



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.

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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.

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 μm² 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 spectrophotometer. 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'-CCGCAATTCMTT-TRAGTTT-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:

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.

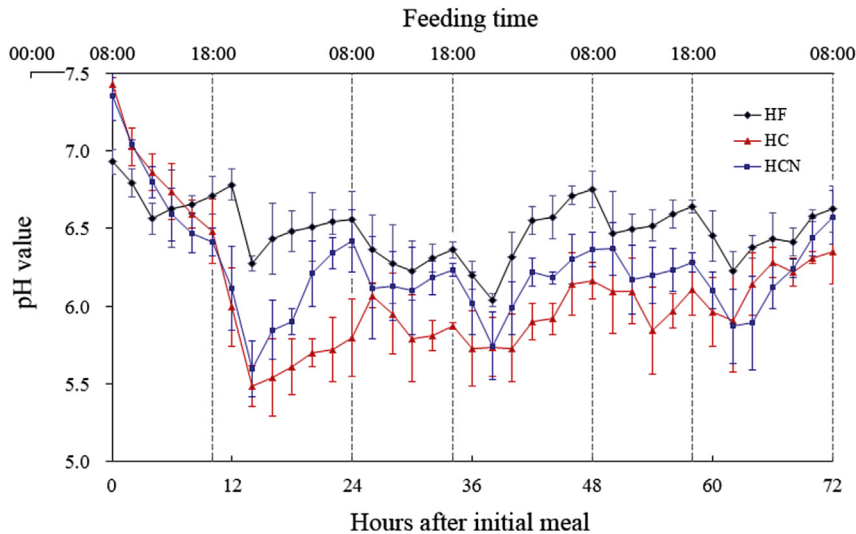


Fig. 1. Effects of diets on ruminal pH. HF = high-forage diet; HC = high-concentrate diet; HCN = HC + niacin diet.

$$Y = \mu + C + P + D + \varepsilon,$$

where Y is the observation for dependent variables, μ is the mean, C , P and D are the effects of cattle, period and dietary, respectively, and ε is the residual error. Very significant differences among treatments were declared at $P < 0.01$, significant differences at $P < 0.05$, and trends at $P < 0.1$.

3. Results

3.1. Effects of diets on feed intakes and ruminal pH

High-forage diet group consumed all of feed offered all the time. But for HC and HCN groups, the feed intakes were only 50% and 40% at the end of the trial, respectively. High-forage diet group had the highest ruminal pH in all groups stabilizing at 5.8 to 6.9 during the whole trial period. From 12 to 24 h, the ruminal pH of HC group below 5.8 lasted about 12 h and then recovered slowly. The ruminal pH of HCN group was the same as that of HC group in the first 14 h, but then it was higher than that of HC group after 14 h (Fig. 1). Supplementation of niacin shortened the duration time of lower pH.

3.2. Operational taxonomic units and α -diversity of ruminal bacterial community

After Miseq sequencing of 9 samples, 190,461 high-quality reads were used for downstream analysis and clustering of sequences yielded 998 OTU at 97% similarity. The mean Chao, ACE and Shannon index estimators of ruminal bacterial community were 807 ± 33 , 813 ± 29 and 4.61 ± 0.26 in Jinjiang cattle, respectively. There were no differences in the Chao and ACE index in all groups ($P > 0.1$) (Table 2). High-forage diet group had the highest OTU and Shannon index in all treatments. Increasing concentrate ratio in diet reduced the number of OTU ($P > 0.1$) and Shannon index ($P < 0.01$). Adding niacin in HC got much lower OTU ($P < 0.1$) and higher Shannon index ($P > 0.1$) than HC.

3.3. Ruminal bacterial composition

Within the bacteria population, 15 phyla, 23 classes, 26 families and 58 genera were observed across all samples of cattle.

Bacteroidetes was the predominant phylum with a relative abundance of 69.13%. *Firmicutes* was the second abundant phylum (18.78%), followed by *Proteobacteria* (6.73%) as shown in Fig. 2A. The 3 phyla represented nearly 95% of total sequences. At the class level (Fig. 2B), *Bacteroidetes* was dominated by *Bacteroidia* (58.16%) and *Sphingobacteria* (7.28%). *Clostridia* class (14.55%) made a significant contribution to the *Firmicutes* phylum, followed by *Negativicutes* (2.59%). Regarding *Proteobacteria* phylum, the major class was *Gamma proteobacteria* (5.79%).

