 2007; Gouvea et al., 2022). In addition to their peripheral action, there is now compelling evidence demonstrating that cytokines cross the blood–brain barrier and reach the feeding-related hypothalamic nuclei, such as the arcuate nucleus (ARC), to regulate appetite and energy homeostasis (Buchanan and Johnson, 2007). Moreover, cytokines have been reported to stimulate energy expenditure (Puigserver et al., 2001) and activate thermogenesis (Garcia et al., 2018), both of which are known to reduce body weight and growth rate (Leibel et al., 1995; Heinitz et al., 2020; Ravussin et al., 2021).

Cold stress also alters the expression of chemokines, leading to complex changes in the immune and inflammatory responses (Straat et al., 2022). Chemokines comprise the largest subfamilies of cytokines based on systemic nomenclature analyses, and they are divided into four groups: CC, CXC, XC, and CX3C (Zlotnik and Yoshie, 2000, 2012; Hughes and Nibbs, 2018). Here, there was a significant P × B × T interaction for the C–C motif chemokine ligands (*Ccl2*, *Ccl5*, and *Ccl20*) (Figures 4A–C), *Cxcl14* Figure 5C), *Ccr2* (Figure 6A), and *Cxcr1* (Figure 6B), but not for *Ccl4* (Figure 4D), *Cxcl12* (Figure 5A), *Xcl1* (Figure 7A), and *Cxcr2* (Figure 6C). Similarly to the cytokines, the mRNA levels of *Ccl5* and the chemokine receptors *Ccr2* and *Cxcr1* were higher in Brahman in November, and the gene expression levels of *Ccl20* and *Cxcl14* were higher in October. In addition, there was a significant effect of period for the gene expression of *Ccl4*, *Cxcl12*, *Xcl1*, and *Cxcr2*, with higher mRNA abundance during November–December for *Ccl4* and *Xcl1* (Figures 4E, 7B) and October for *Cxcl12* and *Cxcr2* (Figures 5B, 6D). These changes in the blood chemokine expression profile coincided with the low air temperatures, with the same interpretation remaining valid for the cytokines. As a supportive example, Sadler and colleagues have shown that cold stress activates the CCR2 axis, primarily through increasing the number of circulating CCR2$^+$ immune cells

TABLE 3 Effects of winter season, breed, and production systems on the feed efficiency (FE) in beef cattle.

Breed[a]	Brahman (*Bos indicus*)		Angus (*Bos taurus*)					
Period (P)[b]/System (PS)[c]	TRT	NAT	TRT	NAT	Three-way ANOVA			
					S. of variation	**MS**	***F* (DFn, DFd)**	***p*-value**
					P	0.1465	79.51	<0.0001
October–November	0.09 ± 0.09	0.10 ± 0.02	0.17 ± 0.01	0.15 ± 0.01	B	0.1323	224.2	<0.0001
November–December	0.19 ± 0.01	0.19 ± 0.01	0.30 ± 0.008	0.24 ± 0.006	PS	0.0507	10.03	0.0143
December–January	0.13 ± 0.007	0.13 ± 0.008	0.20 ± 0.01	0.17 ± 0.006	P × B	0.0229	14.54	0.0284
January–February	0.18 ± 0.01	0.14 ± 0.01	0.21 ± 0.01	0.17 ± 0.01	P × PS	0.0036	0.8202	0.7827
February–March	0.15 ± 0.01	0.07 ± 0.03	0.09 ± 0.02	0.09 ± 0.009	B × PS	0.0012	1.062	0.7049
					P × B × PS	0.0107	2.356	0.2744

Period effect

Oct–Nov	**Nov–Dec**	**Dec–Jan**	**Jan–Feb**	**Feb–Mar**	**One-way ANOVA (*p*-value)**
0.13 ± 0.009ae	0.23 ± 0.007b	0.16 ± 0.005acd	0.18 ± 0.008d	0.10 ± 0.0e	<0.0001

