INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20F–066/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1529
http://www.fspublishers.org

Full Length Article

Ruminal Fermentation Parameters and Microbial Community at Phylum Level Differently Influenced by Forage Types in Bulls

Dong Chen[1*], Qiyuan Tang[2], Su Huawei[4], Muhammad Aziz ur Rahman[5], Duan Qin Wu[3*], Fachun Wan[1] and Weijun Shen[1]

[1]*College of Animal Science and Technology, Hunan Agricultural University, Changsha 410130, China*
[2]*College of Agronomy, Hunan Agricultural University, Changsha 410130, China*
[3]*Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, 410205, China*
[4]*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China*
[5]*Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, 35200, Pakistan*
[*]For correspondence: chendong_326@126.com, wuduanqin@caas.cn
Received 31 March 2020; Accepted 02 May 2020; Published _____

Abstract

The current research aimed to evaluate the changes in the rumen ecosystem at phylum level and ruminal fermentation products by using whole crop corn silage (WCS), whole rice crop silage (WRS), or rice straw (RS) as forage sources in beef cattle ration. Ruminal digesta samples from 10 bulls per treatment were collected on days 60 of the experimental period. Ruminal fermentation pattern revealed that ruminal pH and acetate to propionate concentration was increased in RS fed animals as compared to WCS and WRS fed animals. However, ruminal NH_3-N, isovalerate and valerate concentration was decreased in RS fed animals as compared to other treatments. Results of High-throughput Illumina sequencing of the bacterial 16S rRNA gene revealed distinct ruminal microbiota abundance at the phyla level. Among these phyla, *Firmicutes* was the most abundant phyla with a relative abundance of 48.65% ± 5.99%. The second most abundant phyla was *Bacteroidetes*, with an average abundance of 41.66% ± 4.24%. Furthermore, *Firmicutes* were higher in WRS as compared to WCS and RS treatments, while *Bacteroidetes* were higher in WCS treatment. *Proteobacteria*, *Tenericutes*, *Spirochaetes*, and *Cyanobacteria* were found to be more in RS treatment as compared to other treatments. *Actinobacteria* and *Chloroflexi* were higher in WRS and WCS treatment as compared to RS. Based on the findings of the current study, it is concluded that the microbial community was highly altered by RS. In contrast, it remained mostly similar for the other silages in bulls. © 2020 Friends Science Publishers

Keywords: Nutrition; Rumen fermentation; 16S rRNA gene sequencing; Rumen microorganism; Beef production

Introduction

The rumen of ruminants harbours complex microbial communities those are responsible for digestion and fermentation of ingested feed. Ruminants ration composition is considered as one of the most critical factors those affect the ruminal microorganism (Qiu *et al.* 2020a, b), ruminal fermentation and hence animal productivity (Xia *et al.* 2018a, b, c; Chen *et al.* 2019; Rahman *et al.* 2019; Qiu *et al.* 2020b). Numerous studies have revealed that cattle fed grain-based diet had lower bacterial diversity as compared to forage-rich diet, and these two typical diets possessed distinct communities (Fernando *et al.* 2010; Plaizier *et al.* 2017; Liu *et al.* 2019; Qiu *et al.* 2020b). Similarly, a researcher reported that alteration in concentrate to forage ratio change the composition of the microorganism community in the rumen (Xia *et al.* 2018c) and ruminal pH (Petri *et al.* 2012; Muhammad *et al.* 2016). Moreover, researcher also reported that forage sources also have a significant influence on the ruminal microbial community (Staerfl *et al.* 2012; Whelan *et al.* 2013).

In Europe and developed countries, corn silage or silage of different grasses is being used for beef cattle production (Lengowski *et al.* 2016a). However, in developed countries, crop residues like rice straw, corn stover, corn stalks, and wheat straw are being used for fattening cattle as forage sources (Rahman *et al.* 2019). Effect of feeding silages, hay or crop residues on intake, growth, rumen microbial population and ruminal fermentation parameters was inconsistent in previous studies (Muhammad *et al.* 2016; Rahman *et al.* 2017; Niu *et al.* 2017; Xia *et al.* 2018b, c; Chen *et al.* 2019). For example, in the study of Staerfl *et al.* (2012), Brask *et al.* (2013) and Owens *et al.* (2009), pH was not affected by silage type, while the ruminal pH was decreased with corn silage in the study of Abrahamse *et al.* (2008). Besides, variable results on the production of volatile

fatty acids were observed in various studies depending on the type of forage fed to ruminants (Owens *et al.* 2009; Brask *et al.* 2013).

To our knowledge, most of the *in vitro* studies in the past were carried to evaluate the effect of type of silages and agricultural by-products like wheat straw, rice straw, corn stover) on the ruminal microorganism (Witzig *et al.* 2010a, b; Witzig *et al.* 2015; Liu *et al.* 2016). *In vitro* studies reported the change in abundance of *Firmicutes* and *Bacteroides-Prevotella* (Witzig *et al.* 2010a; Witzig *et al.* 2015) and changes in different microorganisms' populations when incubating grass silage instead of corn silage (Witzig *et al.* 2015; Lengowski *et al.* 2016b). Similarly, *in vitro* studies conducted by (Liu *et al.* 2016) reported a significant difference abundance of dominant genera *Anaeroplasma*, *Butyrivibrio*, *Fibrobacter*, and *Prevotella* between rice straw and alfalfa hay forage type. An *in vivo* study of Lengowski *et al.* (2016a) demonstrated that total bacteria abundance and increase in *Fibrobacter succinogenes* in solids fraction of rumen digesta of dairy cows fed corn silage. They further reported that grass silage feeding to dairy cow increases *Selenomonas ruminantium* and *F. succinogenes* abundance in the liquid portion of rumen digesta and increase the abundance of *Prevotella bryantii, Ruminobacter amylophilus and ruminococci* in both liquid and a solid portion of digesta.

