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Characterization of the rumen microbial community composition of buffalo breeds consuming diets typical of dairy production systems in Southern China

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ABSTRACT

Murrah and Nili-Ravi are water buffalo breeds widely used as dairy animals in Asian countries. In this study, we investigated the diversity of ruminal microbes in six Murrah and six Nili-Ravi water buffaloes consuming diets typical of those used in Southern China which had forage to concentrate ratios of 3.2 and 1.6. After feeding the diets for 25 days, ruminal fluid was sampled by stomach tube before the morning feeding. Bacterial and archaeal 16S rRNA genes and the ciliate protozoal 18S rRNA genes were PCR-amplified from DNA extracted from rumen samples, sequenced using 454 Titanium pyrosequencing, and analyzed using the QIIME software package. Our results showed that, at the phylum level, Bacteroidetes was the predominant bacterial group, accounting for 42–72% of total bacteria, followed by Firmicutes, Fibrobacteres, Proteobacteria and Spirochaetes. At genus level, *Prevotella* dominated, accounting for 22–58% of total bacteria, followed by *Fibrobacter*, *Paludibacter*, and *Ruminococcus*. While there were differences between the bacterial community compositions of different animals, there was no obvious correlation of bacterial community composition at the phylum or genus level with the diets or with buffalo breeds. *Methanobrevibacter*-related organisms were the dominant archaeal group, accounting for around 80% of the total, followed by Methanomassiliicoccales (Rumen Cluster C (RCC), 15%) and *Methanosphaera* (3%). Similar to the bacterial community, there was no obvious correlation of archaeal community profiles with diet or buffalo breed. The ciliate protozoal communities differed between the samples analyzed, and *Entodinium* was the most abundant group of ciliates in every sample, accounting for more than 40% of total protozoa. The second largest ciliate group varied in different samples, with *Isotricha*, *Polyplastron* or *Dasytricha* being the most dominant genera after *Entodinium*.

Abbreviations: DM, dry matter; HC, high concentrate diet; LC, low concentrate diet; MLC, Murrah buffaloes fed low concentrate diet; MHC, Murrah buffaloes fed high concentrate diet; NLC, Nili-Ravi buffaloes fed low concentrate diet; NHC, Nili-Ravi buffaloes fed high concentrate diet; OTUs, operational taxonomic units; RCC, rumen cluster C or Methanomassiliicoccales; VFAs, volatile fatty acids.

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1. Introduction

Water buffaloes (*Bubalus bubalis*) are an important livestock species for milk and meat production worldwide, and are second in milk production only to dairy cows. Buffaloes are adapted to hot climates and high roughage feeding, and consequently they are often farmed in tropical and subtropical regions. In fact, about 90% of the world's buffaloes are found in Asian countries, mainly in subtropical areas (FAO, 2007). There are large physiological, genetic and behavioral differences between buffaloes and dairy cows, which make them an interesting ruminant species for comparative studies. Early nutritional investigations on buffaloes showed that they ingest about the same amount as cattle on a dry matter basis, but they spend around 50% more time ruminating, which results in a larger pool of fine feed particles and a 30% lower mean residence time of particulate matter in the rumen (McSweeney et al., 1989). Other studies have shown that buffaloes digest fibrous feeds more efficiently (Norton et al., 1979), resulting in higher digestibility of organic matter compared to cattle (Franzolin, 1994; Calabrò et al., 2008). Therefore, digestion of fibre in the rumen of buffaloes has attracted some attention, prompting an interest in understanding fibre-degrading microbes from the buffalo rumen, the enzymatic activities involved in lignocellulose breakdown, and the consequences for rumen methanogens and methane production.

The number of studies describing the composition of the rumen microbial communities in buffaloes is small compared with those from other ruminant species such as sheep and cattle. Most of the studies on buffaloes report the occurrence of well-known rumen bacteria and archaea and show strong similarities between rumen microbial profiles of buffaloes and other ruminants (Imai et al., 1995; Calabrò et al., 2005; Wanapat and Cherdthong, 2009; Foiklang et al., 2011; Huws et al., 2012; Lwin et al., 2012). A study of Mediterranean buffaloes on a variety of diets showed that the archaeal populations in the rumen were dominated by sequences associated with *Methanobrevibacter* species, with smaller numbers of sequences associated with *Methanosphaera* and *Methanobacterium* species (Franzolin et al., 2012), typical of methanogen communities found in other ruminants. However, there are several reports from India and Pakistan that indicate that the archaeal communities of Surti and Murrah buffaloes are different, being dominated by members of the genus *Methanomicrobium* (Chaudhary and Sirohi, 2009; Chaudhary et al., 2011; Singh et al., 2011a, 2012). The reasons for these differences are not clear, but diet, buffalo breed or the primers used to amplify methanogen 16S RNA genes may be contributing factors.

In China, native water buffalo have traditionally been used as draught animals, but in recent times there has been a strong drive to develop a dairy buffalo industry, based on imported breeds such as the Murrah and Nili-Ravi. The buffaloes are fed on pastures and by-products of crops and supplemented with some concentrates (Yang et al., 2007). In the southern regions, they are typically fed on fresh or conserved fodder crops, such as corn or cassava and they are also offered varying amounts of grains or waste products from processed plant material. Therefore, depending on the availability of feeds, dairy buffaloes can experience a variety of diets, often varying widely in forage:concentrate ratio, and quite unlike the fibrous diets that wild buffaloes encounter naturally. Comparing the rumen microbial communities of these dairy production buffaloes with those of better studied bovine species, and examining how these communities are affected by diet, is being studied to give a better understanding of the ruminal processes that underpin their unusual utilization of forage material and the rumen conditions that select for different methanogen communities. In the study reported here, Murrah and Nili-Ravi buffalo were fed on two diets of contrasting forage:concentrate ratio to represent the range of diets typically encountered by dairy buffaloes in this region. Rumen content samples were collected and analyzed via barcoded pyrosequencing to define their rumen microbial community composition to allow comparison with previously published data.

2. Materials and methods

2.1. Animals, feed and management

The buffalo feeding experiment was conducted at the buffalo farm of the Buffalo Research Institute, the Chinese Academy of Agricultural Sciences, in Nanning, Guangxi, China, from 1 December 2012 to 10 January 2013. High and low concentrate diets were used to simulate the typical range of feeding that buffaloes experience when farmed in Southern China, and the diet nutritional composition is shown in Table 1. The following AOAC methods (AOAC, 1997) were used to determine diet composition: dry matter, 930.15; crude protein, 928.08; neutral detergent fiber and acid detergent fiber, 973.18. Twelve Murrah and twelve Nili-Ravi buffaloes were separated into high concentrate (HC) and low concentrate (LC) groups, with six of each breed per diet group. The concentrate and forage was offered to buffaloes separately. The high concentrate group was fed 5 kg dry matter (DM)/head/day of concentrate, while the low concentrate group was fed 3 kg DM/head/day of concentrate. The forage portion of the diet was given *ad-libitum*. All the buffaloes were fed in a tie-stall barn and were fed twice a day, at 7:00 am and 4:00 pm. The buffaloes were given the experimental diets for 25 days, during the last 15 days of the experimental period, forage and concentrate feed residues were measured every 2 days to calculate the feed intake. At the end of the feeding period, 3 Murrah and 3 Nili-Ravi with similar body weight and feed intake in each diet group were selected for rumen contents sampling. Rumen contents samples were collected from the animals using stomach tubing after 25 days of receiving the diets.