Breed effect

Period	**Brahman (*B. indicus*)**	**Angus (*B. taurus*)**	**Student's *t*-test (*p*-value)**
October–November	0.096 ± 0.01	0.159 ± 0.01*	<0.0001
November–December	0.196 ± 0.009	0.273 ± 0.007*	<0.0001
December–January	0.134 ± 0.005	0.183 ± 0.007*	<0.0001
January–February	0.161 ± 0.01	0.192 ± 0.01	0.0324
February–March	0.111 ± 0.01	0.091 ± 0.01	0.1626
Overall	0.14 ± 0.01	0.18 ± 0.01	0.0064

Production system effect

Period	**TRT**	**NAT**	**Student's *t*-test (*p*-value)**
October–November	0.13 ± 0.01	0.13 ± 0.01	>0.9999
November–December	0.25 ± 0.01	0.22 ± 0.009*	0.0296
December–January	0.16 ± 0.009	0.15 ± 0.006	0.3591
January–February	0.20 ± 0.01	0.16 ± 0.01*	0.0064
February–March	0.12 ± 0.01	0.08 ± 0.01	0.0064
Overall	0.17 ± 0.01	0.15 ± 0.01	0.1626

[a]Data are the mean ± SEM (n = 50/group).
[b]*B*, breed; *P*, period; *PS*, production system.
[c]*NAT*, natural; *TRT*, traditional with growth-promoting technology. * and different lowercase letters indicate significant differences at P<0.05

or increasing the levels of its ligands, contributing to stress responses and pain/inflammatory hypersensitivity (Sadler et al., 2018). Of particular interest is that the expression levels of the blood *Ccl4* and *Xcl1* genes were significantly upregulated in Brahman compared with the Angus breed (Figures 4F, 7C). Although a direct effect has not been reported yet, CCL4 has been shown to stimulate angiopoitin-2 expression (Lu et al., 2022), which has been demonstrated to reduce appetite, feed intake, and BW gain and to enhance energy expenditure (Kim et al., 2010). Similarly, despite the lack of a direct effect, studies have shown that mice lacking conventional type 1 dendritic cells, which are the primary cells expressing the XCL1 receptor, exhibit an impaired energy expenditure and an increased BW (Hernandez-Garcia et al., 2022). Together, these data suggest that the differential expression

TABLE 4 Effects of winter season, breed, and production systems on the dry matter (DM) intake (in kilograms) in beef cattle.

Breed[a]	Brahman (*Bos indicus*)		Angus (*Bos taurus*)					
Period (P)[b]/system (PS)[c]	TRT	NAT	TRT	NAT	Three-way ANOVA			
					S. of variation	**MS**	***F* (DFn, DFd)**	***p*-value**
					P	108.6	109.0	<0.0001
October–November	5.93 ± 0.05aα	6.11 ± 0.03aα	6.34 ± 0.03aα	6.38 ± 0.04aα	B	792.2	795.2	<0.0001
November–December	7.20 ± 0.09aαβ	7.56 ± 0.17aβ	9.71 ± 0.06bβ	10.01 ± 0.06bβ	PS	12.117	2.125	0.1460
December–January	8.11 ± 0.11aβ	8.23 ± 0.22aγ	11.99 ± 0.12bγ	12.60 ± 0.12bγ	P × B	58.35	58.58	<0.0001
January–February	7.07 ± 0.36aα	7.24 ± 0.43aβ	11.03 ± 0.34bγ	10.96 ± 0.38bβ	P × PS	4.907	4.926	0.0007
February–March	7.45 ± 0.27aα	7.13 ± 0.42aβ	11.29 ± 0.53bγ	11.22 ± 0.29bε	B × PS	8.167	8.199	0.0045
					P × B × PS	9.511	9.548	<0.0001

[a]Data are the mean ± SEM (n = 50/group).
[b]*B*, breed; *P*, period; *PS*, production system.
[c]*NAT*, natural; *TRT*, traditional with growth-promoting technology.
Different letters indicate significant difference at P<0.05

