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INTRODUCTION

Many environmental and dietary factors affect ruminant livestock performance (Ames, 1980; Carberry et al., 2012). Energy used by ruminants is mainly derived from volatile fatty acids (VFA), including acetate, propionate, and butyrate, which are produced by bacterial fermentation of carbohydrates (Barcroft et al., 1944). With the importance of VFA production in ruminants (Bergman, 1990), it is essential to understand how various conditions livestock may be subjected to, such as ambient conditions and diet, can affect VFA production.

With the profound role of VFA production in energy status of the ruminant, and given that heat stress (HS) events can affect ruminal microbial populations (Tajima et al., 2007; Duffy et al., 2018) HS thereby may alter energy substrate available to the animal. Significant reductions in VFA levels have been reported during increased environmental temperature in both ad libitum and limit-fed animals (Weldy et al., 1964; Kelley et al., 1967; Freestone and Lyte, 2010).

We speculate that beta-adrenergic agonists (β-AA) may stimulate the production of VFA to increase the efficiency of nutrient digestion and provide more energy to the animal. However, the influence of HS and β-AA supplementation together on rumen VFA production has not been investigated. Researchers also have observed that β-AA reduce the frequency and intensity of ruminal contractions (Ruckebusch et al., 1983; Brikas et al., 1989; Leek, 2001), which are important to digestion, and others have shown that β-AA increase absorption in the digestive tract (McIntyre and Thompson, 1992). These changes in ruminant digestion attributed to β-AA may lead to changes in VFA production. In addition, β-AA may influence the production of VFA directly to increase the efficiency of digestion and provide more energy to the animal. The objective of this study was to determine the impact of β1 agonists, β2 agonists, and HS on rumen VFA production using lambs as a model for cattle. Our prior work showed that heat stress, but not β-AA alone, changed the overall composition of the ruminant microbiome (Duffy et al., 2018). These changes in the microbiome are postulated to change VFA production. Therefore, we hypothesize that heat stress, β-AA, and the associated interaction decrease the total level of VFA produced.

MATERIALS AND METHODS

Animals and Experimental Design

This study was approved by the institutional animal care and use committee at the University of Nebraska–Lincoln (Project 1300), an AAALAC International-accredited institution. Crossbred Suffolk × Rambouillet lambs averaging ~10 months of age were stratified by body weight and divided into two replicates (replication one =

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39.99 ±1.92 kg; replication two = 37.35 ± 1.92 kg) to accommodate limitations of the thermal chambers (12 stalls). After an acclimation period, all lambs were individually penned and fed identical high-energy diets for 21 d under either thermoneutral (TN; 25 °C, 15% RH) or HS (40 °C, 35% RH) conditions. Each lamb received one of three dietary supplements: no supplement, ractopamine hydrochloride (RH; 0.03996 g/hd/d), or zilpaterol hydrochloride (ZH; 0.025 g/hd/d), delivered in 200 g ground corn added to the ration. Each treatment (environment × supplement) contained eight lambs with the exception of an additional TN lamb fed no supplement. On d 22 lambs were harvested. Contents of the rumen were collected immediately after harvest, flash frozen in liquid nitrogen, and stored at −80 °C until further analysis.

Volatile Fatty Acid Extraction

Rumen fluid and contents collected from each lamb were analyzed for VFA concentrations according to Erwin et al. (1961), with the following modifications. Five grams of each sample was weighed into a flask and 15 ml of 0.5 N H_2SO_4 was added. The flasks were incubated overnight at 4 °C, after which the protocol was followed as suggested. A standard was prepared containing known amounts of VFA, and 2.0 ml of this solution was also combined with 0.5 ml of 25% metaphosphoric acid and 25-mM 2-ethylbutyrate solution. Samples and standards were refrigerated for 30 min and then centrifuged at 10,000 × *g* for 15 min. The supernatant was filtered through a 25-mm Whatman (GE Healthcare Life Sciences, Pittsburgh, PA) syringe filter using a 3-ml BD (Becton, Dickinson and Company, Franklin Lakes, NJ) tuberculin syringe into a 2-ml vial. The samples were analyzed for VFA using a Thermo Scientific Trace 1300 (Thermo Fisher Scientific, Waltham, MA) gas chromatographer.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The model for measurement of VFA included the effects of supplement, environment, replicate, and the interaction between diet and environment. Differences were declared significant at $P < 0.05$, and tendencies noted at $P < 0.10$. If significant differences were determined, Tukey's Honest Significant Difference test was used to evaluate pairwise comparisons for significance. Data are presented as means ± SEM.

