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Ractopamine HCl improved cardiac hypertrophy but not poor growth, metabolic inefficiency, or greater white blood cells associated with heat stress in concentrate-fed lambs¹

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INTRODUCTION

Heat stress decreases livestock performance and well-being (Hahn, 1999; Nienaber and Hahn, 2007), causes metabolic dysfunction that decreases growth efficiency (O'Brien et al., 2010), and alters cardiovascular function (Crandall et al., 2008). Each year, heat stress costs the livestock industry up to \$2.5 billion (St-Pierre et al., 2003). Ractopamine HCl acts as a nutrient repartitioning agent (Beermann, 2002); classified as a β adrenergic agonist (β AA), it shares pharmacological properties with adrenaline (Beermann, 2002). β AA increase muscle mass and decreases fat deposition through unknown mechanisms

(Beermann, 2002). In feedlot cattle, they increase growth efficiency and improve carcass yield and merit (Scramlin et al., 2010; Buntyn et al., 2017), which increases profit and allows more meat to be produced from fewer animals. However, because β AA act via a stress system, it is unclear how the products affect animals under stress conditions. β 1AA and β 2AA can also cause tachycardia, heart palpitations, and arrhythmias (Sears, 2002). We hypothesize that β 1AA combined with heat stress may overstimulate the adrenergic system, resulting in metabolic dysfunction and decreased performance. Sheep are a common model for cattle, and thus, the objective of this study was to determine the impact of ractopamine HCl on health and cardiovascular parameters, growth, and metabolic efficiency in feeder lambs.

MATERIALS AND METHODS

Animals and Experimental Design

This study was approved by the IACUC at the University of Nebraska-Lincoln, which is accredited by AAALAC International. Crossbred Rambouillet wethers averaging 44 kg were used. Lambs were given a 21-d acclimation period then individually penned under randomly assigned thermoneutral (controls; 25 °C, 15% RH) or heat stress (40 °C, 35% RH) conditions for 30 d. Lambs were pair-fed a concentrate finishing diet beginning on day -5. In a 2 × 2 factorial, lambs also received no supplement or ractopamine HCl (60 mg) delivered

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once daily via oral capsule bolus. Blood samples were collected on day -1, 2, 7, 14, 21, and 30. Lambs were euthanized on day 30. Wall thickness for left and right ventricles and septum were measured using a digital caliper (Traceable, Webster, TX). Sartorius muscles were collected for ex vivo studies.

Live Animal Parameters

Body weights (**BW**) were recorded on day 0 and 30 and average daily gain (**ADG**) was calculated. Electrocardiograms (**ECG**) (Cardell 9500, Midmark, Dayton, OH) were performed on day -1, 2, 7, 14, 21, and 30 to measure heart rate and detect arrhythmias. ECG were recorded for 1 min with lead 1 placed over the cervical vertebrae, lead 2 on the fore flank, and the ground lead on the rear flank. Blood pressure was also recorded via a blood pressure cuff (Cardell 9500) on the fetlock of the forelimb. For each reading, 5 replicates were recorded and averaged.

Blood Parameters

Blood collected via jugular venipuncture into EDTA tubes was used to quantify total and differential white blood cell (**WBC**) concentrations using HemaTrue Veterinary Hematology Analyzer (Heska, Loveland, CO). Blood collected into heparin syringes was used to quantify blood glucose, lactate, O₂, CO₂, and HCO₃ using the ABL 90 FLEX blood gas analyzer (Radiometer, Copenhagen, Denmark).

Sartorius Muscle Isolation

Sartorius muscles were collected from both hindlimbs at necropsy. The muscle was dissected tendon-to-tendon and intact longitudinal strips (~400 mg) were used to measure ex vivo glucose uptake and glucose oxidation as previously described (Cadaret et al., 2017). Strips were preincubated at 37 °C for 1 h in gassed (95% O₂, 5% CO₂) Krebs-Henseleit bicarbonate buffer (**KHB**) containing no additive (basal) or 5 mU/mL insulin (Humulin-R, Ely Lilly). Preincubation media also contained 5 mM glucose and 35 mM mannitol, respectively. Strips were then washed in treatment-spiked KHB with no glucose and 40 mM mannitol for 20 min.