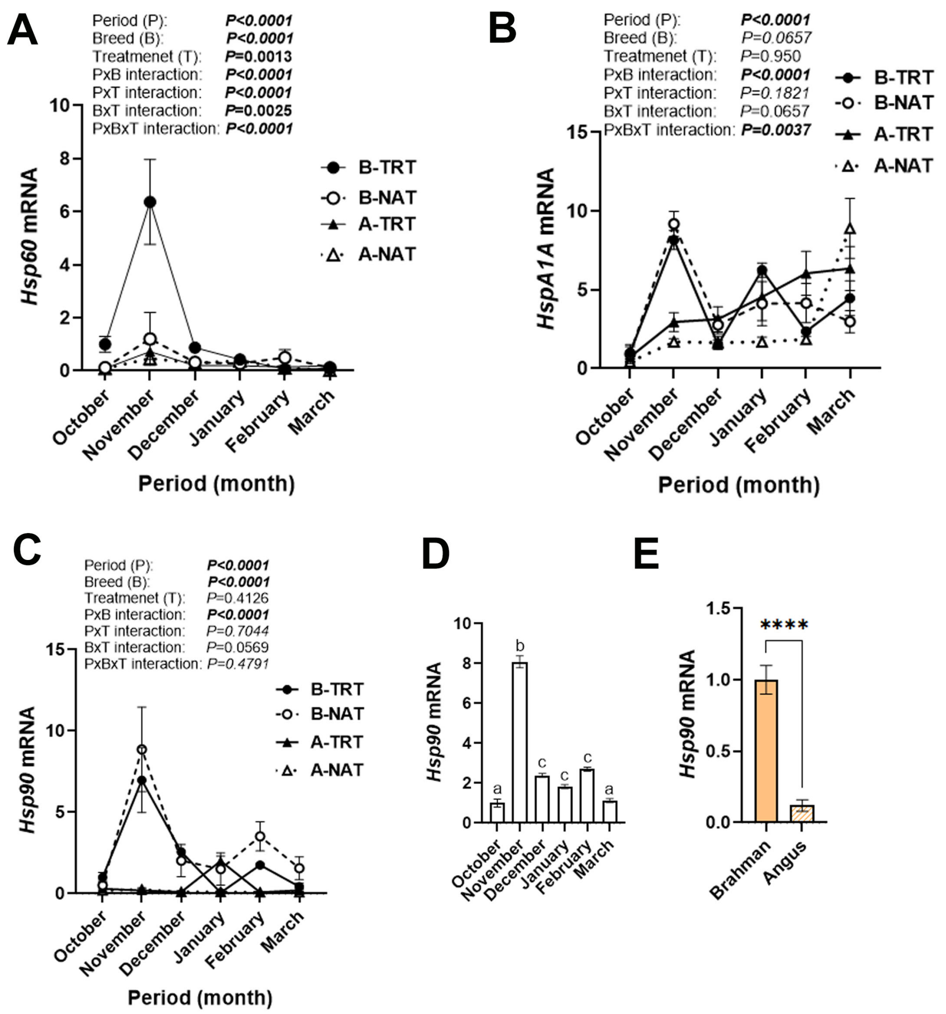

FIGURE 2
Blood expression of the heat shock proteins (HSPs) in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Hsp60* **(A)**, *HspA1A* **(B)**, and *Hsp90* **(C–E)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) using ribosomal r18S as a housekeeping gene. When appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference (p < 0.05). *Four asterisks* indicate significant difference at p < 0.0001. A, Angus; *B*, Brahman; *Hsp*, heat shock protein; *NAT*, natural; *TRT*, traditional with growth-promoting technology.

of chemokines might play a role in the growth differences and physiological adaptive traits between the Brahman and Angus breeds. The administration of growth-promoting technologies (TRT) in the form of implants, RAC, and monensin/tylosin significantly downregulated the expression of the blood *Cxcr2* gene (Figure 6E). The main ligands for this chemokine receptor are the CXC family (CXCL1–3 and CXCL5–8). This change suggests that *Cxcr2* might be involved in ameliorating the feed efficiency in the TRT group compared with the NAT group (Table 3) (Lazennec et al., 2023).