RESULTS

Owing to low detection of VFA, seven animals were removed leaving six to eight lambs per treatment for analysis. Replicate had no impact of VFA production ($P > 0.05$) and was therefore removed from analyses. The ratio of acetate, propionate, and butyrate remained constant among experimental groups, with an average of 75:15:10. Total VFA concentrations did not differ ($P = 0.65$) between TN and HS lambs (11.88 ± 1.12 mM and 12.6 ± 1.12 mM, respectively). Total VFA concentrations were decreased ($P < 0.05$) in RH-fed (10.0 ± 1.37 mM) and ZH-fed (11.6 ± 1.42 mM) compared to unsupplemented lambs (15.12 ± 1.32 mM). RH-fed lambs had reduced ($P = 0.05$) isobutyrate compared to nonsupplemented lambs.

A significant supplement–environment interaction ($P < 0.05$) was found for acetate, propionate, and total VFA production. RH-fed HS lambs produced less ($P < 0.05$) acetate than unsupplemented HS lambs (Figure 1A). Propionate production was less ($P < 0.05$) in HS lambs fed RH or ZH and in unsupplemented TN lambs compared to unsupplemented HS lambs (Figure 1B). In addition, unsupplemented TN lambs produced less propionate than unsupplemented HS lambs ($P = 0.02$). Finally, HS lambs fed RH or ZH produced less ($P < 0.05$) total VFA than unsupplemented HS lambs, and TN lambs fed RH but not ZH produced less ($P < 0.05$) total VFA than unsupplemented TN lambs (Figure 1G). There was no difference in total VFA production between unsupplemented TN and unsupplemented HS lambs.

DISCUSSION

Owing to VFA concentrations being determined in ruminal content diluted in H_2SO_4 rather than directly from rumen fluid, VFA production was lower but proportionate to other study findings. Previous reports (Weldy et al., 1964; Kelley et al., 1967; Freestone and Lyte, 2010) have shown HS reduces VFA levels while our study revealed a significant interaction between environment and supplement. Control lambs in HS tended to have a higher level of VFAs than the others. HS events may affect rumen functionality by altering blood flow to the rumen (Crandall et al., 2008), which may consequently affect rumen epithelium and its ability to absorb nutrients (Storm et al., 2012). Therefore, we hypothesize that animals fed the control diet in the HS environment were not producing more totals VFAs but rather the environment altered the ability of the rumen to uptake VFAs

