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Erin M. Duffy

Shauna M. Tietze

Allison L. Knoell

Nirosh D. Aluthge

Samodha C. Fernando

See next page for additional authors

Authors

Erin M. Duffy, Shauna M. Tietze, Allison L. Knoell, Nirosh D. Aluthge, Samodha C. Fernando, Ty B. Schmidt, Dustin T. Yates, and Jessica L. Petersen

Rumen bacterial composition in lambs is affected by β-adrenergic agonist supplementation and heat stress at the phylum level[1]

Erin M. Duffy,[2] Shauna M. Tietze, Allison L. Knoell, Nirosh D. Aluthge, Samodha C. Fernando, Ty S. Schmidt, Dustin T. Yates, and Jessica L. Petersen,[2]

Department of Animal Science, University of Nebraska–Lincoln, NE 68583-0908

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INTRODUCTION

The rumen has several important physiological functions, including absorption, transport, metabolic activity, and host protection (Roh et al., 2007). The rumen is extensively researched due to the importance of ruminants in agriculture and the major role played by rumen microbes in nutrient utilization and health of the ruminant animal. The microbial community of the rumen is altered by diet (McCann et al., 2014), age (Jami et al., 2013), and environment.

Heat stress is an important environmental stressor that can reduce efficiency in ruminant livestock. Alternatively, β-adrenergic agonists (**β-AA**) increase value due to increased efficiency in muscle growth (Buntyn et al., 2016). Currently, there are two β-AA approved for use in livestock in the United States: zilpaterol HCl (**ZHCl**), a β2 agonist, and ractopamine HCl (**RHCl**), a β1 agonist (Delmore et al., 2010; Boler et al., 2012). The β2 agonist increases muscle glucose oxidation (Barnes et al. 2017; Cadaret et al. 2017). However, the mechanism behind β1 action is poorly understood, and there is a need to understand the impact of β-AA fed during heat stress.

With the profound role of the rumen microbiome in nutrient utilization and animal health, it is possible that heat stress events can impact the microbiota by decreasing metabolic activity and fermentation, thus decreasing heat produced and energy provided to the animal. Additionally, β-AA may influence the microbiome to increase nutrient digestion, providing more energy to the animal in the form of volatile fatty acids. However, the influence of concurrent heat stress and β-AA supplementation on the rumen microbiome has not been investigated. Therefore, the objective of this study was to determine the impact that β1 agonists, β2 agonists, and heat stress have on the rumen bacterial community.

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. Columbia–Suffolk crossbred lambs were purchased commercially. After a 3-wk acclimation, individually penned lambs were fed identical high-energy diets for 21 d and housed under either thermoneutral (25 °C, 15% relative humidity) or heat stress (40 °C,

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[2]Corresponding author: jessica.petersen@unl.edu

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35% relative humidity) conditions. Each lamb received one of three dietary supplements: no supplement, RHCl (0.03996 g/hd/d), or ZHCl (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 lamb in the thermoneutral group fed the control diet. On day 22, lambs were harvested. Contents of the rumen were collected immediately after harvest and flash frozen in liquid nitrogen until further analysis.

DNA Isolation

DNA was isolated from the rumen contents of all lambs in the study using the Mobio PowerSoil Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). The V4 region of the 16S rDNA gene specific to eubacterial communities was then amplified using the Terra PCR Direct Polymerase Mix Kit (Takara Bio USA) and 515F and 806R primers (Illumina; Kozich et al., 2013). PCR conditions were as follows: 3 min at 98 °C for initial denaturation, 25 cycles of 30 s at 98 °C, 30 s at 55 °C, and 45 s at 68 °C; the profile was terminated after a final 4-min hold at 68 °C. Amplification and product size were verified by agarose gel electrophoresis. PCR products were normalized to 2 ng/μL using the Invitrogen SequalPrep Normalization Plate kit (Fredrick, MD), and libraries were prepped, normalized, and pooled (Eppendorf epMotiom; M5073, Germany). The pooled libraries were purified as described in the NucleoSpin Gel and PCR Clean-Up kit (Clontech Laboratories Inc., CA), and size selected (380 bp) using the Pippin Prep (Sage Science, Inc.). Finally, the quality and quantity of purified products were verified using Agilent 2100 Bioanalyzer high sensitivity DNA chips (Agilent Technologies). Resulting amplicons were sequenced using the Illumina MiSeq platform using a V2 500 cycle kit with the dual-index sequencing strategy according to Kozich et al. (2013).

Microbial Community Analysis

Raw sequence reads were quality filtered as described by Anderson et al. (2016). In Quantitative Insights Into Microbial Ecology (QIIME) (ver.19.1), sequences with one or more errors in the barcode were removed (Caporaso et al., 2010). The paired-end reads were processed in Mothur (Schloss et al., 2009) to improve operational taxonomic unit (**OTU**) classification (Edgar, 2013). After reverse complementation in Mothur, sequences were processed using a custom pipeline (Fernando Lab, UNL), chimera identification and removal, and OTUs selected based on 97% sequence similarity (Paz et al., 2018). Target OTU sequences generated previously were aligned, and OTUs aligning outside the 16s gene eliminated. Taxonomic classification was performed using QIIME via GreenGenes database (ver.13_8).

An overall (total) and core measurable microbiome were determined based on supplementation and environment. The core measurable microbiome was defined as OTUs present in at least 50% of the samples in each subset.