Environmental cold stress has been shown to affect blood cells by decreasing their deformability and increasing their membrane rigidity (Brenner et al., 1999; Teleglow et al., 2021). In this study, there was a significant P × B × T interaction for *Cldn*1, but not for the *Ocln* gene expression (Figures 8A, B). The abundance of *Ocln* mRNA was significantly affected by period, with increasing levels from November to reach a maximum in January, and then a decline for the rest of the study period (Figure 8C). Although it is still not known in what specific blood cells these tight junction proteins are expressed, the upregulation of *Ocln* during the cold period indicates potential damage to the cell integrity. It is possible that these tight junction proteins are released from the damaged blood–brain barrier or other endothelial barriers outside of the blood vessels (Ballabh et al., 2005; Andersson et al., 2021). Although it is challenging to the traditional view, tight junction proteins have also been found to be expressed in certain white blood cells, such as leukocytes (Mandel et al., 2012). If this case is true for cattle, the upregulation of the *Ocln* gene expression suggests a potential role in

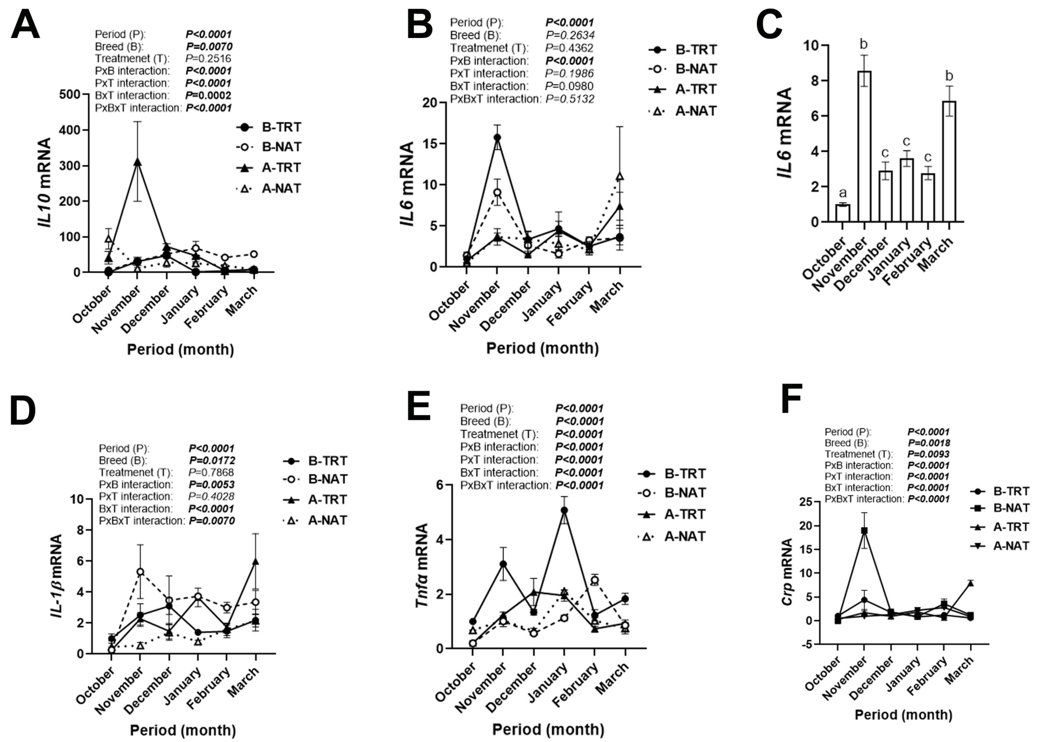

FIGURE 3
Blood expression of the anti- and pro-inflammatory cytokines in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *IL10* **(A)**, *IL6* **(B, C)**, and *IL-1β* **(D)**, *Tnfα* **(E)**, and *Crp* **(F)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method as described in "*Materials and methods*." Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference (p < 0.05). *A*, Angus; *B*, Brahman; *IL*, interleukin; *NAT*, natural; *TRT*, traditional with growth-promoting technology.