Compared with HF group, HC group increased the percentage of *Bacteroidia* phylum ($P < 0.1$), and decreased the proportions of *Spirochaetes* ($P < 0.1$). There was no difference in the percentage of *Bacteroidia* phyla between HCN and HC groups ($P > 0.1$). But the relative abundance of *Proteobacteria* in HCN group ruminal fluid was 3.1 times higher than that in HC group ($P < 0.1$), and 26% higher than that in HF group. High-concentrate diet group also increased the percentage of *Bacteroidia* class ($P < 0.1$) and decreased the proportions of *Spirochaetes* class ($P < 0.1$). In HCN group, the percentage of *Clostridia* class was lower and *Negativicutes* class was higher than those in HF group ($P < 0.1$). Compared with HC, the addition of niacin increased the percentage of 2 classes encompassing *Negativicutes* and *Spirochaetes* ($P < 0.1$).

The diet had a number of effects on ruminal bacteria families (Table 3). Compared with HF, HC increased the percentage of *Prevotellaceae* ($P < 0.05$), and decreased the proportions of *Lachnospiraceae* ($P < 0.1$). In HCN group, the percentage of *Acidaminococcaceae* was higher ($P < 0.1$), and the proportions of *Lachnospiraceae* ($P < 0.05$) and *Ruminococcaceae* ($P < 0.1$) were

Table 2
Estimators of diversity with Jinjiang cattle's ruminal bacteria.

Item	Dietary treatments			P-value ¹		
	HF	HC	HCN	P1	P2	P3
Observed OTU ²	724	705	627	ns	*	+
Chao	836	832	772	ns	ns	ns
ACE	841	818	762	ns	ns	ns
Shannon index	4.96	4.35	4.52	**	**	ns

HF = high-forage diet; HC = high-concentrate diet; HCN = HC + niacin diet; OTU = operational taxonomic units.

¹ P1 = HC vs. HF; P2 = HCN vs. HF; P3 = HC vs. HCN; "ns" means $P > 0.1$; "+" means $P < 0.1$; "*" means $P < 0.05$; "**" means $P < 0.01$.

² At 97% similarity.

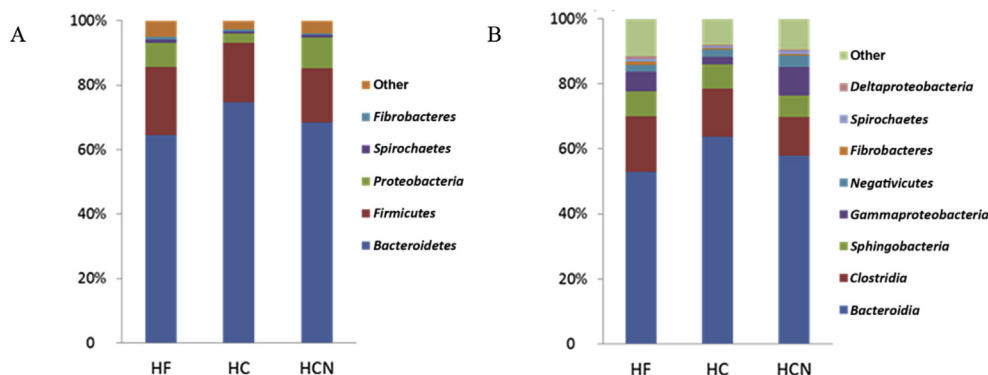


Fig. 2. Percentage contribution of sequences (%) evaluated at the phylum (A) and class (B) levels to the total number of sequences in the database. HF = high-forage diet; HC = high-concentrate diet; HCN = HC + niacin diet.

lower than those in HF group. Compared with HC alone, adding niacin increased the percentage of *Acidaminococcaceae*, *Succinivibrionaceae* and *Spirochaetaceae* ($P < 0.1$), and decreased the proportions of *Prevotellaceae* and *Lachnospiraceae* ($P < 0.1$).

Table 3
Percent abundance of taxa to the rumen bacteria affected by dietary changes.