Based on the review, it is clear that the type of silages and forage type have a variable influence on ruminal microorganisms' community, and consequently, on fermentation parameters. In the current study we used, rice straw, whole crop rice silage and whole crop corn silage in the diet of bulls, because rice straw is abundant, cheap and is the major forage source for animals in the tropical zones of the world and other two forages, are recognized for its better quality and is used globally as forage in ruminant production. Therefore, this study was planned to further investigate the ruminal microbiota variation by using whole crop rice silage (WCS), whole rice crop silage (WRS), or rice straw (RS) as forage source in the feed of bulls. The focus of the study was mainly to check the influence of WCS, WRS on the ruminal bacterial community at the phylum level, and fermentation characteristics *in vivo*. We hypothesized that WCS, WRS, and RS would differentially affect temporal fluctuations of bacterial species at the phylum level and thus fermentation products *in vivo*.

Materials and Methods

Experimental design, animal management, and diet

This study was part of a larger experiment investigating the effect of replacing rice straw (RS) and whole plant corn/maize silage (WCS) with whole-plant rice crop silage (WRS) on the performance parameters of growing Angus bulls. Animals used in the current study had body weight (both determined at the beginning of the study) 272.43 ± 21.80 kg. An equal amount of concentrate was supplemented with above-said forage sources. The concentrate was made according to the nutritional requirements recommended by the Nutritional Research Council (NRC) of USA. The amount was enough to maintain the minimum daily requirement of the animals. It was ensured that the amount of concentrate was enough to maintain a daily weight gain of half kg per day. During the experimental trial first ten days were considered as an adaptation period, and the experiment was finished after 70 days. Before the start of the experiment, all the animals were weighed and tagged for identification. De-worming and vaccination programs were ensured before the start of the experiment.

The WCS was transported from Hunan Deren Animal Husbandry Co., Ltd. The Whole-plant rice was harvested at the milky ripe stage from Yueyang City, Hunan Province, China. A commercial harvester was used to harvest the Whole-crop rice, and harvested whole-crop rice was wilted for one day before ensiling. The wilted whole rice crop was ensiled at Hunan Deren Animal Husbandry Co., Ltd. site for 60 days before animal feeding. After the opening of ensiled crops, both WCS and WRS were chopped with a commercial cutter to the size of about 2–3 cm length before every feeding time throughout the experimental trial. Rice straw was supplied by Hunan Tianhua Industrial Co., Ltd. A 1.75 kg of concentrate per day per cattle was offered to each animal. Silages were offered ad-libitum, and the feed intake was recorded every day. The feeding time to the experimental animals was twice a day i.e., at 8:00 and 14:00, and 5 to 10% orts were ensured throughout the experimental trial.

Sample collection

Ruminal samples were collected after two h following the morning feeding (08:00) on day 6o of the experimental period, as described in our recent study (Chen *et al.* 2020). In the current experiment, nearly 100 mL of rumen digesta samples were orally collected by using a mouth tube. After collection, nearly ~50 mL of rumen liquid was stored at −20°C for rumen bacterial 16S rRNA analysis. Nearly ~50 mL of rumen liquid was filtered for pH determination. The filtered ruminal fluid samples were further centrifuged at 2000 × g for 15 min at 4°C, and supernatant of ruminal fluid was used for ruminal volatile fatty acid and ammonia nitrogen (NH_3-N).

Chemical analytical procedures

In the current study, the chemical composition of experimental diets was determined using the standard procedure of AOAC. Organic portion of feedstuff was calculated using the following formula:

Organic matter= 100 - the percentage of ash

Neutral detergent fiber and acid detergent fiber fraction in feed, orts, and fecal samples were measured by using Ankom Fiber Analyzer. For determination of NDF and ADF official method of Vansoest *et al.* (1991) was followed. Crude protein determination was carried out following the

procedure of Kjeldahl (AOAC 1990; method 990.03). All proximate analysis procedure along with NDF and ADF determination was completely or partially followed by standard methods as described in previous studies (Su *et al.* 2013; Li *et al.* 2014; Zhang *et al.* 2015; Wang *et al.* 2016; He *et al.* 2018; Sharif *et al.* 2018; Chen *et al.* 2020). The concentration of various rumen volatile fatty acids was determined by using high-performance gas chromatography, as described in the recent study (Chen *et al.* 2020). The rumen liquid NH_3–N was determined following the procedure described by (Bremner and Keeney 1965) by using a spectrophotometer.

DNA extraction and 16S rRNA pyrosequencing

The procedure used for DNA extraction and 16S rRNA pyrosequencing is fully described in our published work (Chen *et al.* 2020). In brief, a 1.5 mL of rumen fluid was centrifuged at 1000 × g for 10 min. After eradicating the sediment of the centrifuged rumen sample, the clear supernatant extract was eliminated by second centrifugation at 12000 × g for 10 min. Then, a commercial kit was used to extract the DNA from rumen fluid. After that, obtained DNA was further quantified using a Qubit 3.0 Fluorometer. Barcoded primers were used to amplify bacterial 16S rRNA genes of the V3-V4 region from extracted DNA. PCR reactions were performed in triplicate 25 μL mixture containing 2.5 μL of TransStart Buffer, two μL of dNTPs, one μL of each primer, and 20 ng of template DNA. Then, Illumina MiSeq platform (San Diego, C.A., U.S.A.) was used to purify the PCR products after an initial check of size and specificity by agarose gel electrophoresis. Finally, high-throughput sequencing was carried out by using the Illumina MiSeq platform (San Diego, C.A., U.S.A.) following the manufacture's protocol.

Pyrosequencing data analyses

The detailed procedure of pyrosequencing data analyses is given in our recent study (Chen *et al.* 2020). QIIME (Version 1.9.1) was used to filter the raw reads and to remove low-quality sequences. FLASH (Version 1.2.7) was used to merge the filtered data into tags. Furthermore, the merged sequences with high quality were identified by QIIME. Moreover, for the removal of chimeric tags, the Uchime algorithm (Edgar *et al.* 2011) was applied in Usearch software (Version 8.1.1861). Uclust algorithm in QIIME (Version 1.9.1) was used to clustered the resulting tags of each sample into operational taxonomic units (OTUs) at the level of 97% similarity. QIIME (Version 1.9.1) and the GreenGene database (Release 13_8_99) (DeSantis *et al.* 2006) were used to select the representative sequence for each OTU and to annotate the taxonomic information. QIIME (Version 1.9.1) was used to calculate richness estimates and diversity indices, including Chao1, Observed OTUs, Good's coverage, phylogenetic diversity whole tree (PD whole tree), and Shannon's index.