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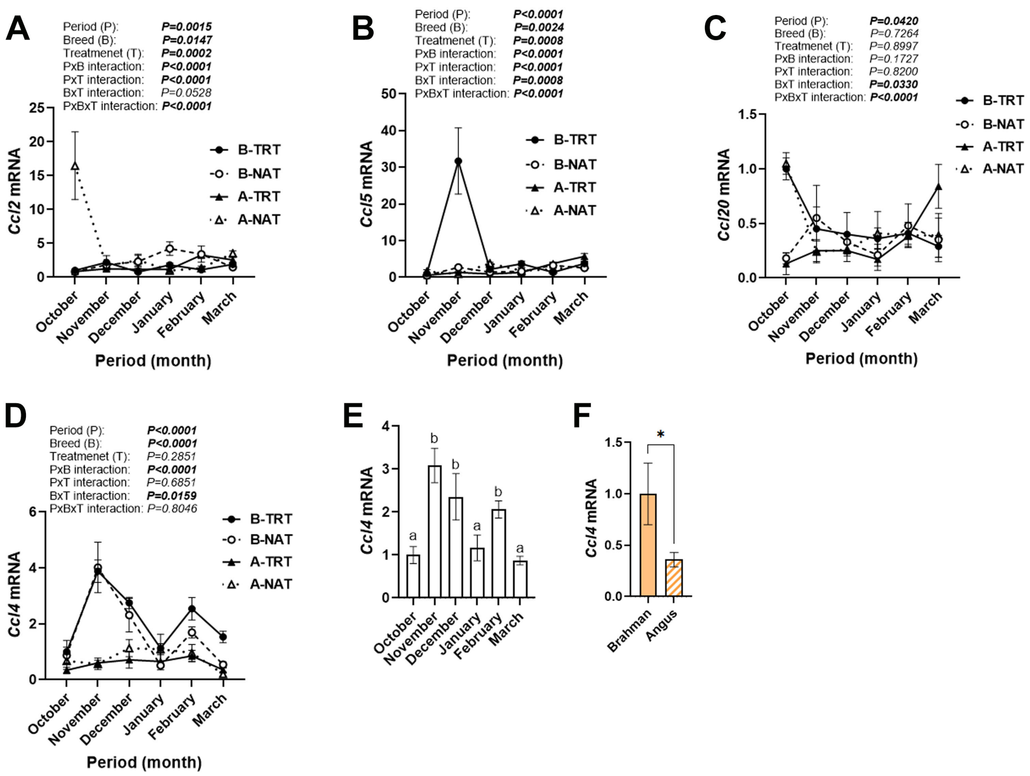

FIGURE 4
Circulating expression of the C–C motif chemokine ligands in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Ccl2* **(A)**, *Ccl5* **(B)**, and *Ccl20* **(C)**, and *Ccl4* **(D–F)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference (p < 0.05). *Asterisk* indicates significant difference at p < 0.05. A, Angus; *B*, Brahman; *Ccl*, C–C motif chemokine ligand; *NAT*, natural; *TRT*, traditional with growth-promoting technology.