Classification	Dietary treatments			P-value ¹		
	HF	HC	HCN	P1	P2	P3
<i>Bacteroidetes</i>	64.67	74.59	68.42	+	ns	ns
<i>Bacteroidia</i>	52.88	63.76	57.84	+	ns	ns
<i>Prevotellaceae</i>	36.26	53.16	41.87	*	ns	+
<i>Prevotella</i>	28.97	49.47	36.76	*	ns	+
<i>Paraprevotella</i>	3.08	1.03	1.48	**	**	+
<i>Porphyromonadaceae</i>	1.89	1.82	2.70	ns	ns	ns
<i>Paludibacter</i>	1.17	1.31	1.39	ns	ns	ns
<i>Rikenellaceae</i>	2.28	1.15	1.29	ns	ns	ns
<i>Rikenella</i>	2.28	1.15	1.29	ns	ns	ns
<i>Sphingobacteria</i>	7.73	7.51	6.59	ns	ns	ns
<i>Sphingobacteriaceae</i>	3.93	3.47	1.33	ns	ns	ns
<i>Treponema</i>	0.51	0.22	0.44	*	ns	+
<i>Firmicutes</i>	21.20	18.4	16.75	ns	ns	ns
<i>Clostridia</i>	17.03	14.67	11.94	ns	+	ns
<i>Ruminococcaceae</i>	9.33	7.60	7.06	ns	+	ns
<i>Clostridium_IV</i>	1.77	1.33	0.89	ns	ns	ns
<i>Acetivibrio</i>	1.11	1.01	1.37	ns	ns	+
<i>Ruminococcus</i>	1.53	0.85	0.56	*	*	ns
<i>Saccharofermentans</i>	0.63	0.49	0.36	ns	ns	ns
<i>Sporobacter</i>	0.53	0.27	0.34	*	*	ns
<i>Lachnospiraceae</i>	4.89	3.79	2.74	+	+	+
<i>Lachnospiraceae_incertain_sedis</i>	0.68	0.33	0.27	+	+	ns
<i>Butyrivibrio</i>	0.81	0.81	0.65	ns	ns	ns
<i>Clostridiales_Incertae_Sedis_XIII</i>	0.50	0.51	0.36	ns	ns	ns
<i>Anaerovorax</i>	0.50	0.49	0.36	ns	*	+
<i>Negativicutes</i>	2.06	2.15	3.56	ns	+	+
<i>Acidaminococcaceae</i>	1.62	1.60	2.56	ns	+	+
<i>Succiniclasicum</i>	1.62	1.60	2.56	ns	+	+
<i>Proteobacteria</i>	7.59	3.07	9.53	ns	ns	+
<i>Gammaproteobacteria</i>	6.12	2.41	8.85	ns	ns	ns
<i>Succinivibrionaceae</i>	2.03	0.57	2.08	ns	ns	+
<i>Succinivibrio</i>	0.92	0.31	1.60	ns	ns	ns
<i>Ruminobacter</i>	0.97	0.05	0.30	ns	ns	ns
<i>Spirochaetes</i>	0.91	0.60	0.92	+	ns	+
<i>Spirochaetes</i>	0.91	0.60	0.92	+	ns	+
<i>Spirochaetaceae</i>	0.88	0.58	0.90	ns	ns	+
<i>Fibrobacteres</i>	0.95	0.55	0.43	ns	ns	ns
<i>Fibrobacteria</i>	0.95	0.55	0.43	ns	ns	ns
<i>Fibrobacteraceae</i>	0.95	0.55	0.43	ns	ns	ns
<i>Fibrobacter</i>	0.95	0.55	0.43	ns	ns	ns

HF = high-forage diet; HC = high-concentrate diet; HCN = HC + niacin diet.

¹ P1 = HC vs. HF; P2 = HCN vs. HF; P3 = HC vs. HCN; "ns" means $P > 0.1$; "+" means $P < 0.1$; "*" means $P < 0.05$; "**" means $P < 0.01$; "****" means $P < 0.0001$.

At the genera level (Table 3), HC increased the percentage of *Prevotella* ($P < 0.05$), and decreased the proportions of *Paraprevotella* ($P < 0.01$), *Ruminococcus* ($P < 0.05$), *Treponema* ($P < 0.05$), *Sporobacter* ($P < 0.05$) and *Lachnospiraceae incertain_sedis* ($P < 0.1$) compared with HF. In HCN group, the percentage of *Succiniclasicum* was higher ($P < 0.1$), and the proportions of *Paraprevotella* ($P < 0.01$), *Ruminococcus* ($P < 0.05$), *Anaerovorax* ($P < 0.05$), *Sporobacter* ($P < 0.05$) and *L. incertain_sedis* ($P < 0.1$) were lower than those in HF. Compared with HC group, HCN group increased the percentage of *Succiniclasicum*, *Paraprevotella*, *Acetivibrio* and *Treponema* ($P < 0.1$), and decreased the proportions of *Prevotella* and *Anaerovorax* ($P < 0.1$).

4. Discussion

It was observed the duration time of ruminal pH less than 5.8 were about 12 h in HC group. Thus, based on the indication of SARA defined by Kleen and Cannizzo (2012), we considered the SARA occurred in the cattle fed HC in this study. Some researchers reported that niacin supplementation could alleviate ruminal acidosis by inhibiting the proliferation of *Streptococcus bovis*, producing more NAD^+ to inhibit the activity of lactate dehydrogenase resulting in reducing lactic acid production (Yang et al., 2013; Zhang et al., 2014). In our study, adding niacin shortened the duration time of lower pH for HC.

