Fish oil and dexamethasone administration partially mitigates heat stress-induced changes in circulating leukocytes and metabolic indicators in feedlot wethers

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INTRODUCTION

Heat stress and its economic impact are becoming an increasingly common concern for livestock producers due to climate change. Heat stress-induced deficits in average daily gain and feed efficiency of livestock have been primarily attributed to lower dry matter intake (Bernabucci et al., 2010). However, our recent studies demonstrate that these negative outcomes are not explained solely by reduced nutrient consumption (Barnes et al., 2019; Swanson et al., 2020). Indeed, pair-feeding thermoneutral livestock with heat-stressed livestock revealed that there are physiological mechanisms independent of intake that decrease growth and efficiency. A key culprit identified by these studies was chronic systemic inflammation characterized by elevated levels of circulating leukocytes and TNF-α, as well as increased circulating triglycerides and cholesterol that was consistent with lipid dysregulation (Barnes et al., 2019; Swanson et al., 2020). Additional studies by our lab have reported that inflammatory mediators such as the cytokines TNF-α and IL-6 influence muscle growth and metabolism (Cadaret et al., 2017; Posont et al., 2018), and thus, the present study aimed to investigate the effectiveness of targeting heat stress-induced inflammation through administration of the anti-inflammatory synthetic steroid, dexamethasone (DEX), and through oral supplementation of fish oil, which is high in anti-inflammatory ω-3 polyunsaturated fatty acids that have been shown to reduce circulating white blood cells in cattle (Didara et al., 2015) and TNF-α in piglets (Yao et al., 2012). The objective of this study was to assess the effects of DEX and fish oil administration over a 30-d period in heat-stressed wethers on hematological factors, blood gases, rectal temperatures, and respiration rates. We hypothesized that targeting systemic inflammation with these products during chronic heat stress would improve indicators of health and metabolic efficiency.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln and was performed at the UNL Animal Science Complex, which is accredited by AAALAC International. Sixteen crossbred Rambouillet wethers averaging 41.2 kg were purchased commercially and given a 21-d acclimation period during which they were housed at 25 °C and 15% relative humidity (RH) and transitioned to a pelleted lamb grower/finisher ration (Complete B30; Purina). For the study, wethers were stratified by body mass, individually penned in indoor environmental chambers, and housed under thermoneutral conditions of 25 °C, 15% RH (controls) or heat stress conditions of 40 °C, 35% RH for 12 h/d and 35 °C, and 35% RH for 12 h/d for 30 d. Heat-stressed wethers were randomly assigned to receive no intervention (HS, placebo...
boluses and injections; \( n = 4 \)), DEX injections (0.15 mg/kg, IM) every 3 d (DEX, placebo boluses; \( n = 4 \)), or twice-daily fish oil boluses (FO, 1,200 mg containing 360 mg eicosapentaenoic acid; injection boluses; \( n = 4 \)). Fish oil doses (i.e., 2,400 mg/d) were extrapolated from Toral et al. (2016), who fed ~3,900 mg/d to sheep averaging 82.5 kg. Controls received placebo boluses and injections and were pair-fed to the average intake of HS, which were fed ad libitum. Respiratory rates were measured 3 h after heat was initiated on days −2, 2, 7, 15, and 22. Body temperatures were taken when heat was initiated (0900 h) on days –5, –2, 0, 6, 15, and 29. Blood was collected via jugular venipuncture into EDTA vacutainer tubes (6 mL) and heparinized syringes (0.5 mL) on days −3, 3, 9, 21, and 30, and at necropsy. Total and differential blood counts were performed with a Hematrue Veterinary Hematology Analyzer (Heska). Blood gas and metabolites were determined with an ABL90 FLEX (Radiometer). A completely randomized experimental design was utilized. Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Institute) with day as a repeated measure. Animal was considered the experimental unit. Fisher’s least significant difference test was used for pairwise comparisons among experimental groups. Significance was declared at \( P < 0.05 \), and tendencies were declared at \( P < 0.1 \). Data are presented as mean ± standard error.