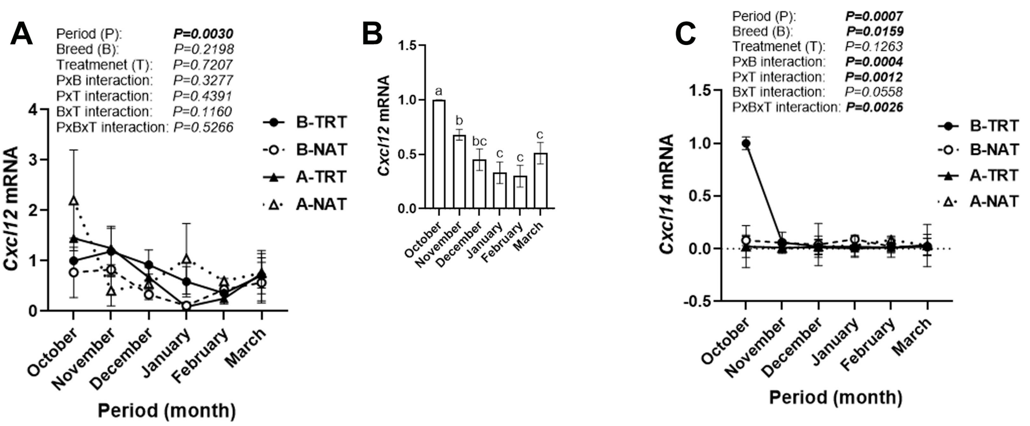

FIGURE 5
Circulating expression of the C–X–C motif chemokine ligands in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Cxcl12* **(A, B)** and *Cxcl14* **(C)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference (p < 0.05). *A*, Angus; *B*, Brahman; *Cxcl*, C–X–C motif chemokine ligand; *NAT*, natural; *TRT*, traditional with growth-promoting technology.