Ex Vivo Glucose Uptake

Glucose uptake was measured by the incorporation of [³H]2-deoxyglucose as described in Cadaret

et al. (2017). Muscle was incubated at 37 °C for 20 min in treatment-spiked KHB media with 1 mM [³H]2-deoxyglucose (300 μCi/mmol) and 39 mM [1-¹⁴C] mannitol (1.25 μCi/mmol), washed 3 times in PBS, weighed, and lysed in 2 M NaOH at 37 °C for 1 h. UltimaGold scintillation fluid (Perkin-Elmer) was added to the lysate. Specific activity of ³H and ¹⁴C was measured by liquid scintillation with a Beckman-Coulter 1900 TA (Brea, CA).

Ex Vivo Glucose Oxidation

Glucose oxidation was determined by oxidation of [¹⁴C-U]D-glucose (Cadaret et al., 2017). Muscle strips were placed in sealed dual-well chambers and incubated at 37 °C for 2 h in treatment-spiked KHB with 5 mM [¹⁴C-U]D-glucose (0.25 μCi/mmol). In the adjacent well, 2 M NaOH was placed to capture CO₂. After 2 h, wells were cooled at -20 °C for 2 min, 2 M HCl was added to the media through the seal, and plates were placed at 4 °C for 1 h. Muscle strips were washed and weighed, and NaOH was mixed with UltimaGold scintillation fluid to determine specific activity of ¹⁴CO₂ via liquid scintillation. Specific activity of all media was also determined.

Statistical Analysis

All data were analyzed by ANOVA using the Mixed procedure of SAS 9.4 (SAS Institute, Cary, NC) to determine the effects of ambient condition, dietary supplement, and their interaction. Repeated measures were used for serial measurements. Animal was considered the experimental unit. Data are presented as mean ± standard error, and significance was $\alpha = 0.05$.

RESULTS

Growth

There were no differences between groups or supplements for initial or final BW. ADG tended to be ~34% less ($P = 0.08$) in heat-stressed lambs than controls (0.21 vs. 0.32 kg/d).

White Blood Cells

Total WBC were increased ($P < 0.05$) in heat-stressed lambs compared to thermoneutral controls (Figure 1A) but did not differ between supplement groups. An ambient condition × supplement interaction was observed ($P < 0.05$) for blood lymphocytes (Figure 1B). Lymphocytes were greater ($P <$

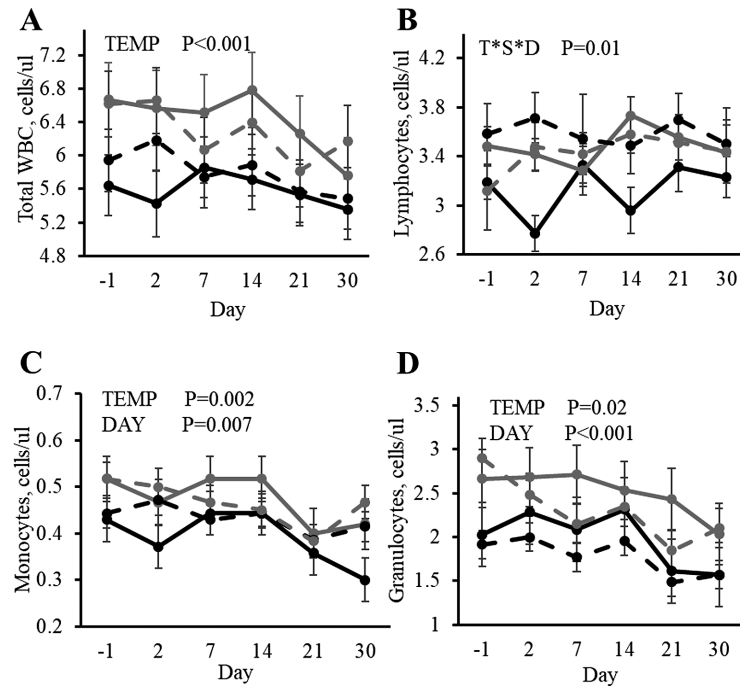


Figure 1. Circulating white blood cells in heat-stressed lambs fed ractopamine HCl for 30 d. Blood concentrations of total white blood cells (A), lymphocytes (B), monocytes (C), and granulocytes (D) are shown for day -1, 2, 7, 14, 21, and 30. *P*-values are shown for effects of ambient temperature (TEMP), supplement (SUPP), day (DAY), and the interaction (T * S * D), where significant. Controls, dark line; heat-stressed lambs, light line; unsupplemented, solid line; ractopamine HCl-fed, dotted line.

