Alterations in the fecal microbiome of lactating dairy cows during experimentally induced heat stress

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Introduction

Heat stress costs the dairy industry over $1.2 billion annually as a result of decreased milk production. Mastitis incidence is higher during the warmer months of the year, presumably due to a seasonal increase in Gram-negative organisms in the bedding of dairy cows. It is currently unknown if the increased environmental load of Gramnegative organisms during warmer months is associated with cows shedding more of these organisms in their feces, which has been documented in laying hens. Our objective was to evaluate the effects of heat stress on the fecal microbiome of lactating dairy cows. We hypothesized there would be a decrease in the relative abundance of Firmicutes and an increase in the relative abundance of Bacteroidetes in the feces of dairy cows during heat stress; specifically, an increase in Gram-negative environmental mastitis pathogens.

Materials and Methods

Six Holstein cows (averaging 175 days in milk, 1.5 parities and 36.3 kg/d of milk) were housed in tie stalls in an environmental chamber, milked twice-daily and provided feed and water ad libitum. Cows were allowed 5d acclimation to the chambers (d-5 to 0) with Temperature Humidity Index (THI) set at ~65 and were then subjected to constant heat stress for 16d (d0 to 16; THI~76), followed by a 9d recovery period (d16 to 24; THI~65). Fecal samples were collected per rectum on d-1, 0, 6, 13, 16, 20 and 24. Samples were immediately frozen at -20°C. Fecal DNA was extracted using PowerFecal kits (Qiagen), the V4 hypervariable region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform, and operational taxonomic units (OTUs) were assigned to the SILVA database using BLAST based on a 97% nucleotide identity, to evaluate richness and composition of fecal bacterial populations. To evaluate pathogen profiles over time, an ANOVA with time as the repeated measure was used. The specific environmental pathogens that were profiled included: *Klebsiella*, *Enterobacter*, *Escherichia/Shigella*, *Enterococcus*, and *Streptococcus*.

Results

The relative abundance of the Firmicutes or Bacteroidetes, or their ratio, did not differ between the acclimation and heat stress periods (p>0.01). There were more Firmicutes during heat stress on d6 and 13 than at d24 (p<0.001). There were more Bacteroidetes in the recovery period compared to d-1, 6, and 13 (p<0.001).The ratio of Firmicutes to Bacteroidetes was higher during d6 and 13 compared to the recovery period (p<0.001). There were no *Klebsiella* or *Enterobacter* identified. There was no difference in the relative abundance of *Escherichia/Shigella* between the acclimation and heat stress periods (p>0.05). There were fewer *Escherichia/Shigella* in the recovery period compared to d0 and d13 (p<0.05). There was no difference in the relative abundance of *Enterococcus* (p>0.05). The relative abundance of *Streptococcus* was higher on d13 compared to d0 and d20 (p<0.05). The relative abundance of *Streptococcus* did not differ between the acclimation and recovery periods.

Significance

Contrary to our hypothesis, Firmicutes were more prevalent during heat stress. The prevalence of Bacteroidetes was greater during the recovery period than during heat stress, perhaps due to a delay in the effect of heat stress on the fecal microbiome. Evaluating the changes during extended periods of heat stress may be informative. The ratio of Firmicutes to Bacteroidetes was higher during heat stress compared to the recovery period but there was no difference between acclimation and heat stress or recovery. Most of the environmental pathogens were not more abundant during heat stress, however the prevalence of *Streptococcus* did increase with heat stress. We conclude that the relative abundance of some environmental pathogens that commonly cause mastitis were altered in the feces of heat stressed dairy cows.