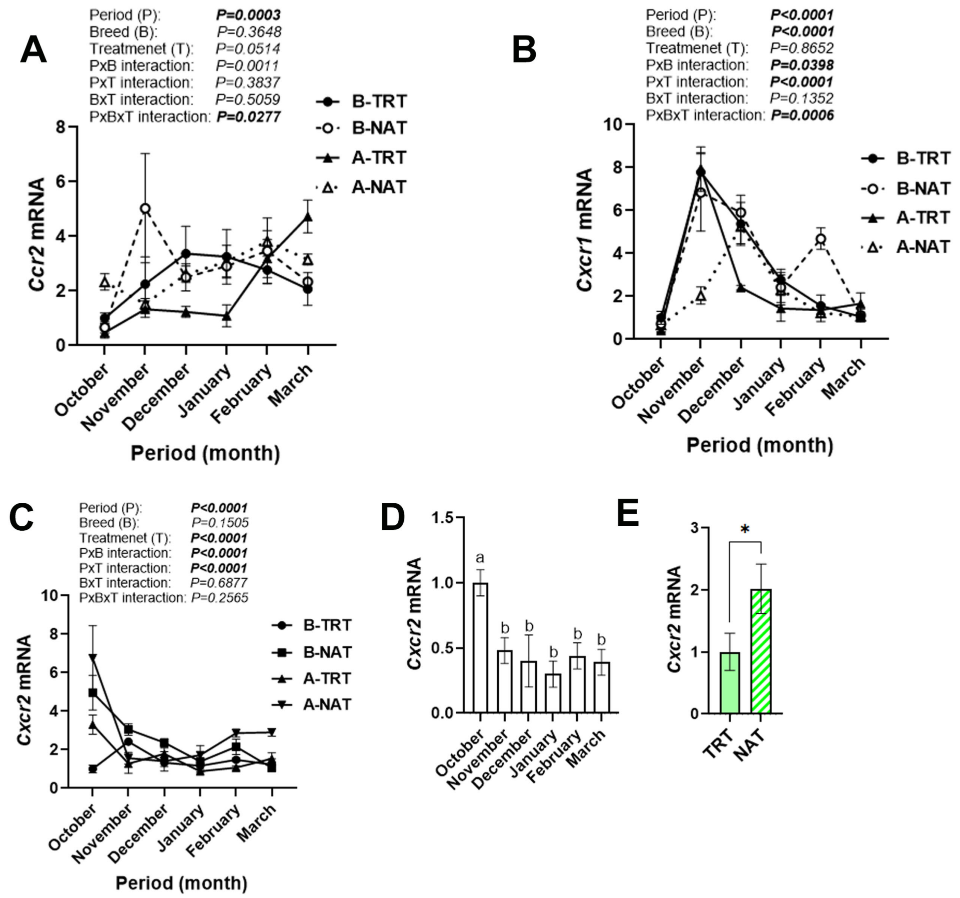

FIGURE 6
Circulating expression of the chemokine receptors in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Ccr2* **(A)**, *Cxcr1* **(B)**, and *Cxcr2* **(C–E)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method as described in "*Materials and methods*." Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference ($p < 0.05$). *Asterisk* indicates significant difference at $p < 0.05$. A, Angus; *B*, Brahman; *Ccr2*, C–C motif chemokine receptor 2; *Cxcr*, C–X–C motif chemokine receptor; *NAT*, natural; *TRT*, traditional with growth-promoting technology; *Xcl1*, X–C motif chemokine ligand 1.

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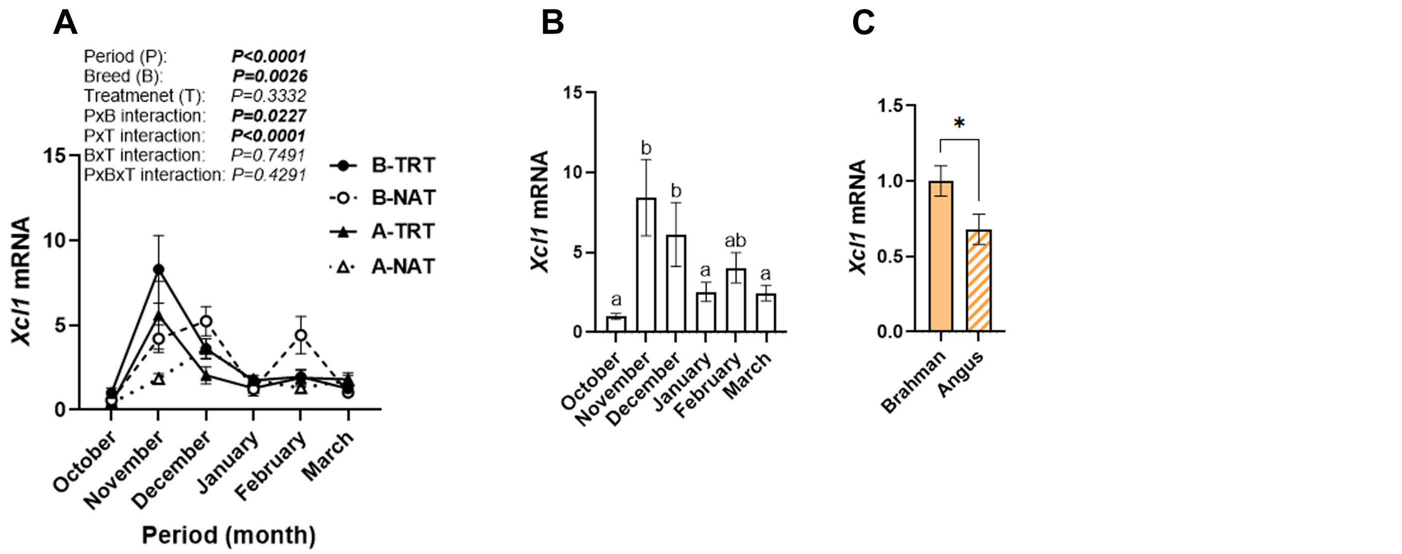

FIGURE 7
Circulating expression of the X–C motif chemokine ligand 1 (XCL1) in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Xcl1* **(A–C)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method as described in "*Materials and methods*." Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (n = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference (p < 0.05). *A*, Angus; *B*, Brahman; *NAT*, natural; *TRT*, traditional with growth-promoting technology; *Xcl1*, X–C motif chemokine ligand 1.