0.05) in all heat-stressed lambs and in controls fed ractopamine HCl compared to nonsupplemented controls on day 2. Lymphocytes were also greater ($P < 0.05$) in all heat-stressed lambs than all controls on day 14. Monocytes and granulocytes were greater ($P < 0.05$) in heat-stressed lambs than controls but were not different between supplements (Figure 1C and D, respectively).

Cardiovascular Parameters

An ambient condition \times day interaction was observed ($P < 0.05$) for heart rate (Figure 2A). Heart rate was less ($P < 0.05$) in heat-stressed lambs compared to controls on day 7, 14, and 21. Systolic blood pressure was lower ($P < 0.05$) and diastolic blood pressure tended to be less ($P < 0.10$) in heat-stressed lambs compared to controls. Mean arterial pressure was also less ($P < 0.05$) in heat-stressed lambs compared to controls (Figure 2B). No arrhythmias were observed in ECG readouts from any lambs throughout the study. Ambient condition \times supplement interactions were observed ($P < 0.05$) for left ventricle wall thickness proper and per BW. Both were increased ($P < 0.05$) in all heat-stressed lambs compared to controls, but to a lesser ($P < 0.05$) degree in those fed ractopamine HCl. Right ventricle and septum wall thicknesses proper and per BW tended to be increased ($P < 0.10$) in heat-stressed

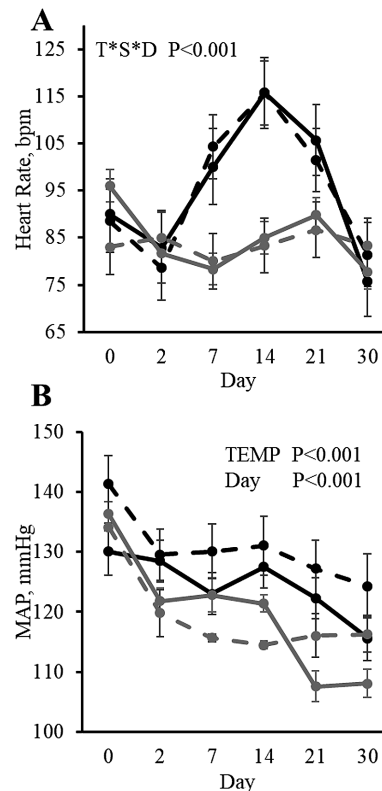


Figure 2. Cardiovascular parameters in heat-stressed lambs fed ractopamine HCl for 30 d. Heart rate (A) and mean arterial blood pressure (B) are shown for day -1, 2, 7, 14, 21, and 30. *P*-values are shown for effects of ambient temperature (TEMP), supplement (SUPP), day (DAY), and the interaction (T * S * D), where significant. Controls, dark line; heat-stressed lambs, light line; unsupplemented, solid line; ractopamine HCl-fed, dotted line.

lambs. An ambient condition \times supplement interaction was observed ($P < 0.05$) for left ventricle thickness/septum thickness, which was greater ($P < 0.05$) in all heat-stressed lambs, but to a lesser ($P < 0.05$) degree in those fed ractopamine HCl.

Blood Gases and Metabolites

Blood glucose concentrations did not differ due to ambient conditions or supplement (Figure 3A). Blood lactate tended to be lower ($P < 0.10$) and glucose-to-lactate ratios were greater ($P < 0.05$) in lambs fed ractopamine HCl than nonsupplemented lambs (Figure 3B and C, respectively). Blood O₂ partial pressure and hemoglobin-bound O₂ were increased ($P < 0.05$) in heat-stressed lambs compared to controls on day 2, 7, 14, 21, and 30 but did not differ between supplements. Hemoglobin-bound CO₂ was increased ($P < 0.05$) in heat-stressed lambs compared to controls but decreased ($P < 0.05$) in ractopamine-supplemented lambs compared to nonsupplemented lambs (Figure 3G).

O₂ were increased ($P < 0.05$) in heat-stressed lambs compared to controls (Figure 3D and E, respectively). They were also increased ($P < 0.05$) in ractopamine-supplemented compared to nonsupplemented lambs. Ambient condition \times day interactions were observed ($P < 0.05$) for blood CO₂ partial pressure and HCO₃ (Figure 3F and H, respectively). Both were increased ($P < 0.05$) in all heat-stressed lambs compared to controls on day 2, 7, 14, 21, and 30 but did not differ between supplements. Hemoglobin-bound CO₂ was increased ($P < 0.05$) in heat-stressed lambs compared to controls but decreased ($P < 0.05$) in ractopamine-supplemented lambs compared to nonsupplemented lambs (Figure 3G).