**RESULTS**

**Rectal Temperatures and Respiration Rates**

Experimental group × day interactions were observed (\( P < 0.05 \)) for respiration rates and rectal temperatures. Respiration rates did not differ among groups on day –2 but were greater (\( P < 0.05 \)) for HS than for controls on days 2, 7, 15, and 22 (Figure 1). Respiratory rates did not differ among HS, DEX, and FO on day 2, 7, or 22 but were intermediate (\( P < 0.05 \)) for DEX and FO between controls and HS on day 15. Rectal temperatures did not differ among groups on day –5, –2, or 0 but were greater (\( P < 0.05 \)) for HS, DEX, and FO than for controls on days 6, 15, and 24.

**Blood Gases and Electrolytes**

Experimental group × day interactions were observed (\( P < 0.05 \)) for blood glucose, partial pressure of \( \text{CO}_2 \), pH, hemoglobin, and blood \( \text{Na}^+ \). Blood glucose was less (\( P < 0.05 \)) for HS, DEX, and FO than for controls on day 3 but greater (\( P < 0.05 \)) for HS, DEX, and FO than controls on day 21 (Figure 2). Blood lactate and partial pressure of \( \text{O}_2 \) did not differ among groups for any day. Blood partial pressure of \( \text{CO}_2 \) was less (\( P < 0.05 \)) for HS than controls on days 3, 9, 21, and 30 and was intermediate (\( P < 0.05 \)) for DEX and FO between controls and HS on days 3 and 9. Oxyhemoglobin and carboxyhemoglobin were greater (\( P < 0.05 \)) for HS but not DEX or FO compared with controls. Blood pH was greater (\( P < 0.05 \)) for HS than controls on days 3, 9, and 21 and for DEX and FO on days 3 and 9 compared with controls. Blood \( \text{HCO}_3^- \) was less (\( P < 0.05 \)) for HS but not DEX or FO compared with controls. Blood \( \text{Na}^+ \) was greater (\( P < 0.05 \)) for HS but not DEX or FO than for controls. Blood \( \text{K}^+ \) tended to be greater (\( P < 0.1 \)) for HS but not DEX or FO than for controls. Blood \( \text{Ca}^{2+} \) was less (\( P < 0.05 \)) for HS and FO and was intermediate (\( P < 0.05 \)) for DEX compared with controls.

**Hematology**

No experimental group × day interactions were observed for any hematology parameter. Total white blood cells and monocytes were greater (\( P < 0.05 \)) for HS, DEX, and FO than controls.
(Table 1). Granulocytes and granulocyte-to-lymphocyte ratios were greater \((P < 0.05)\) for HS and DEX but not FO than for controls. Lymphocytes did not differ among groups. Mean corpuscular volume was less \((P < 0.05)\) for DEX and greater \((P < 0.05)\) for FO compared with controls but did not differ between HS and controls. Mean corpuscular hemoglobin concentration was less \((P < 0.05)\) for HS, DEX, and FO than for controls. Red blood cell concentrations did not differ between HS and controls but were less \((P < 0.50)\) for DEX and FO than for HS and controls. Platelets were less \((P < 0.05)\) for FO than for HS, DEX, and controls.

**DISCUSSION**

In this study, we found that supplementing anti-inflammatory fish oil and DEX was effective in improving several health and metabolic indicators diminished by heat stress. Moreover, this was independent of dietary intake, which was equivalent across all groups. Heat stress induced hyperthermia and hyperventilation, as expected, but targeting previously observed systemic inflammation with fish oil and DEX moderated hyperventilation later in the heat-stress period. Hyperventilation is not only a primary mode of heat dissipation but is also directly associated with respiratory alkalosis (Hales, 1973). When animals hyperventilate to dissipate heat, they indirectly alter their blood acid–base balance by exhaling \(\text{CO}_2\) faster than it is synthesized by the tissues, which accounts for reduced \(\text{CO}_2\) levels observed in our heat-stressed wethers. Reduced blood \(\text{CO}_2\) relative to \(\text{HCO}_3\) stimulates the removal of blood \(\text{HCO}_3\) by the kidneys, which was evident by lower blood \(\text{HCO}_3\) in our heat-stressed wethers.