The diversity of ruminal bacteria should be reduced in HC (Zened et al., 2013). In our study, HC group had lower OTU and Shannon index than HF group. The Shannon index of HCN group was higher than that of HC group, but lower than that of HF group. It was suggested niacin supplementation could increase ruminal bacterial diversity caused by HC, for its nutritional functions of bacteria that could not produce niacin.

Bacteroidetes, *Firmicutes* and *Proteobacteria* were the dominant phyla in cattle whatever the diet was. And, the effects of HC on the composition of ruminal bacterial at phylum lever were found in our study. *Bacteroidetes*, a kind of gram-negative bacteria contributing to the degradation of protein and polysaccharides, increased as the proportion of concentrate increased in the diet, as a same result of the studies of Fernando et al. (2010) and Petri et al. (2013). In contrast, Mao et al. (2013) reported a decreased percentage of *Bacteroidetes* in the Holstein suffered from SARA (sampled on day 21). One reason maybe was that the increasing *Bacteroidetes* promoted the glycolysis of carbohydrate, and produced more VFA, resulting in the decrease of ruminal pH, which could accelerate the death and dissolution of gram-negative bacteria. Therefore, the growth trend of *Bacteroidetes* always was found at the beginning of fermentation (Tajima et al., 2001). At the genus level, some studies of lactating cow revealed that the numbers of *Prevotella*, a major

amylolytic genus (Huws et al., 2011), increased significantly during mildly grain-induced SARA (Tajima et al., 2001; Khafipour et al., 2009). In this study, the percentage of *Prevotella* increased in the cattle fed HC. Many researchers reported that an excess of grain introduced into the rumen caused the cellulolytic bacteria to decrease greatly (Hungate et al., 1952; Petri et al., 2013; Mao et al., 2013). In our study, we also observed a decrease in the percentage of *Ruminococcus*, a major cellulolytic genus (Bryant and Small, 1956), and *Treponema*, a genus involved in the degradation of soluble fibers (Bekele et al., 2011). And, the *Paraprevotella* was significantly and negatively affected by HC. *Paraprevotella* had been firstly identified in human's faeces and produced succinic and acetic acids as end products of glucose metabolism (Morotomi et al., 2009).

Besides, our results revealed that the addition of niacin had a positive effect on *Proteobacteria*, but had no effects on *Bacteroidetes* and *Firmicutes* phyla compared with HC. We also observed that the addition of niacin tended to decrease the abundance of *Prevotella* significantly, suggesting less degradation of starch in HF. *Succiniclasicum* was fairly common in the ruminal bacterial community. Van Gylswyk (1995) detected *Succiniclasicum* fermenting succinic acid to propionate. Niacin tended to increase the relative abundance of *Succiniclasicum*, which would suggest that the addition of niacin tended to promote the accumulation of propionic acid in HC. The addition of niacin also had positive effects on some minor or poorly known genera (such as *Acetivibrio* and *Treponema*), but had no effect on main cellulolytic genus (such as *Ruminococcus*, *Butyrivibrio* and *Fibrobacter*), which was consistent with the results of Pinloche et al. (2013) about probiotic yeast addition. *Treponema* was a commonly detected bacterial group in the rumen that was conductive to in the degradation of soluble fibers (Bekele et al., 2011). It was reported that most of cellulolytic genus was attached to the chyme and had less number in the rumen fluid (Feng, 2006). Thus, the difference in cellulolytic genus caused by niacin was not found in our study. In addition, the addition of niacin tended to increase the abundance of *Acetivibrio*, a mesophilic, gram-positive bacterium, known for its efficient degradation of crystalline cellulose (Saddler and Khan, 1981; Xu et al., 2003; Bareket D et al., 2012).

5. Conclusions

This study provided the detailed account of the effects of HC and the niacin supplementation on ruminal microbe. Bacterial profile was primarily predominated by *Bacteroidetes*, *Firmicutes* and *Proteobacteria* in *Jinjiang* cattle whatever the diet was. The SARA caused by HC in the cattle was observed. High-concentrate diet supplemented with or without niacin significantly decreased effects on the α -diversity of ruminal microbial community. High-concentrate diet significantly increased the abundance of starch utilizing bacteria (like *Prevotella*), and decreased the abundance of *Paraprevotella*, *Sporobacter* and some fibrolytic bacteria (such as *Ruminococcus* and *Treponema*). And, the addition of niacin tended to reverse the symptoms of SARA in terms of inhibiting starch utilizing and stimulating fibrolytic degradation by decreasing the percentage of *Prevotella*, and increased the abundance of *Succiniclasicum*, *Acetivibrio* and *Treponema*.

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