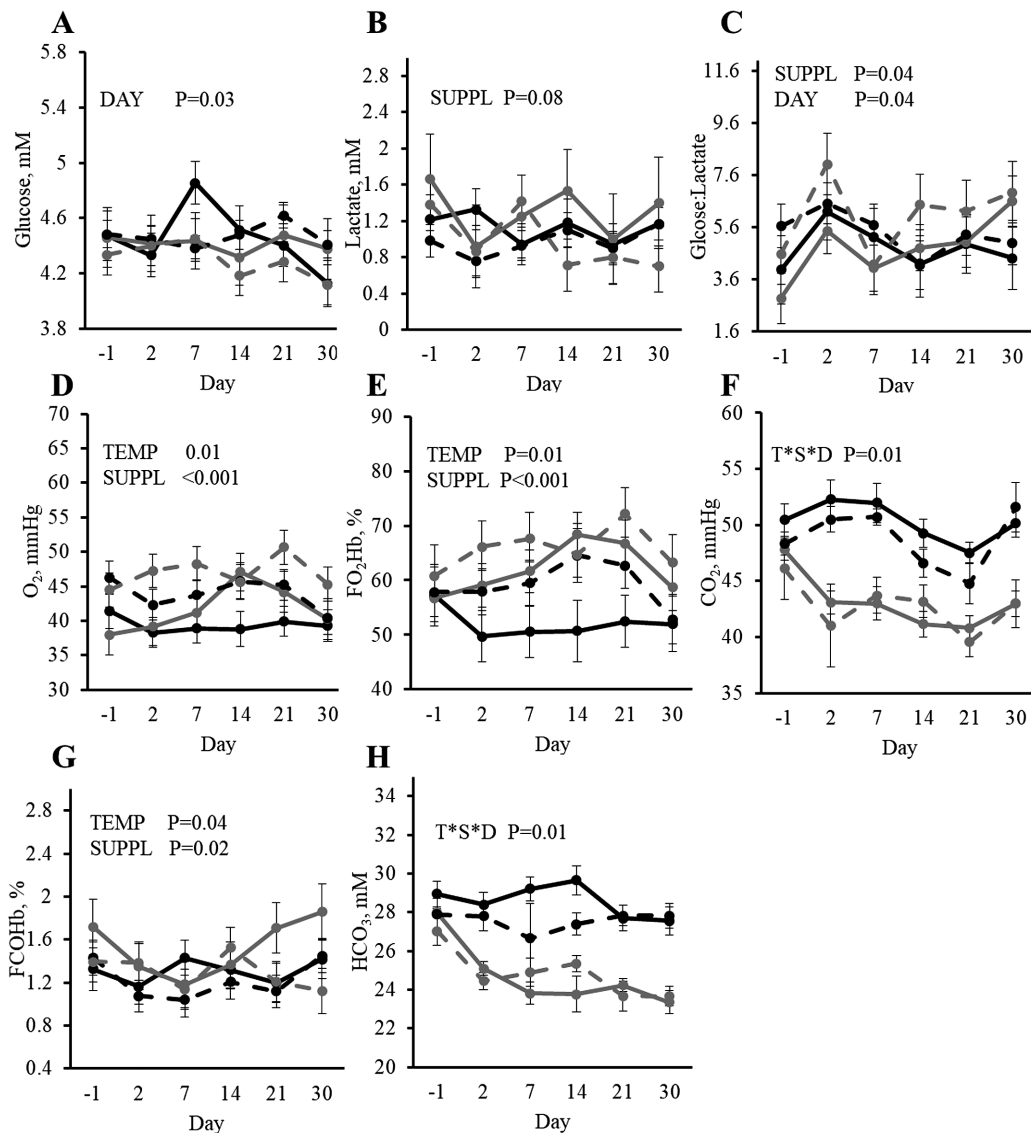


Figure 3. Blood gases and metabolites in heat-stressed lambs fed ractopamine HCl for 30 d. Blood values for glucose (A), lactate (B), glucose:lactate (C), O₂ partial pressure (D), hemoglobin-bound O₂ (E), CO₂ partial pressure (F), hemoglobin-bound CO₂ (G), and HCO₃ (H) are shown for day -1, 2, 7, 14, 21, and 30. P-values are shown for effects of ambient temperature (TEMP), supplement (SUPPL), day (DAY), and the interaction (T * S * D), where significant. Controls, dark line; heat-stressed lambs, light line; unsupplemented, solid line; ractopamine HCl-fed, dotted line.