Statistical analyses

The collected data of rumen pH and ruminal fermentation parameters were statistically analyzed with the MIXED procedure of SPSS Version 18 (SPSS, Chicago, IL, USA) according to the model

$$Yijklm = \mu + Gi + C\,(G)ij + Pk + \tau l + Dm + \tau Pkl + eijklm$$

The microbial data were analyzed with the general linear model procedure in SPSS Version 18 according to the model

$$Yijkl = \mu + Gi + C\,(G)ij + Pk + \tau l + \tau Pkl + eijkl$$

Fisher's LSD was used to compare the means and to check the statistical difference in the means. Statistical differences were considered at $P < 0.05$ of significance. Differences between treatments at $0.05 \leq P \leq 0.10$ were considered a trend toward significance.

Results

Illumina sequence

A total of 30 samples from three groups were used to generate the Raw reads by Illumina MiSeq PE250 sequencing. Quality trimming, pair-end joining, and chimeric filtering were used for downstream analyses of raw reads to obtain a total of 1,758,219 high quality joined reads. A total average of 57,276 raw Tags was obtained with an average of 48,031 effective Tags per sample (Supplementary Table 1), with an average length of 410 bp, which were assigned to 2,474 operational taxonomic units (OTUs) of rumen bacterial base on a 97 similarity cut-off.

Ruminal parameters

Variation in ruminal fermentation parameters of bulls fed WRS, WCS, and RS are presented in Table 1. Ruminal fermentation parameters revealed that feeding RS as a forage source to the bulls increased the ruminal pH as compared to two types of silages ($P < 0.05$). However, ruminal NH_3-N concentration was decreased in RS fed animals as compared to bulls fed WCS and WRS ($P < 0.05$). The total volatile fatty acids were the same in all the animals fed WRS, WCS, and RS experimental treatments ($P > 0.05$). Ruminal acetate concentration was not also influenced by experimental treatments in bulls ($P > 0.05$). Ruminal fermentation parameter results also revealed that propionate concentration was also the same in animals fed WRS, WCS, and RS experimental treatments ($P > 0.05$). Similar to propionate, butyrate level was also not affected by experimental treatments ($P > 0.05$). Results of ruminal fermentation parameters explored that isovalerate concentration was decreased in RS as compared to WRS and WCS ($P < 0.05$). Similarly, the concentration of valerate concentration was decreased in RS as compared to WRS and WCS ($P < 0.05$). Furthermore, ruminal fermentation parameters results explored that acetate to propionate ratio was higher in RS

Table 1: Rumen parameters of growing beef cattle fed different forage sources

Parameter	Dietary treatment [a]			SEM[b]	*P*-value
	RS	WCS	WRS		
pH	7.63[a]	7.14[b]	7.41[ab]	0.073	0.014
NH_3-N (mg/dL)	2.17[b]	4.09[a]	4.70[a]	0.273	＜0.001
TVFA (mmol/L)	1694.17	2020.11	1815.92	147.206	0.669
Acetate (mmol/L)	1465.11	1457.78	1312.98	109.131	0.807
Propionate (mmol/L)	269.52	322.38	283.38	23.280	0.369
Isobutyrate (mmol/L)	14.11[b]	24.48[a]	22.61[a]	1.634	0.016
Butyrate (mmol/L)	121.61	165.07	148.30	11.654	0.318
Isovalerate (mmol/L)	17.58[b]	37.24[a]	34.10[a]	2.624	0.002
Valerate (mmol/L)	8.24[b]	16.16[a]	14.55[a]	1.158	0.008
Acetate/Propionate	5.44[a]	4.53[b]	4.69[b]	0.086	＜0.001

Mean values in the same row with different letters (a, b, c) differ ($P < 0.05$).
[a] RS, diet with rice straw as main forage source; WCS, diet with whole crop corn silage as main forage source; WRS, diet with whole crop rice silage as main forage source.
[b] Standard error of mean

Table 2: Phylum-level composition of the rumen bacteria influenced by different forage source in rumen of bulls

Phylum	Relative abundance (%)				SEM	P
	All	WRS	RS	WCS		
Firmicutes	48.65	52.67[a]	47.87[b]	47.01[b]	1.76	0.015
Bacteroidetes	41.66	38.18[b]	41.20[b]	43.02[a]	1.41	0.076
Proteobacteria	1.34	1.08[b]	2.29[a]	1.29[b]	0.37	0.008
TM7	1.43	1.27[b]	1.71[a]	1.26[b]	0.15	0.038
Fibrobacteres	0.89	0.31[b]	0.89[a]	1.73[a]	0.41	0.001
SR1	1.21	1.36	1.21	0.99	0.11	0.745
Tenericutes	1.05	0.92[b]	1.32[a]	0.96[b]	0.13	0.010
Spirochaetes	0.74	0.43[b]	0.93[a]	0.69[b]	0.14	0.015
Cyanobacteria	0.20	0.09[b]	0.41[a]	0.19[b]	0.09	0.029
Actinobacteria	0.12	0.14[a]	0.09[b]	0.16[a]	0.02	0.080
Elusimicrobia	0.07	0.03[b]	0.07[b]	0.15[a]	0.04	0.011
WPS-2	0.07	0.07	0.04	0.12	0.02	0.085
Euryarchaeota	0.13	0.19	0.11	0.14	0.02	0.151
Chloroflexi	0.08	0.14[a]	0.07[b]	0.10[a]	0.02	0.007

Mean values in the same row with different letters (a, b, c) differ ($P < 0.05$)
[a] RS, diet with rice straw as main forage source; WCS, diet with whole crop corn silage as main forage source; WRS, diet with whole crop rice silage as main forage source
[b] Standard error of mean

treatment as compared to other dietary treatments ($P < 0.05$).

