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Short Communication

Niacin alters the ruminal microbial composition of cattle under high-concentrate condition

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ABSTRACT

To understand the effects of niacin on the ruminal microbial ecology of cattle under high-concentrate diet condition, Illumina MiSeq sequencing technology was used. Three cattle with rumen cannula were used in a 3 × 3 Latin-square design trial. Three diets were fed to these cattle during 3 periods for 3 days, respectively: high-forage diet (HF; forage-to-concentrate ratio = 80:20), high-concentrate diet (HC; forage-to-concentrate ratio = 20:80), and HC supplemented with 800 mg/kg niacin (HCN). Ruminal pH was measured before feeding and every 2 h after initiating feeding. Ruminal fluid was sampled at the end of each period for microbial DNA extraction. Overall, our findings revealed that subacute ruminal acidosis (SARA) was induced and the α-diversity of ruminal bacterial community decreased in the cattle of HC group. Adding niacin in HC could relieve the symptoms of SARA in the cattle but the ruminal pH value and the Shannon index of ruminal bacterial community of HCN group were still lower than those of HF group. Whatever the diet was, the ruminal bacterial community of cattle was dominated by *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. High-concentrate diet significantly increased the abundance of *Prevotella*, and decreased the abundance of *Paraprevotella*, *Sporobacter*, *Ruminococcus* and *Treponema* than HF. Compared with HC, HCN had a trend to decrease the percentage of *Prevotella*, and to increase the abundance of *Succiniclasticum*, *Acetivibrio* and *Treponema*. Increasing concentrate ratio could decrease ruminal pH value, and change the ruminal microbial composition. Adding niacin in HC could increase the ruminal pH value, alter the ruminal microbial composition.

1. Introduction

The health and high production performance of ruminants rely on equilibrium of ruminal microbial ecosystem (Welkie et al., 2010; Kim et al., 2011). The diet is one of the most critical influences on the ruminal microbes (Larue et al., 2005). In recent years, concentrate feed was widely served as an energy supplement in finishing cattle and high yield dairy cow production. However, the ruminal environment should change with the increase of the concentrate ratio resulting in the change of ruminal bacterial community (Zened et al., 2013; Petri et al., 2013). And in turn the changing ruminal bacterial should lead to a metabolic disturbance and cause the ruminal environment to change. Han et al. (2011) reported as the dietary non-fiber carbohydrate:neutral detergent fiber ratio increased, the ruminal pH decreased and the rumen starch decomposing bacteria, *Lactobacillus*, *Megasphaera elsdenii* and *Selenomonas ruminantium* number tended to increase, and the lactic acid bacteria proliferated particularly. Tajima et al. (2001) observed an increase in the number of *Prevotella* in the rumen of lactating cow during subacute ruminal acidosis (SARA). Similarly, Khafipour et al. (2009) found the proportion of *Bacteroidetes* decreased in the rumen as a result of SARA in dairy cattle.

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The B-vitamin niacin function as a component of the coenzyme NAD(H) and NADP(H), which plays a critical role in the metabolism of carbohydrate, lipids and protein. It was reported that niacin supplementation could improve the production performance of high-performing ruminants (Shields et al., 1983; Drackley et al., 1998), particularly with high-concentrate diet (HC) relying on regulating the ruminal lactic acid metabolism and stabilizing the ruminal pH (Zhang et al., 2014). Besides, niacin could promote the growth of ruminal microbes, maintain the stability of the microbial community, and avoid the accumulation of lactic acid in rumen (Doreau and Ottou, 1996; Yang et al., 2013). In our earlier works, 800 mg/kg niacin (about 6 g/d) supplementation in HC could inhibit the proliferation of Bovine Streptococcus, a main lactic acid-producing bacterium (Zhang et al., 2014). It was also reported an increase in total protozoa (Wang et al., 2002; Doreau and Ottou, 1996), especially the number of *Entodinia* in ruminal fluid due to niacin feeding (Niehoff et al., 2009). To understand more functions of niacin on the ruminant production, this study was conducted to assess the effects of HC supplemented with or without niacin on the bacterial community in the rumen of cattle, using Illumina MiSeq sequencing.

2. Material and methods

2.1. Animals and experimental design

Three ruminal cannulated *Jinjiang* cattle (a native breed of Jiangxi Province in southern China, 400 ± 20 kg body weight) were used in this study. The cattle were used to high-forage diet (HF) with free range model in the past time. Now they were fed higher ratio of concentrate in the diet for the potential for producing higher-quality beef (Zhang and Yuan, 2012).