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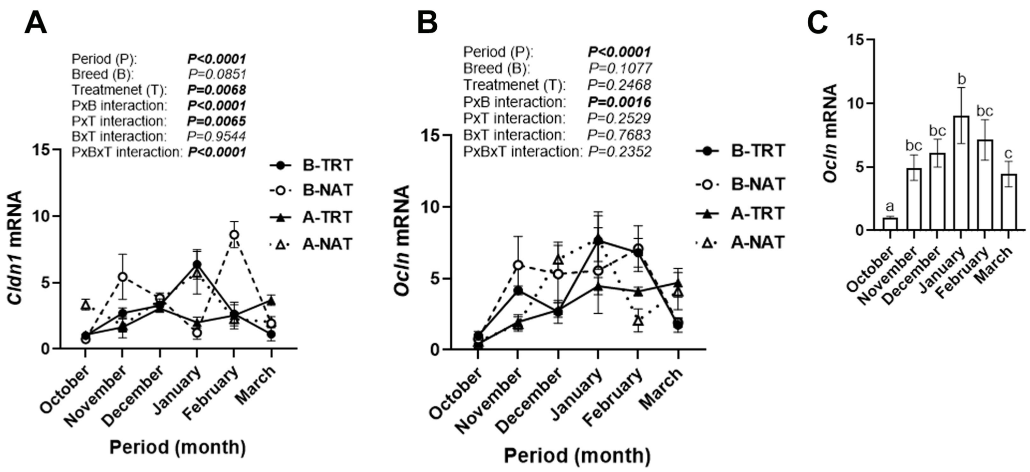

FIGURE 8
Blood expression of the tight junction proteins in cattle breeds (Angus *vs.* Brahman) reared in Colorado under two feeding systems during the winter season. The expression of *Cldn1* **(A)** and *Ocln* **(B, C)** was determined with quantitative PCR (qPCR) and the $2^{-\Delta\Delta Ct}$ method as described in "*Materials and methods*." Ribosomal r18S was used as a housekeeping gene and, when appropriate, Brahman, TRT, and/or October were used as calibrators. If the breed × period × production system interaction is not significant, the main effects were analyzed separately using one-way ANOVA or *t*-test, as appropriate. Data are the mean ± SEM (*n* = 12 steers/breed per period per production system). *Different superscript letters* denote statistical difference ($p < 0.05$). *A*, Angus; *B*, Brahman; *Cldn1*, claudin 1; *NAT*, natural; *Ocln*, occludin; *TRT*, traditional with growth-promoting technology; *Xcl1*, X–C motif chemokine ligand 1.

leukocyte migration into target tissues during cold stress (Du et al., 2010). It is also conceivable that *Ocln* upregulation is indicative of extravasation (Shrestha et al., 2014).

In summary, to the best of our knowledge, this is the first report demonstrating the effect of the winter season (cold stress) and feeding technique on the growth performance and blood expression profile of the stress-, inflammation-, and cellular integrity-related genes in Brahman and Angus cattle. The upregulation of the blood *Hsp90*, *Xcl1*, and *Ccl4* gene expression in Brahman might explain its sensitivity to extreme cold environment and open a new vista for the identification and the development of circulating molecular signatures to monitor stress and/or for marker-assisted selection. The use of GPT affected only the blood expression of *Cxcr2*, which might probably be associated with the effect of anabolic steroids.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Colorado State University, USA. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KS-L: Writing – review & editing. MB: Data curation, Formal analysis, Writing – review & editing. AS-H: Writing – review & editing. PH: Writing – review & editing. EM: Writing – review & editing. LA: Writing – review & editing. CA: Writing – review & editing. MS: Writing – review & editing. SD: Data curation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

Authors MB, LA, CA, and MS are employed by Zinpro Corporation.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author SD declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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