Diversities of rumen microbiota

Diversity metrics are used to estimate the species richness and evenness in a certain sample or a single community. Results of the current study indicated that richness and diversity in the rumen microbiota differed significantly between WCS、WRS, and RS group at Chao 1 ($P < 0.05$), Observed Otus ($P < 0.05$) and Shanno ($P < 0.05$) (Fig. 1).

Rumen bacteria composition at Phylum level

Fig. 2 represents the microorganism compositions at the phylum. At phylum levels, 14 phylas were identified, and these 14 phylas were distributed across all experimental rations (Supplementary Table 2). Among the identified phyla, the least detected phyla were *WPS-2*, and the most abundant phylum was *Firmicutes*, with a relative abundance of 48.65% ± 5.99%. *Bacteroidetes* was the second most abundant phylum, with an average abundance of 41.66% ± 4.24%. Among all of the phyla detected, *Firmicutes, Proteobacteria, TM7, Fibrobacteres, Tenericutes, Spirochaetes, Cyanobacteria, Actinobacteria, Elusimicrobia,* and *Chloroflexi* showed significant in ruminal bacteria community composition and relative abundance between the experimental treatments (Table 2).

Results of relative abundance between experimental treatments explored that that *Firmicutes* abundance was higher in bulls fed WRS diet as compared to bulls fed WCS and RS experimental diet ($P < 0.05$). Overall, *Bacteroidetes* was the second most abundant phylum, with an average abundance of 41.66% ± 4.24%, however in experimental treatments, *Bacteroidetes* were higher in bulls fed WCS treatment as compared to the experimental treatments WRS and RS ($P < 0.05$). In the current study, *TM7* was the third most abundant phyla, and its abundance was higher in bulls fed RS as compared to bulls fed WCS and WRS experimental diets ($P < 0.05$). Similarly, *Proteobacteria* abundance was higher in bulls fed RS experimental diet as compared to other experimental treatments. The phylum *Fibrobacteres* abundance was higher in bulls fed WCS and RS experimental treatments as compared to animals on WRS experimental treatment ($P < 0.05$). *Tenericutes* phylum abundance was higher in the rumen of animals, which were on RS experimental treatment as compare to WCS and WRS experimental treatment ($P < 0.05$). Similarly, *Spirochaetes* were higher in bulls fed RS experimental diets as compare to WCS and WRS experimental diets ($P < 0.05$). Moreover, *Cyanobacteria* phylum abundance was also higher in animals, which were on RS experimental treatments as compared to WCS and WRS experimental treatments ($P < 0.05$).

In contrast to phylums *Tenericutes, Spirochaetes,* and *Cyanobacteria*, the abundance of *Actinobacteria* was lower RS treatment as compared to animals that were on experimental treatments WCS and WRS. The phylum *Elusimicrobia* was higher in animals fed WCS experimental treatment as compared to animals fed RS and WRS experimental treatments. Similar to *Actinobacteria,* phylum *Chloroflexi* abundance was lower in bulls fed RS treatment as compared to animals, which were on experimental treatments WCS and WRS.

Discussion

In the current study, similar ruminal pH, NH_3-N, isovalerate, valerate, and acetate to propionate ratio were observed between two types of silages. Similar rumen metabolites in WCS and WRS are similar to the findings of the Ki *et al*. (2009), who reported that replacing WCS with WRS does not affect rumen metabolites of the lactating dairy cow. Takahashi *et al*. (2007) also reported similar volatile fatty acids and pH in cows fed either Sudan grass hay or WCS. However, in the current study, ruminal pH increased in the animals received diet contained RS. The ruminal pH of the

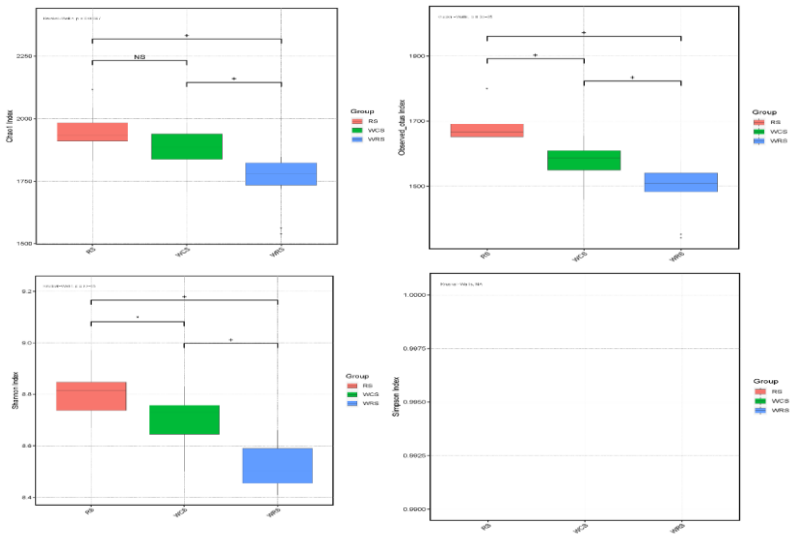

Fig. 1: Community richness estimates (Chao1 and Observed OTUs) and diversity indices (Shannon and Simpson) for different treatments (n=10). *, + between boxes differ significantly ($P < 0.05$)

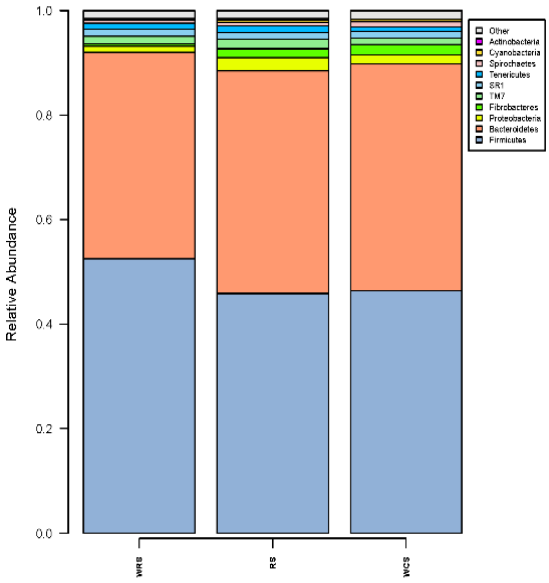

Fig. 2: Bacterial community structure variation in different stages. The relative abundance species of bacteria at the phylum level is shown. Lower the top 10 abundance of the phyla were merged into others. Each bar represents the relative abundance of each sample. Each color represents a particular phylum. The numbers associated with the sample names indicate the replication of group

bulls fed RS was at a higher level (7.63). The higher pH in the rumen was probably because of the consumption of rice straw led to a high salivation rate, which led to high ruminal pH of bulls (Muhammad *et al.* 2016). Kim *et al.* (2000) reported similar results that feeding rice straw to sheep increased the ruminal pH as compared to corn silage. Similarly, Kim *et al.* (2000) also reported that feeding wormwood (*Artemisia montana*) silage instead of rice straw to ruminants results in reduced pH of silage fed animals due to the presence of lactic acid in silages. The higher ruminal pH could be explained by the theory of higher crude fiber, NDF, and ADF in rice straw as compared to both silages. It has been reported that higher crude fiber, NDF, and ADF enhance the effective fiber and more effective fiber are known to increase the ruminal pH by enhancing chewing and salivation (Muhammad *et al.* 2016; Rahman *et al.* 2017, 2019). The lower ruminal NH_3-N in the bulls fed RS is also consistent with the study of Kim *et al.* (2000), who reported reduced ruminal NH_3-N in sheep fed RS compared to WCS. The reduced ruminal NH_3-N results in reduced cellulolytic bacteria growth and reduce the fiber digestion in rumen (Kim *et al.* 2000) and hence the growth of the bulls. Generally, structural carbohydrates are mainly fermented into acetate, and nonstructural carbohydrates produce more propionate (Qiu *et al.* 2020a), which should be reflected in the current study. In the current study, RS contained higher structural carbohydrase as compared to WCS and RCS that should increase acetate concentration. Furthermore, WCS and RCS should increase the propionate concentration in the current study as compared to RS. Similar propionate and acetate concentration in all experimental treatments could be explained by variation in intake. It has been reported that voluntary intake of structural and nonstructural carbohydrates is adjusted by ruminates to reduce the chance of acidosis (Xia *et al.* 2018c; Chen *et al.* 2019). Therefore, it could be assumed that bulls adjusted their voluntary intake of structural carbohydrates in the current study and resulted in similar acetate and propionate concentration in the rumen. Kim *et al.* (2006) also reported similar acetate concentrations and total volatile fatty acid concentration in the rumen of sheep fed rice straw or wormwood silage. If acetate and propionate concentration was the same in all experimental treatments in the current study, the acetate to propionate ratio

should be the same in all experimental treatments. The current study results revealed that acetate to propionate ratio was higher in RS fed animals. The contradiction in results could be explained by the numerical increase in acetate concentration, and numerical decreased in propionate concentration in the RS diet of the bulls. Similar results have been reported in the study of Qiu *et al.* (2020a) that explained that acetate to propionate ratio increased as dietary nutrient density decreased, which is in accordance with the fact that the acetate to propionate ratio increased as the supply of structural carbohydrate increased in bulls fed RS. Branched-chain fatty acids usually derive from the degradation of crude protein and have been used as an indicator of ruminal protein fermentation (Qiu *et al.* 2020a). In the current study, branched-chain fatty acids like isobutyrate and isovalerate were increased in the rumen of bulls fed WRS and WCS diets that would lead to better performance of bulls. Qiu *et al.* (2020a) reported that increasing the concentrate concentration in fattening bulls increased the of isobutyrate and isovalerate concentration in the rumen. The previous study reported an elevated valerate concentration as the proportion of dietary concentrate increased (Qiu *et al.* 2020a). In this study, higher valerate concentration in silages could be explained by the more nonstructural carbohydrates in both silages as compared to RS.

Diversity metrics are used to estimate the species richness and evenness in a certain sample or a single community (Tucker *et al.* 2017). In this study, rumen samples from bulls fed RS showed higher diversity as compared to two types of silage, which is similar with many reports (Plaizier *et al.* 2017; Xia *et al.* 2018c; Qiu *et al.* 2019; Qiu *et al.* 2020b) in which highly fermentable carbohydrates-based diet decreased microbial diversity. Similar findings have also been reported by Qiu *et al.* (2020a) that increasing the effective fiber or forage concentration of the diet of steers increase the microbiota diversity. These differences may be explained by the well-established theory that ruminal pH has a large impact on rumen bacterial diversity. In the current study, rumen samples from bulls fed silage showed lower ruminal pH (Table 1) showed lower diversity as compared to RS fed animals had higher ruminal pH, which is in line with previous reports (Wang *et al.* 2009; Kim *et al.* 2016) in which grain-based contained high fermentable carbohydrates decrease microbial diversity. Lv *et al*. (2020) also stated that ruminal microorganism diversity of growing lambs fed low energy diets was higher because of higher ruminal pH.

In the current study, *Firmicutes* and *Bacteroidetes* dominated about 90% of the bacterial composition, with 48.65% ± 5.99% and 41.66% ± 4.24%, respectively, in the rumen. It has been reported that *Bacteroidetes and Firmicutes* are also present in the gut of humans, mice, and pigs (Ley *et al.* 2005, 2006; Guo *et al.* 2008). It has also been reported that *Firmicutes and Bacteroidetes* in mice are associated with energy-harvesting abilities (Ley *et al.* 2005, 2006). Interestingly, a recent study on ruminants also represents that the energy-rich diets in steers result in a higher abundance of *Firmicutes* (Qiu *et al.* 2020a). In the current study, the higher abundance of *Firmicutes* in bulls fed WRS was due to lower ruminal pH because the previous study reported that lower ruminal pH increased the proportion of *Firmicutes* in bacterial composition (Kim *et al.* 2016). *Bacteroidetes* are known to help the digestion of complex carbohydrates, and also ferment organic matter (Jiang *et al.* 2019). However, in the current study, *Bacteroidetes* were higher in WCS (contained lesser structural carbohydrates as compared to RS) as compared to WRS and RS that represents *Bacteroidetes* correlation with nonstructural carbohydrates. These findings are opposite with the findings of Cui *et al*. (2019), who reported that the diet with high fiber in lamb's rumen increased *Bacteroidetes* abundance. C*ellulolytic* bacteria are comparatively abundant in rumen of lambs with low metabolizable diet, and similar observations were found in the gut of humans on higher fiber diets, indicating that their metabolic function may be vital in low energy diets. However, in the current study, lower diversity of *cellulolytic* bacteria *Bacteroidetes* is unknow and indicates *Bacteroidetes* role in the fermentation of both structural and nonstructural carbohydrates of ruminants. However, further research work is required to justify this theory.

Proteobacteria played an important role in the rumen metabolism despite the relatively low abundance and was frequently observed in nonstructural carbohydrates rations (Fernando *et al.* 2010; Petri *et al.* 2012; Qiu *et al.* 2019; Qiu *et al.* 2020a). However, the present study showed the opposite result wherein a high abundance of *Proteobacteria* was observed in bulls fed RS diets contained higher contents of fiber. Our findings of a higher abundance of *Proteobacteria* in fiber-rich diets are consistent with the findings of Qiu *et al.* (2020a), who reported that certain species in the phylum *Proteobacteria* might also actively take part in the digestion of fiber. Therefore, based on current study findings and the study of Qiu *et al.* (2020a), it could be assumed that certain species in the phylum *Proteobacteria* may also actively take part in the digestion of fiber, but further studies are needed to confirm this assumption and certain species. *Fibrobacteres* is well known for its vital role in degrading cellulose, and they are commonly detected in the fiber-rich diet (Cui *et al.* 2019). In the current study, RS diet had more structural carbohydrates and represents the higher abundance of *Fibrobacteres,* which is similar to the findings of Qiu *et al.* (2020a) who reported that fiber-rich diet had a higher abundance of *Fibrobacteres* in the rumen of steers fed high fibrous diet. If it was the case, the WCS diet should have a lesser abundance of *Fibrobacteres* in the current experiment, which is contrary to this theory. The similar abundance of *Fibrobacteres* in WCS and RS could be explained by lesser fiber intake in the RS diet compared to RS, as represented in our recent paper (Chen *et al.* 2019). *Spirochaetes* have a minor role in rumen fiber degradation and usually abundant in the fiber-rich diet (Liu *et al.* 2016), which is consistent with current study findings. It has been reported that the abundance of *Tenericutes* reduced at a

higher rate because of their intolerance to low rumen pH (Loor *et al.* 2016). In the current study, the decrease in *Tenericutes* in WCS and WRS could be attributed to lower rumen pH linked with WCS and WRS (Table 1). Qiu *et al.* (2020a) reported that *Fibrobacteres, Kiritimatiellaeota,* and *Cyanobacteria* are positively correlated were with NDF, and ADF contents of diet and *Cyanobacteria* participate in degrading plant polysaccharides. The higher abundance of *Cyanobacteria* in bull's rumen fed RS diet is the agreement with the study of Qiu *et al.* (2020a), who reported an abundance of *Cyanobacteria* in steers rumen fed fibrous diet. *Actinobacteria* is considered a beneficial bacterium that has a vital role in increasing the immune system, improving the gut barrier, and reducing enteric pathogens (Fukuda *et al.* 2011). In the current study, *Actinobacteria* increased in bulls fed the diet contained silages due to lower ruminal pH caused by silage in the rumen (Table 1). Loor *et al.* (2016) reported that the *Actinobacteria* level at lower ruminal pH increased, which is involved in starch fermentation. Higher *Chloroflexi* has been reported in the cecal microbial communities of goats fed diets contained a higher amount of fermentable carbohydrates. Similar results have been reported (Derakhshani *et al.* 2017) in dairy cows. In the current study, a higher abundance of *Chloroflexi* in WCS and WRS could be justified by higher fermentable carbohydrates in WCS and WRS as compare to RS diet of the bulls.

Conclusion

Current study findings revealed that the microbial community at phyla level was highly altered by RS, whereas remained mostly similar for the other silages in bulls. The findings of current work suggest that the different forages and bacterial communities have a role in adapting host biological parameters in beef cattle. Furthermore, the richness of both *Firmicutes* and *Bacteroidetes* in the rumen of bulls will be beneficial for discovering the structure of rumen microorganisms for future beef cattle rumen microbiota research with different types of forages.

Acknowledgements

This study was supported by 'National Key Research and Development Program of China (2018YFD0501800)','The Key Research and Development Project of Hunan Province, China (2017NK1020)', 'National Modern Agricultural Technology System in China (CARS-01 and CARS-37)', 'The Technology Development Project of Local Government Guided by Central Government of China (YDZX20184300004784)' and 'Hunan Modern Agricultural Technology System of Herbivora'.

Author Contributions

Dr. Dong Chen and Prof. Qiyuan Tang conceptualize the experiment. Dr. Dong Chen, Prof. Fachun Wan and Prof. Weijun Shen handled experimental animals, collected samples, and analyzed the samples for fermentation parameters. Dr. Su Huawei and Dr. DuanQin Wu carried out DNA extraction and 16S rRNA pyrosequencing. Prof. Qiyuan Tang and Dr. DuanQin Wu did pyrosequencing data analyses. Dr. Chen Dong and Dr. M. Aziz ur Rahman analyzed data, prepared the original draft and finished the manuscript.

References

Abrahamse PA, B Vlaeminck, S Tamminga, J Dijkstra (2008). The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. *J Dairy Sci* 91:4778–4792AOAC (1990). *Official methods of analysis*, 15th edn. Association of Official Analytical Chemists, Washington DC, USA

Brask M, P Lund, ALF Hellwing, M Poulsen, MR Weisbjerg (2013). Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. *Anim Feed Sci Technol* 184:67–79

Bremner JM, DR Keeney (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. *Anal Chim Acta* 32:485–495

Chen D, J Yan, W Shen, Y Song, X Lan, K Yi, AUR Muhammad (2020). Effect of inclusion of HMBi in the ration of goats on feed intake, nutrient digestibility, rumen bacteria community and blood serum parameters. *J Anim Physiol Anim Nutr* 2020:1–11 https://doi.org/10.1111/jpn.13270

Chen D, C Yuanwei, Z Huabing, X Kequan, T Jing, T Qiyuan, MA Rahman (2019). Effects of replacing whole-plant corn silage with whole-plant rice silage and rice straw on growth performance, apparent digestibility and plasma parameters in growing angus cross bred beef cattle. *Intl J Agric Biol* 22:1116–1122

Cui K, M Qi, S Wang, Q Diao, N Zhang (2019). Dietary energy and protein levels influenced the growth performance, ruminal morphology and fermentation and microbial diversity of lambs. *Sci Rep* 9; Article 16612

Derakhshani H, HM Tun, FC Cardoso, JC Plaizier, E Khafipour, JJ Loor (2017). Linking peripartal dynamics of ruminal microbiota to dietary changes and production parameters. *Front Microbiol* 7; Article 2143

DeSantis TZ, P Hugenholtz, N Larsen, M Rojas, EL Brodie, K Keller, T Huber, D Dalevi, P Hu, GL Andersen (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72:5069–5072

Edgar RC, BJ Haas, JC Clemente, C Quince, R Knight (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200

Fernando SC, HT Purvis, FZ Najar, LO Sukharnikov, CR Krehbiel, TG Nagaraja, BA Roe, U DeSilva (2010). Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl Environ Microbiol* 76:7482–7490

Fukuda S, H Toh, K Hase, K Oshima, Y Nakanishi, K Yoshimura, T Tobe (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469:543–547

Guo X, X Xia, R Tang, J Zhou, H Zhao, K Wang (2008). Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. *Lett Appl Microbiol* 47:367–373

He Y, W Niu, Q Qiu, C Xia, T Shao, H Wang, Q Li, Z Yu, Z Gao, MAU Rahman, H Su, B Cao (2018). Effect of calcium salt of long-chain fatty acids and alfalfa supplementation on performance of Holstein bulls. *Oncotarget* 9:3029–3042

Jiang X, X Liu, S Liu, Y Li, HB Zhao, YG Zhang (2019). Growth, rumen fermentation and plasma metabolites of Holstein male calves fed fermented corn gluten meal during the postweaning stage. *Anim Feed Sci Technol* 249:1–9

Ki KS, MA Khan, WS Lee, HJ Lee, SB Kim, SH Yang, KS Baek, JG Kim, HS Kim (2009). Effect of replacing corn silage with whole crop rice silage in total mixed ration on intake, milk yield and its composition in holsteins. *Asian-Aust J Anim Sci* 22:516–519

Kim S, A Adesogan, J Kim, Y Ko (2006). Influence of replacing rice straw with wormwood (*Artemisia montana*) silage on feed intake, digestibility and ruminal fermentation characteristics of sheep. *Anim Feed Sci Technol* 128:1–13

Kim SC, JH Kim, CH Kim, JC Lee, YD Ko (2000). Effects of whole crop corn ensiled with cage layer manure on nutritional quality and microbial protein synthesis in sheep. *Asian-Aust J Anim Sci* 13:1548–1553

Kim YH, R Nagata, N Ohtani, T Ichijo, K Ikuta, S Sato (2016). Effects of dietary forage and calf starter diet on ruminal ph and bacteria in holstein calves during weaning transition. *Front Microbiol* 7; Article 1575

Lengowski MB, M Witzig, J Mohring, GM Seyfang, M Rodehutscord (2016a). Effects of corn silage and grass silage in ruminant rations on diurnal changes of microbial populations in the rumen of dairy cows. *Anaerobe* 42:6–16

Lengowski, MB, KHR Zuber, M Witzig, J Möhring, J Boguhn, M Rodehutscord (2016b). Changes in rumen microbial community composition during adaption to an *in vitro* system and the impact of different forages. *PLoS One* 11; Article e0150115

Ley, RE, DA Peterson, JI Gordon (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124:837–848

Ley RE, F Bäckhed, P Turnbaugh, CA Lozupone, RD Knight, JI Gordon (2005). Obesity alters gut microbial ecology. *Proc Natl Acad Sci* 102:11070–11075

Li L, Y He, MAU Rahman, B Cao (2014). Effects of different dietary energy and rumen-degradable protein levels on rumen fermentation, nutrients apparent digestibility and blood biochemical constituents of chinese crossbred yellow bulls. *Pak Vet J* 34:367–371

Liu C, H Wu, S Liu, S Chai, Q Meng, Z Zhou (2019). Dynamic alterations in yak rumen bacteria community and metabolome characteristics in response to feed type. *Front Microbiol* 10; Article 1116

Liu J, M Zhang, C Xue, W Zhu, S Mao (2016). Characterization and comparison of the temporal dynamics of ruminal bacterial microbiota colonizing rice straw and alfalfa hay within ruminants. *J Dairy Sci* 99:9668–9681

Loor JJ, AA Elolimy, JC McCann (2016). Dietary impacts on rumen microbiota in beef and dairy production. *Anim Front* 6:22–29

Lv X, K Cui, M Qi, S Wang, Q Diao, N Zhang (2020). Ruminal microbiota and fermentation in response to dietary protein and energy levels in weaned lambs. *Animals* 10; Article 109

Muhammad AU, CQ Xia, BH Cao (2016). Dietary forage concentration and particle size affect sorting, feeding behaviour, intake and growth of Chinese holstein male calves. *J Anim Physiol Anim Nutr* 100:217–223

Niu W, Y He, C Xia, MAU Rahman, Q Qiu, T Shao, Y Liang, L Ji, H Wang, B Cao (2017). Effects of replacing *Leymus chinensis* with whole-crop wheat hay on Holstein bull apparent digestibility, plasma parameters, rumen fermentation, and microbiota. *Sci Rep* 7; Article 2114

Owens D, M McGee, T Boland, P O'Kiely (2009). Rumen fermentation, microbial protein synthesis, and nutrient flow to the omasum in cattle offered corn silage, grass silage, or whole-crop wheat. *J Anim Sci* 87:658–668

Petri RM, RJ Forster, W Yang, JJ McKinnon, TA McAllister (2012). Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. *J Appl Microbiol* 112:1152–1162

Plaizier JC, S Li, AM Danscher, H Derakshani, PH Andersen, E Khafipour (2017). Changes in microbiota in rumen digesta and feces due to a grain-based subacute ruminal acidosis (SARA) challenge. *Microb Ecol* 74:485–495

Qiu Q, C Gao, MAU Rahman, B Cao, H Su (2020a). Digestive ability, physiological characteristics, and rumen bacterial community of holstein finishing steers in response to three nutrient density diets as fattening phases advanced. *Microorganisms* 8; Article 335

Qiu Q, X Qiu, C Gao, AUR Muhammad, B Cao, H Su (2020b). High-density diet improves growth performance and beef yield but affects negatively on serum metabolism and visceral morphology of Holstein steers. *J Anim Physiol Anim Nutr* 2020; doi 10.1111/jpn.13340

Qiu Q, Y Zhu, X Qiu, C Gao, J Wang, H Wang, Y He, MAU Rahman, B Cao, H Su (2019). Dynamic variations in fecal bacterial community and fermentation profile of Holstein steers in response to three stepwise density diets. *Animals* 9; Article 560

Rahman MAU, C Xia, L Ji, B Cao, H Su (2019). Nutrient intake, feeding patterns, and abnormal behavior of growing bulls fed different concentrate levels and a single fiber source (corn stover silage). *J Vet Behav* 33:46–53

Rahman MAU, X Chuanqi, S Huawei, C Binghai (2017). Effects of hay grass level and its physical form (full length *vs*. chopped) on standing time, drinking time, and social behavior of calves. *J Vet Behav* 21:7–12

Sharif M, M Shoaib, MAU Rahman, F Ahmad, SU Rehman (2018). Effect of distillery yeast sludge on growth performance, nutrient digestibility and slaughter parameters in Japanese quails. *Sci Rep* 8; Article 8418

Staerfl SM, JO Zeitz, M Kreuzer, CR Soliva (2012). Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. *Agric Ecosyst Environ* 148:111–120

Su H, Y Wang, Q Zhang, F Wang, Z Cao, MAU Rahman, B Cao, S Li (2013). Responses of energy balance, physiology, and production for transition dairy cows fed with a low-energy prepartum diet during hot season. *Trop Anim Health Prod* 45:1495–1503

Takahashi T, M Maehara, Y Zhang, T Motobayashi, Y Ishii, S Kanda, H Itabashi (2007). Effect of Feeding of whole crop rice silage on milk production, rumen fermentation, blood metabolites and feeding behavior of lactating cows. *Anim Sci* 78:45–55

Tucker CM, MW Cadotte, SB Carvalho, TJ Davies, S Ferrier, SA Fritz, R Grenyer, MR Helmus, LS Jin, AO Mooers (2017). A guide to phylogenetic metrics for conservation, community ecology and macroecology. *Biol Rev* 92:698–715

Vansoest PJ, JB Robertson, BA Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74:3583–3597

Wang C, A Muhammad, Z Liu, B Huang, B Cao (2016). Effects of ensiling time on banana pseudo-stem silage chemical composition, fermentation and in sacco rumen degradation. *J Anim Plant Sci* 26:339–346

Wang Y, M Xu, F Wang, Z Yu, J Yao, L Zan, F Yang (2009). Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. *Livest Sci* 122:48–52

Whelan SJ, FJ Mulligan, JJ Callan, B Flynn, KM Pierce (2013). Effect of forage source and a supplementary methionine hydroxyl analogue on rumen fermentation parameters in lactating dairy cows offered a low crude protein diet. *Anim Feed Sci Technol* 183:62–66

Witzig M, J Boguhn, M Zeder, J Seifert, M Rodehutscord (2015). Effect of donor animal species and their feeding on the composition of the microbial community establishing in a rumen simulation. *J Appl Microbiol* 119:33–46

Witzig M, J Boguhn, S Kleinsteuber, I Fetzer, M Rodehutscord (2010a). Effect of the corn silage to grass silage ratio and feed particle size of diets for ruminants on the ruminal *Bacteroides-Prevotella* community *in vitro*. *Anaerobe* 16:412–419

Witzig M, J Boguhn, S Kleinsteuber, I Fetzer, M Rodehutscord (2010b). Influence of the maize silage to grass silage ratio and feed particle size of rations for ruminants on the community structure of ruminal Firmicutes *in vitro*. *J Appl Microbiol* 109:1998–2010

Xia C, MAU Rahman, H Yang, T Shao, Q Qiu, H Su, B Cao (2018a). Effect of increased dietary crude protein levels on production performance, nitrogen utilisation, blood metabolites and ruminal fermentation of Holstein bulls. *Asian-Aust J Anim Sci* 31:1643–1653

Xia C, Y Liang, S Bai, Y He, AUR Muhammad, H Su, B Cao (2018b). Effects of harvest time and added molasses on nutritional content, ensiling characteristics and *in vitro* degradation of whole crop wheat. *Asian-Aust J Anim Sci* 31:354–362

Xia CQ, AUR Muhammad, W Niu, T Shao, Q Qiu, S Huawei, B Cao (2018c). Effects of dietary forage to concentrate ratio and wildrye length on nutrient intake, digestibility, plasma metabolites, ruminal fermentation and fecal microflora of male Chinese Holstein calves. *J Integr Agric* 17:415–427

Zhang X, MAU Rahman, Z Xue, X Wang, Y He, B Cao (2015). Effect of post-pubertal castration of wannan cattle on daily weight gain, body condition scoring and level of blood hormone. *Intl J Agric Biol* 17:334–338