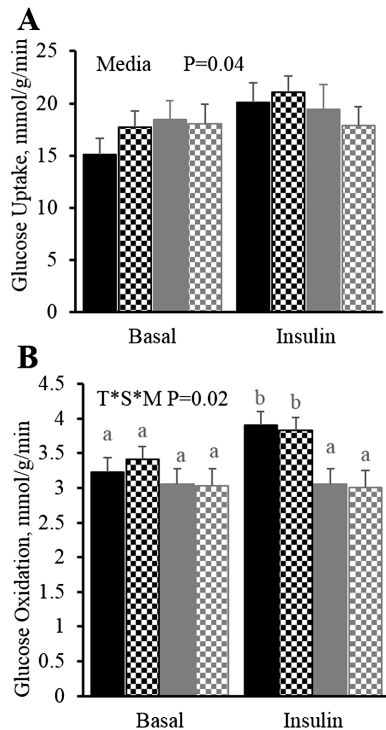


Figure 4. Ex vivo glucose metabolism in primary skeletal muscle from heat-stressed lambs fed ractopamine HCl for 30 d. Glucose uptake (A) and glucose oxidation (B) were measured in basal and insulin-spiked media. *P*-values are shown for effects of ambient temperature (TEMP), supplement (SUPP), media (MEDIA), and the interaction (T * S * M), where significant. ^{a,b}Means with different superscripts differ (*P* < 0.05). Controls, dark bars; heat-stressed lambs, light bars; unsupplemented, solid bars; ractopamine HCl-fed, checkered bars.

Skeletal Muscle Glucose Metabolism

There were no differences between groups or dietary supplements for ex vivo skeletal muscle glucose uptake (Figure 4A). As expected, insulin-spiked media increased (*P* < 0.05) glucose uptake in muscle from all lambs compared to basal media. An ambient condition × media interaction was observed (*P* < 0.05) for glucose oxidation (Figure 4B). In basal media, skeletal muscle glucose oxidation did not differ between groups or supplement. In controls, insulin-spiked media increased (*P* < 0.05) glucose oxidation by ~219% over basal. In heat-stressed lambs, glucose oxidation rates in insulin-spiked media did not differ from basal.

DISCUSSION

Even with improved management, heat stress is still a major barrier to sustainable livestock production (Nienaber and Hahn, 2007). In this study, we found that heat stress decreased ADG and indices of metabolic efficiency, increased circulating

leukocytes, and altered cardiovascular function. In general, these outcomes were neither compounded nor improved by supplementation with the β1AA, ractopamine HCl. Heat stress decreased growth only modestly in this study. However, this reduction was a direct effect of heat stress and not mediated by reduced intake, as thermoneutral lambs were pair-fed with the heat-stressed lambs. The decreases in heart rate in heat-stressed lambs were somewhat surprising, as the literature indicates that heat stress typically increases heart rate (Crandall et al., 2008). Conversely, heat stress-induced reductions in blood pressure have been shown in previous work to be due to decreased blood volume and a shift from central circulation to peripheral circulation (Crandall et al., 2008). Glucose oxidation was decreased by heat stress, which is consistent with decreased metabolic efficiency in feedlot cattle (O'Brien et al., 2010). The β1 agonist ractopamine HCl did not increase metabolic efficiency in our lambs, unlike β2 agonists in previous studies (Beermann, 2002). Together, our findings show that heat stress impairs metabolic function and alters indices of health and well-being, resulting in an overall decrease in production efficiency. Overall, ractopamine HCl did not improve metabolic efficiency in the lambs and its effects on growth and well-being were minimal. Although it increases growth in finishing cattle, other studies have shown little or no effect of ractopamine HCl in finishing lambs (López-Carlos et al., 2010; Scramlin et al., 2010). We previously found that β2 agonists increase growth and metabolic efficiency through increased skeletal muscle glucose oxidation (Barnes et al., 2017), and ractopamine HCl may have some benefit on growth and/or metabolic efficiency in cattle by binding to β2 adrenoceptors at a low affinity.

IMPLICATIONS

Stress conditions and growth enhancing technology each have considerable impacts on economic sustainability. If we are able to identify the mechanisms by which each affect skeletal muscle and other tissues, we will be able to better stimulate increased growth and feed efficiency. Additionally, identifying negative compounding effects of the β1AA ractopamine HCl together with stress will allow for better management decisions to be made, such as using this product differently in certain climates or times of the year.

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