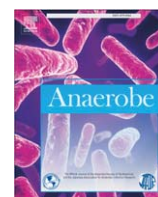




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An rRNA-based analysis for evaluating the effect of heat stress on the rumen microbial composition of Holstein heifers

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ABSTRACT

We performed a set of heifer feeding trials to investigate the effect of heat and humidity stresses on the rumen bacterial molecular diversity of Holstein heifers (Tajima K, Nonaka I, Higuchi K, Takusari N, Kurihara M, Takenaka A, *et al.* Anaerobe 2007;13:57–64). To further characterize the response of the microbial community to the physiological changes caused by the stresses, we evaluated changes in the ruminal bacterial community composition in the same trials by applying an RNA-based method (sequence-specific small-subunit (SSU) rRNA cleavage method), which was optimized for a comprehensive description of the predominant bacterial groups inhabiting the rumen. Four Holstein heifers were kept at three temperatures (20 °C, 28 °C, 33 °C) in a climatic chamber for two weeks each, and rumen fluid samples were obtained on the last day of each temperature experiment. For quantitative detection, we applied a set of 15 oligonucleotide probes, including those targeting taxa comprised of uncultured rumen bacteria (URB) belonging to phylum *Firmicutes*, to the RNAs extracted from the fluid samples. The relative populations of the *Clostridium coccooides*–*Eubacterium rectale* group, and the genus *Streptococcus* increased, and that of the genus *Fibrobacter* decreased in response to increasing temperature both in the first (nine months old, 80% relative humidity) and second (15 months old, 60% relative humidity) experiments. In addition, the population of a defined URB group was higher at 33 °C than at 20 °C in the second trial, whereas one of the other URB groups showed a decreasing trend with the temperature rise. These results indicate that the exposure to heat affects the population levels of specific bacterial groups in the ruminal microbial community.

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1. Introduction

Ruminant animals are recognized to be among the least tolerant to heat stress, which is caused by a combination of environmental factors (ambient temperatures, relative humidity, solar radiation, air movement) [1,2]. The physiological responses of cattle to heat stress have been well described [3,4], and include increased body temperature [5], increased respiratory rate [6], decreased feed intake [5,7], and increased water intake [8]. These responses have detrimental effects on milk production, reproduction, and health in lactating dairy cows [9–11]. Although a number of studies have

been conducted to characterize and alleviate the effects, little information has been available for rumen microbial community, which plays a critical role in the digestion of ingesta in the rumen, in relation to these physiological changes caused by heat stress.

We previously assessed the effect of temperature and humidity on the rumen bacterial diversity of Holstein heifers [12]. In the feeding trial, the ruminal microbial composition, determined by 16S rRNA gene cloning, was significantly changed at elevated environmental temperatures and humidity, along with decreases in dry matter intake, body weight gain and an increase in the digestibility of the feed with rising temperature. However, since the previous experiment lacked quantitative data on the respective bacterial groups, it is still uncertain how the microbial composition changed along with the change in the environmental conditions. We therefore aimed to further investigate how the rumen microbial community responds to heat-stress. For this purpose, we monitored the change in the ruminal bacterial community at the group

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