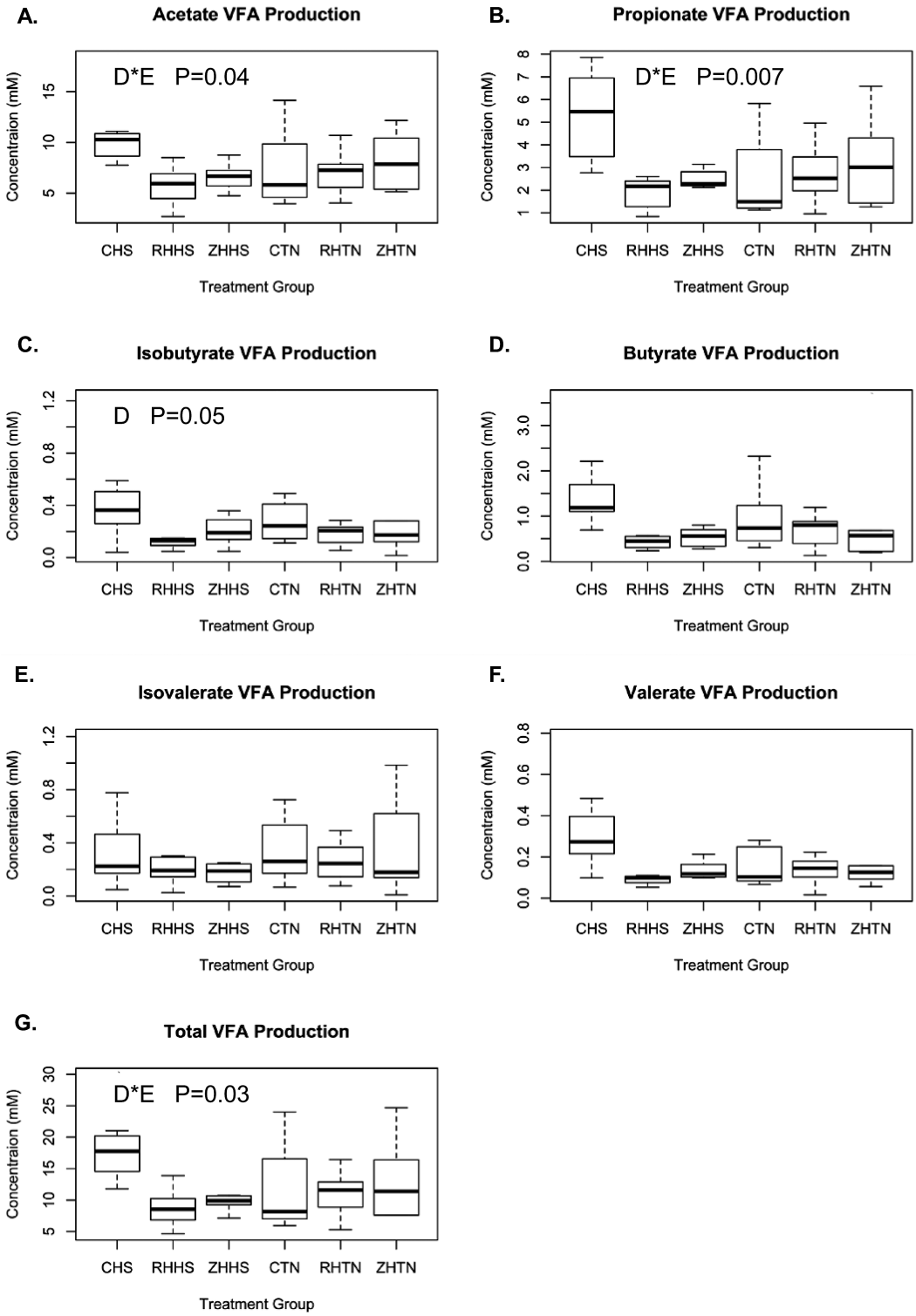

Figure 1. Production of (A) acetate, (B) propionate, (C) isobutyrate, (D) butyrate, (E) isovalerate, (F) valerate, and (G) total VFA in lambs subjected to one of six treatments in a 3 × 2 factorial of diet: no supplement (C), ractopamine HCl (RH), zilpaterol HCl (ZH), and environment: heat stress (HS), thermoneutral (TN). *P*-values are shown for the effects of diet (D), environment (E) or the interaction (D*E) when significant.

produced, leaving them unabsorbed. Reduced absorption of VFAs leads to reduced energy uptake by the animal which can reduce overall efficiency impacting livestock production systems (Bergman, 1990; Doreau et al., 1997).

Changes in VFA attributed to HS have been hypothesized to be caused by changes in the bacterial composition in response to HS itself or due to decreased feed intake and rumination, in turn decreasing buffering agent entering the rumen

(Hyder et al., 2017). In addition, temperature and humidity have a notable effect on the rumen microbial composition which may lead to changes in VFA production (Tajima et al., 2007). A significantly different microbial composition was present in these lambs comparing HS to TN conditions (Duffy et al., 2018), therefore we expected to see a clear difference in VFA as well. However, these results suggest that the response due to the interaction between environment and supplementation is more complex. Our results could be attributed to a change in the metabolic properties of microbial fermentation. By the microbes changing their metabolic function they were able to combat HS and maintain adequate ruminal fermentation.

A study observing the effect of ZH on ruminal fermentation in finishing steers showed no impact of the supplement on VFA production (Romero et al., 2009). ZH, however, increases muscle glucose oxidation (Barnes et al., 2017; Cadaret et al., 2017), suggesting its mode of action is elsewhere rather than in the gastrointestinal tract. There was a significant interaction in the production of propionate and total VFA in ZH-fed lambs in the HS environment, suggesting that ZH together with HS cause a negative impact on propionate and total VFA. In addition, the interaction between environment and RH supplement for production of acetate and propionate and the effect of RH on total VFA production was results were similar to Walker et al. (2007) who attributed decreased VFA production to RH. These results suggest that the interaction between supplementation with β-AA and the environment fails to support efficient ruminal fermentation, which can decrease animal performance. Changes in ruminal fermentation may be caused by poor ruminal contractions which can cause feed to pass through the rumen quickly, not allowing for microbes to come into contact with feedstuffs. Conversely, the decreased amount of VFA in ruminal contents attributed to the interaction of environment and supplement in this study may cause VFAs to be metabolized and absorbed more rapidly, leaving less unabsorbed in the contents collected.

The observed change in total VFA is contrary to Walker and Drouillard (2010) in which RH added directly to buffered ruminal fluid had no impact on VFA levels, showing that RH does not affect microbial growth or fermentation end products when grown in pure cultures. RH supplementation did not change bacterial composition in these sheep (Duffy et al., 2018); however, RH and the interaction of RH with environment impacted production of VFAs. This implicates another mechanism, besides the change in the bacterial community itself in altered VFA production. The relative reduction in total VFAs when fed β-AA may therefore be suggestive of decreased microbial activity, or, conversely, that the microbial population is using the VFAs as an energy substrate, not allowing for normal amounts to be released. In addition, the reduction in VFA production in this study suggests that production depends on the overall anatomy and function of the rumen in vivo which would explain why in vitro studies saw no reduction in the production of VFA.

IMPLICATIONS

Improving animal efficiency and decreasing loss are major concerns for livestock producers, therefore any reliable and economical means to do so will be readily implemented by the industry. This study showed that β-AA fed during HS have a negative impact on VFA production, resulting in a negative impact on nutrient uptake and utilization in the animals. Ongoing work is underway to compare transcriptional changes of the host rumen across treatments as well as changes to observed microbial communities to better understand how overall changes to the rumen affect animals in HS environments, fed β-AA, or both.

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