Statistical Analysis

Sequence reads were analyzed using bioinformatics pipelines from UPARSE (Edgar et al., 2011), QIIME, and Mothur. Reads per sample were rarefied across samples, and a Good's coverage test was performed. Alpha diversity estimators Chao1 and observed OTUs were calculated for the overall rumen microbiome in QIIME. To select taxa that were significantly different in abundance between supplementation and environment, the bioinformatics tool linear discriminate analysis effect size (**LEfSe**) (Segata et al., 2011) was used with default parameters of $P \leq 0.05$ and a linear discriminant analysis (LDA) score ≥ 2.

RESULTS

Rumen Bacterial Richness and Bacterial Composition

One of the 49 samples was removed due to insufficient reads (594). Rarefaction to the lowest depth (7,000 reads) resulted in 1,565 OTUs. Rarefaction curves showed a diminishing rate of OTU identification at ~6,000 sequences per sample, and the Good's coverage test showed all samples were able to characterize ~98% of the bacterial community.

Based on taxonomic analysis of the reads, regardless of environment and supplementation, *Bacteroidetes* and *Firmicutes* were the predominant phyla within the core microbiome accounting for 48.5% and 33.1% of the total reads, respectively (Figure 1).

Bacterial Community Composition Associated with Environment

Between the thermoneutral and heat stress groups fed the control diet, three phyla were

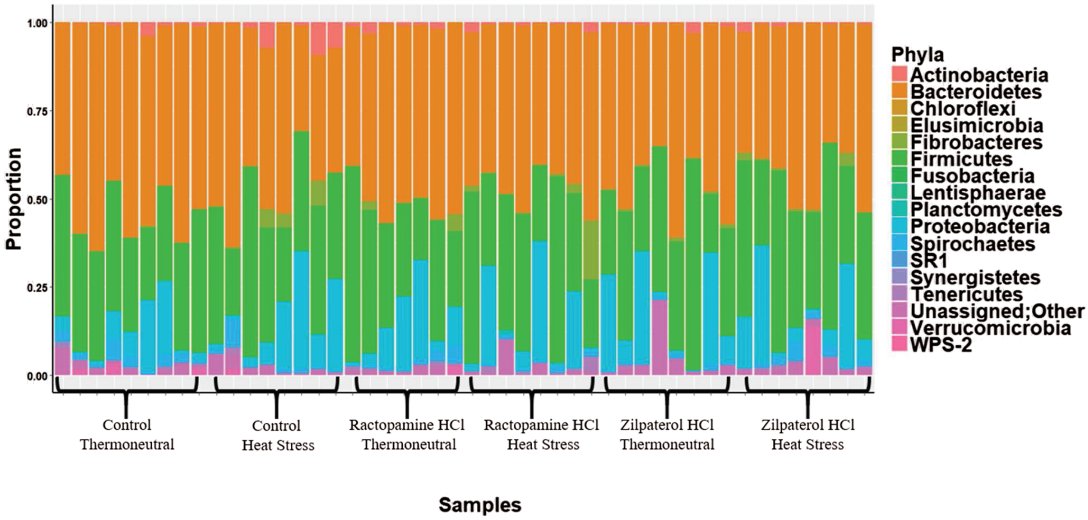

Figure 1. Phylum level classification of the bacterial community composition depending on environment and supplementation.

differentially ($P \leq 0.05$) abundant; *Bacteroidetes* were more abundant ($P \leq 0.05$) in the thermoneutral lambs, while *Fibrobacters* and *Actinobacteria* were more abundant ($P \leq 0.05$) in heat-stressed lambs. Additionally, among lambs supplemented with RHCl, *Elusibacteria* were more abundant ($P \leq 0.05$) in heat stress conditions than thermoneutral conditions. No differences between environmental conditions were observed in lambs fed ZHCl.

Bacterial Community Composition Associated with Supplementation

Among thermoneutral lambs, *Fibrobacteres* and *Bacteroidetes* differed ($P \leq 0.05$), with *Bacteroidetes* more abundant ($P \leq 0.05$) in lambs fed the control diet and *Fibrobacters* more abundant ($P \leq 0.05$) in lambs fed ZHCl. Also, among thermoneutral lambs, the abundance of *Bacteroidetes* was higher ($P \leq 0.05$) in the nonsupplemented lambs compared with those fed RHCl.

DISCUSSION

The general characterization of the core microbial communities from this study shows the overall microbial composition was similar to those seen previously (Petri et al. 2013; Paz et al. 2016). Additionally, diet appeared to have a lesser role in determining the microbial community composition than heat stress. The thresholds for making changes in community compositions are currently unknown, but temperature and β-AA supplementation may act directly or indirectly via changes in the physiology of animals in response to heat stress conditions and diet.

At the phylum level, the microbial communities differed by treatment. Additionally, *Bacteroidetes* was more abundant in the thermoneutral group fed the control diet compared with those supplemented with β-AA. This is different than Tajima et al. (2007), who observed no obvious effects of heat stress on rumen composition at the phylum level. Our findings indicate that supplementation with β-AA works within the rumen to create an environment that alters the relative competitive advantage of other phyla. Although this study produced significant changes at the phylum level of classification, more research must be done at the family level to determine discrete differences in rumen microbial composition.

IMPLICATIONS

In this study, we show that β-AA supplementation and heat stress affect the ruminal microbiome of wethers. Despite these changes, the two primary phyla *Bacteroidetes* and *Firmicutes* remained the same. Further examination is necessary to identify the overall impact these changes have on the animal and how this information could be utilized to improve production efficiency and animal health. Ongoing work will compare transcriptional changes of the host rumen across treatments as well as to the observed microbial communities.

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