**Figure 2.** Blood gases and \(\text{HCO}_3\) in heat-stressed wethers receiving DEX or fish oil.

**Table 1.** Total and differential blood counts in heat-stressed wethers receiving injectable DEX or oral fish oil

| Parameter                        | Controls        | Heat stress    | DEX            | Fish oil       | \(P\)-value |
|----------------------------------|-----------------|----------------|----------------|----------------|-------------|
| White blood cells, cells/µL      | 5.59 ± 0.14<sup>a</sup> | 6.5 ± 0.22<sup>b</sup> | 7.16 ± 0.23<sup>c</sup> | 6.13 ± 0.22<sup>b</sup> | <0.01       |
| Lymphocytes, cells/µL            | 3.33 ± 0.09     | 3.2 ± 0.12     | 3.65 ± 0.15    | 3.48 ± 0.13    | NS          |
| Monocytes, cells/µL              | 0.41 ± 0.02<sup>a</sup> | 0.53 ± 0.02<sup>b</sup> | 0.6 ± 0.03<sup>c</sup> | 0.49 ± 0.03<sup>c</sup> | <0.01       |
| Granulocytes, cells/µL           | 2.01 ± 0.1<sup>a</sup> | 2.93 ± 0.14<sup>b</sup> | 2.98 ± 0.12<sup>c</sup> | 2.26 ± 0.15<sup>c</sup> | <0.01       |
| Granulocyte: Lymphocyte          | 0.61 ± 0.03<sup>a</sup> | 0.92 ± 0.03<sup>b</sup> | 0.86 ± 0.05<sup>c</sup> | 0.66 ± 0.05<sup>c</sup> | <0.01       |
| MCV, fl                          | 27.78 ± 0.22<sup>a</sup> | 27.91 ± 0.33<sup>b</sup> | 27.1 ± 0.32<sup>c</sup> | 28.59 ± 0.36<sup>c</sup> | 0.05        |
| MCHC, %                          | 39.3 ± 0.16<sup>a</sup> | 38.31 ± 0.26<sup>b</sup> | 38.94 ± 0.16<sup>c</sup> | 38.74 ± 0.3<sup>c</sup> | 0.03        |
| Red blood cells, cells/µL        | 11.24 ± 0.22<sup>a</sup> | 11.26 ± 0.21<sup>b</sup> | 10.38 ± 0.19<sup>c</sup> | 10.29 ± 0.12<sup>c</sup> | <0.01       |
| Platelets, #/µL                  | 326 ± 17<sup>a</sup> | 325 ± 21<sup>b</sup> | 346 ± 26<sup>c</sup> | 251 ± 12<sup>c</sup> | <0.01       |
| MPV, fl                          | 5.35 ± 0.03<sup>a</sup> | 5.37 ± 0.04<sup>b</sup> | 5.33 ± 0.04<sup>c</sup> | 5.19 ± 0.04<sup>c</sup> | 0.02        |

<sup>a,b,c</sup>Means with differing superscripts differ \((P < 0.05)\).

DEX = Dexamethasone; MCV = mean cell volume; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume; NS = not significant.
The loss of HCO₃⁻ is particularly harmful to growth and feed efficiency in ruminants, as HCO₃⁻ is a key pH buffer in the rumen (O’Brien et al., 2010). Together, these observations indicate that our heat-stressed wethers were likely suffering from respiratory alkalosis. The ability of fish oil and DEX to moderate hyperventilation and thus lower the risk of respiratory alkalosis is almost certainly attributable to their suppression of inflammation, as demonstrated in previous studies (Lemoine et al., 2019; Mikolka et al., 2019). This improvement coincided with better average daily gain and feed efficiency for heat-stressed animals receiving fish oil or DEX. Our assessment of systemic inflammation in this study was not robust. However, heat stress-induced increases in circulating granulocytes and granulocyte-to-lymphocyte ratios, which are associated with inflammatory diseases (Gūragaç and Demirer, 2016), were moderated by fish oil. DEX was surprisingly ineffective at reducing circulating leukocytes and in fact even increased the circulating concentration of monocytes and total white blood cells. However, this did not translate into a reduction in growth and feed efficiency.

**IMPLICATIONS**

These data show that targeting systemic inflammation in heat-stressed livestock is an effective method for improving growth performance. This occurs in part through improved respiration rates, which are crucial for efficient heat dissipation.

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