Cattle were assigned to 3 treatments: HF (forage-to-concentrate ratio = 80:20), HC (forage-to-concentrate ratio = 20:80), and HCN (HC supplemented with 800 mg/kg niacin) in a 3 × 3 Latin-square design. We used straw as forage, and the composition of the concentrate is listed in Table 1. Diets were formulated according to the China Feeding Standard of Beef Cattle (NY/T815-2004). The niacin used in this study was produced by Tianjin Zhongrui Pharmaceutical Co, Ltd., China (Assay ≥ 99%). Cattle were kept in individual stalls. They had free access to water and received 10 kg of dry matter daily in 2 equal meals at 08:00 and 18:00.

Table 1
Ingredients and chemical composition of concentrate diets (DM basis).

Item	Content
Ingredients, %	
Corn	66.00
Wheat bran	17.00
Soybean meal	4.00
Cottonseed meal	10.00
NaCl	0.60
Limestone	0.80
$CaCO_3$	0.60
Pre-mix[1]	1.00
Chemical composition[2]	
Nemf, MJ/kg	6.32
Crude protein	13.87
Ca	0.47
P	0.30

Nemf = total net energy.
[1] Pre-mix provided per kilogram diet: 0.4 g Cu, 2.50 g Fe, 1.50 g Mn, 3.00 g Zn, 0.01 g Se, 0.05 mg I, 0.01 g Co, 25.00 g Mg, 300,000 IU of vitamin A, and 1,000 IU of vitamin E.
[2] The values of chemical composition were calculated.

The trial included 14 days of washout sub period during which all cattle received HF, and then 3 days of treatment period in which each cattle received 1 of the 3 experimental diets. The cattle were fed HC or HCN after fasting for 24 h which was designed by Goad et al. (1998) to enhance the stress of feed changing.

2.2. Sample collection

Ruminal pH was determined before feeding and every 2 h after initiating feeding. A ruminal fluid of 50 mL was sampled from each cattle at the end of trial (72 h after initiating feeding). Whole liquid fraction was collected from the ventral region of the rumen, and strained through the 200 μm^2 stainless steel membrane (collected). All samples were immediately stored at −80 °C until further analysis.

2.3. DNA extraction and MiSeq sequencing

Total genomic DNA of the samples was extracted by using the TIANamp Bacteria DNA Kit (TransGen Biotech, China) according to the manufacturer's instructions. DNA purity and concentration were analyzed by spectrophotometric quantification and NanoDrop ND-1000 spectrophotomer. The extracted quality qualified DNA were stored −20 °C for the follow-up testing. To analyze the taxonomic composition of the bacterial community, universal primers (515F 5′-GTGCCAGCMGCCGCGG-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′) targeting the V4 to V5 region of 16S rRNA gene were chosen for the amplification and subsequent pyrosequencing of the Polymerase Chain Reaction (PCR) products. The PCR amplification was performed in a 20 μL reaction system by using TransGen AP221-02: TransStart Fastpfu DNA Polymerase (TransGen Biotech, China). The amplification was implemented in an ABI GeneAmp 9700 (ABI, USA) under the following conditions: 95 °C for 2 min, 25 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min, 10 °C until halted by user. All PCR products were visualized on agarose gels (2% in TBE buffer) containing ethidium bromide, and purified by using AxyPrep DNA Gel Extraction Kit (AXYGEN, USA). The DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double stranded DNA assay (Promega, USA), Before pyrosequencing, we equally mixed the DNA samples, selected the DNA samples that meet the requirements and then stored the prepared DNA samples in the TE buffer, loaded a 1.5 mL nonstick tube, sealed with the parafilm, and made marks. We send the samples using dry ice using MiSeq on Illumina MiSeq PE250 to Major bio Bio-Pharm Technology Co., Ltd., Shanghai, China.

2.4. Taxonomical classification

After MiSeq sequencing, sequence reads were preprocessed according to the tags with no ambiguous base pairs. The primers were removed, and the sequences were trimmed to remove low quality sequences. After that, sets of sequences with greater than or equal to 97% identity were defined as operational taxonomic units (OTU). Operational taxonomic units were assigned to a taxonomy using the Ribosomal Database Project (RDP) Naive Bayes classifier. From these, the Chao, ACE and the Shannon index were calculated by mothur.

2.5. Statistical analysis

The relative abundance of bacteria in the rumen fluid was analyzed by ANOVA, using the General Linear Model of SPSS (version 17.0), according to the model shown below: