SCIENTIFIC REPORTS
nature research

Check for updates

OPEN

The effect of a diet based on rice straw co-fermented with probiotics and enzymes versus a fresh corn Stover-based diet on the rumen bacterial community and metabolites of beef cattle

Yongqiang Hu[1,4], Yuyong He[1,4], Shan Gao[1], Zhiqiang Liao[2], Tao Lai[1], Haimei Zhou[1], Qianlin Chen[3], Lingyu Li[1], Haijun Gao[2] & Wei Lu[1]✉

Improvement of the food value of rice straw is urgently required in rice crop growing areas to mitigate pollution caused by rice straw burning and enhance the supply of high-quality forages for ruminants. The aims of the present study were to compare the effects of fresh corn Stover and rice straw co-fermented with probiotics and enzymes on rumen fermentation and establish the feasibility of increasing the rice straw content in ruminant diets and, by extension, reducing air pollution caused by burning rice straw. Twenty Simmental hybrid beef cattle were randomly allotted to two groups with ten cattle per group. They were fed diets based either on rice straw co-fermented with probiotics and enzymes or fresh corn Stover for 90 days. Rumen fluid was sampled with an esophageal tube vacuum pump device from each animal on the mornings of days 30, 60, and 90. Bacterial diversity was evaluated by sequencing the V4–V5 region of the 16S rRNA gene. Metabolomes were analyzed by gas chromatography/time-of-flight mass spectrometry (GC–TOF/MS). Compared to cattle fed fresh corn Stover, those fed rice straw co-fermented with probiotics and enzymes had higher ($P < 0.05$) levels of acetic acid and propionate in rumen liquid at d 60 and d 90 respectively, higher ($P < 0.05$) abundances of the phyla Bacteroidetes and Fibrobacteres and the genera *Ruminococcus*, *Saccharofermentans*, *Pseudobutyrivibrio*, *Treponema*, *Lachnoclostridium*, and *Ruminobacter*, and higher ($P < 0.05$) concentrations of metabolites involved in metabolisms of amino acid, carbohydrate, and cofactors and vitamins. Relative to fresh corn Stover, rice straw co-fermented with probiotics and enzymes resulted in higher VFA concentrations, numbers of complex carbohydrate-decomposing and H_2-utilizing bacteria, and feed energy conversion efficiency in the rumen.

It is of great significance to vigorously develop ruminant production in the main grain producing areas to increase the amount of crop straw in ruminant diet, reduce the pollution caused by crop straw burning, and increase the income of farmers and herdsmen. However, feeding large amounts of untreated crop straw to ruminants will reduce their performance and expel large amounts of methane, which not only reduces the energy efficiency of ruminant diet, but also adversely affects the climate[1]. Therefore, the key problem to be solved is how to improve the effective fermentation of crop straw and reduce the formation of methane in the rumen while using crop straw to develop ruminant production.

[1]Jiangxi Province Key Laboratory of Animal Nutrition/Engineering Research Center of Feed Development, Jiangxi Agricultural University, Nanchang, China. [2]Yichun Institute of Agriculture Science in Jiangxi Province, Yichun, China. [3]Yichun Institute of Agricultural Varieties, Yichun, China. [4]These authors contributed equally: Yongqiang Hu and Yuyong He. ✉email: lw20030508@163.com

Items	Sampling time	Fermented rice straw group (Group A)	Fresh corn Stover group (Group B)	*P*
pH	d 30 of feeding	6.12 ± 0.06	6.19 ± 0.07	0.495
	d 60 of feeding	6.18 ± 0.05	6.22 ± 0.05	0.634
	d 90 of feeding	6.31 ± 0.02	6.45 ± 0.11	0.257
Lactic acid (mM)	d 30 of feeding	0.30 ± 0.12	0.37 ± 0.08	0.664
	d 60 of feeding	0.65 ± 0.12	0.72 ± 0.09	0.640
	d 90 of feeding	0.44 ± 0.09	0.49 ± 0.20	0.834
Acetic acid (mM)	d 30 of feeding	38.12 ± 3.19	38.26 ± 3.30	0.976
	d 60 of feeding	49.29 ± 3.29	36.30 ± 3.22	0.022
	d 90 of feeding	48.22 ± 6.93	30.38 ± 2.63	0.043
Propionate (mM)	d 30 of feeding	9.00 ± 0.83	8.70 ± 0.90	0.809
	d 60 of feeding	16.61 ± 1.33	11.82 ± 1.07	0.023
	d 90 of feeding	10.39 ± 1.33	5.99 ± 0.47	0.014
Butyric acid (mM)	d 30 of feeding	7.93 ± 0.50	8.04 ± 0.81	0.907
	d 60 of feeding	10.34 ± 1.09	7.87 ± 0.82	0.108
	d 90 of feeding	4.80 ± 0.57	3.82 ± 0.55	0.255
Acetic:propionic	d 30 of feeding	4.26 ± 0.14	4.46 ± 0.24	0.499
	d 60 of feeding	2.98 ± 0.13	3.08 ± 0.12	0.596
	d 90 of feeding	4.62 ± 0.15	5.06 ± 0.17	0.092

Table 1. pH, lactic acid, and VFA in rumen fluid of cattle fed fermented rice straw- or fresh corn Stover-based diets.

During rumen fermentation, Firmicutes are the main H_2 producing bacteria and Bacteroidetes are the net H_2 utilizers[2], a decrease in Firmicutes abundance and an increase in Bacteroidetes abundance can effectively reduce the production of methane[3]. Studies also reported that an increase of *Prevotella* genus can decrease methane production[3], because *Prevotella* can increase propionate level and inhibit methanogenesis[4]. In addition, an increase in fumarate or some amino acids (aspartate, valine, leucine, isoleucine, glutamate) in rumen fluid benifits the production of propionate[3]. Low rumen pH favors propionate producing bacteria, but inhibits other microorganisms[5]. Starch and neutral detergent fiber (NDF) content and NDF digestibility of diet can affect the fermentation characteristics in rumen[6], because dietary starch increases propionate production and decreases CH_4[7], and dietary NDF enhances the production of acetic acid and methane. Dietary fiber is a major energy source for ruminants in many parts of the world[8]. Thus, the efficient conversion of fiber in the rumen is vital to ruminant production. If ruminants could more efficiently utilize rice straw as a roughage source, then feed shortages and air pollution from rice straw burning and methane emission could be substantially mitigated.

Rice straw and fresh corn Stover are the common crop straws, which consist mainly of complex lignocellulose polymers, pectin, silica, and wax[9]. The cuticle-wax-silica layer and lignin hinder microbial and enzyme access to cellulose and hemicellulose[10,11]. Compared with corn Stover, rice straw has lower nutrient digestibility in the rumen as it has a thick outer cuticle-wax silica-layer and high lignin content[12,13]. For this reason, rice straw is usually burned instead of being used as the main constituent of ruminant diets.

Biological degradation is an increasingly popular alternative to crop straw pretreatment, because it decomposes cellulosic polymers to cellulose that can then be digested by various cellulases and hemicellulases[14,15]. Previous study by Wadhwa et al.[16] showed that the intake and nutritive value of naturally fermented rice straw with urea were superior to those of untreated rice straw. However, there is little information on the effects of rice straw co-fermented with probiotics and enzymes on the rumen microbiome and metabolome. Fresh corn Stover is also used as ruminant roughage in China and it has higher feed values than untreated rice straw[17,18]. Here, we fermented rice straw with a mixture of *Bacillus subtilis*, *Enterococcus faecalis*, cellulase, and xylanase for 14 d. We then conducted an experiment to compare the influences of rice straw co-fermented with probiotics and enzymes with fresh corn Stover on beef cattle. Rumen fluid samples were collected to (1) compare rumen fermentation characteristics in cattle fed fermented rice straw with those fed fresh corn Stover; (2) investigate the differences in the compositions of H_2-producing and utilizing bacteria and metabolites of the rumen fluid of cattle fed fermented rice straw with those fed fresh corn Stover; and (3) explore the relationship between the differential bacteria and differential metabolites in rumen fluid.

Results

Dry matter intake. During experiment, each day beef cattle had an average dry matter intake of (7.95 ± 0.08) kg in fermented rice straw group and (7.85 ± 0.10) kg in fresh corn Stover group, respectively, and no significant difference was observed between two groups ($P = 0.433$).

Rumen pH, lactic acid, and VFA. Table 1 shows that beef cattle fed fermented rice straw based diet had lower pH value and lower lactic acid concentration in rumen fluid compared to beef cattle fed fresh corn Stover based diet at d 30 ($P = 0.495$ and $P = 0.664$), d 60 ($P = 0.634$ and $P = 0.640$) and d 90 ($P = 0.257$ and $P = 0.834$),

Items	Sampling time	Fermented rice straw group (Group A)	Fresh corn Stover group (Group B)	*P*
Chao 1	d 30 of feeding	1,101.80 ± 26.19	1,069.80 ± 11.50	0.296
	d 60 of feeding	1,095.80 ± 35.33	1,072.00 ± 32.44	0.633
	d 90 of feeding	1,139.80 ± 47.27	1,099.60 ± 21.14	0.460
Shannon	d 30 of feeding	5.07 ± 0.07	5.19 ± 0.04	0.167
	d 60 of feeding	4.92 ± 0.09	4.75 ± 0.09	0.194
	d 90 of feeding	5.13 ± 0.12	5.10 ± 0.07	0.869

Table 2. Comparison of alpha diversity of bacteria in rumen fluid.

respectively. Beef cattle fed fermented rice straw based diet had higher rumen concentrations of acetic acid and propionate than those fed fresh corn Stover based diet at d 60 ($P = 0.022$ and $P = 0.023$) and d 90 ($P = 0.043$ and $P = 0.014$), respectively.

Bacterial community in rumen fluid. The sequence data produced in this experiment have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number SRP140749. Data in Table 2 showed that there was no significant differences in Chao 1 estimator and Shannon estimator of rumen fluid bacteria between cattle in fermented rice straw group and cattle in fresh corn Stover group at d 30 ($P = 0.296$ and $P = 0.167$), d 60 ($P = 0.633$ and $P = 0.194$) and d 90 ($P = 0.460$ and $P = 0.869$), respectively.

The composition and relative abundance of rumen fluid bacteria at the phylum level are depicted in Fig. 1a, Firmicutes and Bacteroidetes were the most dominant phyla (Table 3). Firmicutes and Bacteroidetes accounted for 28.12–40.63% and 52.15–63.37% of the total reads respectively in the rumen liquid of beef cattle fed fermented rice straw based diet, but for beef cattle fed fresh corn Stover based diet, Firmicutes and Bacteroidetes in rumen liquid represented 30.10–42.76% and 48.69–61.28% of the total reads respectively. Compared with the fresh corn Stover-fed cattle, the fermented rice straw-fed cattle had a lower relative abundance of Spirochaetae at d 30 ($P = 0.018$) (Fig. 1b), lower relative abundances of Actinobacteria ($P = 0.033$) and higher relative abundances of Fibrobacteres ($P = 0.035$) respectively at d 60 (Fig. 1c). At d 90, cattle fed fermented rice straw had lower relative abundances of Actinobacteria ($P = 0.001$) and Firmicutes ($P = 0.030$), higher relative abundances of Bacteroidetes ($P = 0.013$), Verrucomicrobia ($P = 0.022$) and Elusimicrobia ($P = 0.041$) than those fed fresh corn Stover respectively (Fig. 1d).

The composition and relative abundance of rumen fluid bacteria at the genus level are depicted in Fig. 2a. Rumen fluid bacteria with relative abundances > 0.5% and common to the cattle at various sampling times were: unclassified, *Prevotella, Succiniclasticum, Butyrivibrio, Ruminococcus,* and *Saccharofermentans*. Cattle fed fermented rice straw had higher relative abundances of *Ruminococcus* ($P = 0.023$) and *Lachnoclostridium* ($P = 0.020$), lower relative abundances of *Ruminiclostridium* ($P = 0.007$) at d 30 compared to those fed fresh corn Stover (Fig. 2b). At d 60, fermented rice straw-fed cattle had higher relative abundances of *Saccharofermentans* ($P = 0.036$), *Treponema* ($P = 0.043$) and *Ruminobacter* ($P = 0.043$) respectively, lower relative abundances of *Selenomonas* ($P = 0.035$), *Olsenella* ($P = 0.030$), *Desulfobulbus* ($P = 0.041$), *Desulfovibrio* ($P = 0.035$), *Denitrobacterium* ($P = 0.001$) and *Enhydrobacter* ($P = 0.010$) than those fed fresh corn Stover respectively (Fig. 2c). At d 90, cattle fed fermented rice straw had higher relative abundances of *Pseudobutyrivibrio* ($P = 0.008$) and lower relative abundances of *Acetitomaculum* ($P = 0.004$), *Mogibacterium* ($P = 0.022$), *Marvinbryantia* ($P = 0.009$), *Syntrophococcus* ($P = 0.014$), *Atopobium* ($P = 0.010$), *Olsenella* ($P = 0.022$), *Desulfobulbus* ($P = 0.048$) and *Howardella* ($P = 0.004$) than those fed fresh corn Stover respectively (Fig. 2d).

To assess relative differences between the fermented rice straw- and fresh corn Stover-fed cattle in terms of the predicted function of the microbiota in their rumen fluid, Tax4FUN was used to analyze their KEGG pathways. At the second KEGG level, no differences were detected between treatment groups in terms of the functions of their rumen fluid microbiota at d 30. At d 60, the rumen fluid of cattle fed fermented rice straw had higher relative abundances of microbiota involved in metabolism of terpenoid and polyketide ($P = 0.033$), amino acid metabolism ($P = 0.036$) and translation ($P = 0.049$) (Fig. 3a). At d 90, the rumen fluid of cattle fed fermented rice straw had higher relative abundances of microbiota involved in cell growth and death ($P = 0.040$) (Fig. 3b). At the third KEGG level, the rumen fluid of cattle fed fermented rice straw had higher relative abundances of microbiota involved in histidine metabolism ($P = 0.002$), cysteine and methionine metabolism ($P = 0.021$) and lysine biosynthesis ($P = 0.022$) at d 60 (Fig. 4). At d 90, the rumen fluid of cattle fed fermented rice straw had lower relative abundances of microbiota involved in glycolysis/gluconeogenesis ($P = 0.013$), *D*-alanine metabolism ($P = 0.044$) and propanoate metabolism ($P = 0.047$) (Fig. 5).

Identified differential metabolites and metabolic pathways. The metabolites identified in the rumen fluid samples from cattle fed fermented rice straw and fresh corn Stover are listed in Table 4. Sixteen metabolites were identified including thirteen from rumen fluid sampled at d 30 and three from rumen fluid sampled at d 90. Fourteen compounds (benzoic acid, hydroxycinnamic acid, azelaic acid, phenylacetic acid, D-arabitol, fumaric acid, salicylic acid, adipic acid, 4-hydroxybenzoic acid, 2-methylglutaric acid, pimelic acid, 3-phosphoglycerate, xanthine, and adenosine) were upregulated and two compounds (threonine and glutamic acid) were downregulated in the rumen fluids of cattle fed fermented rice straw compared with those fed fresh

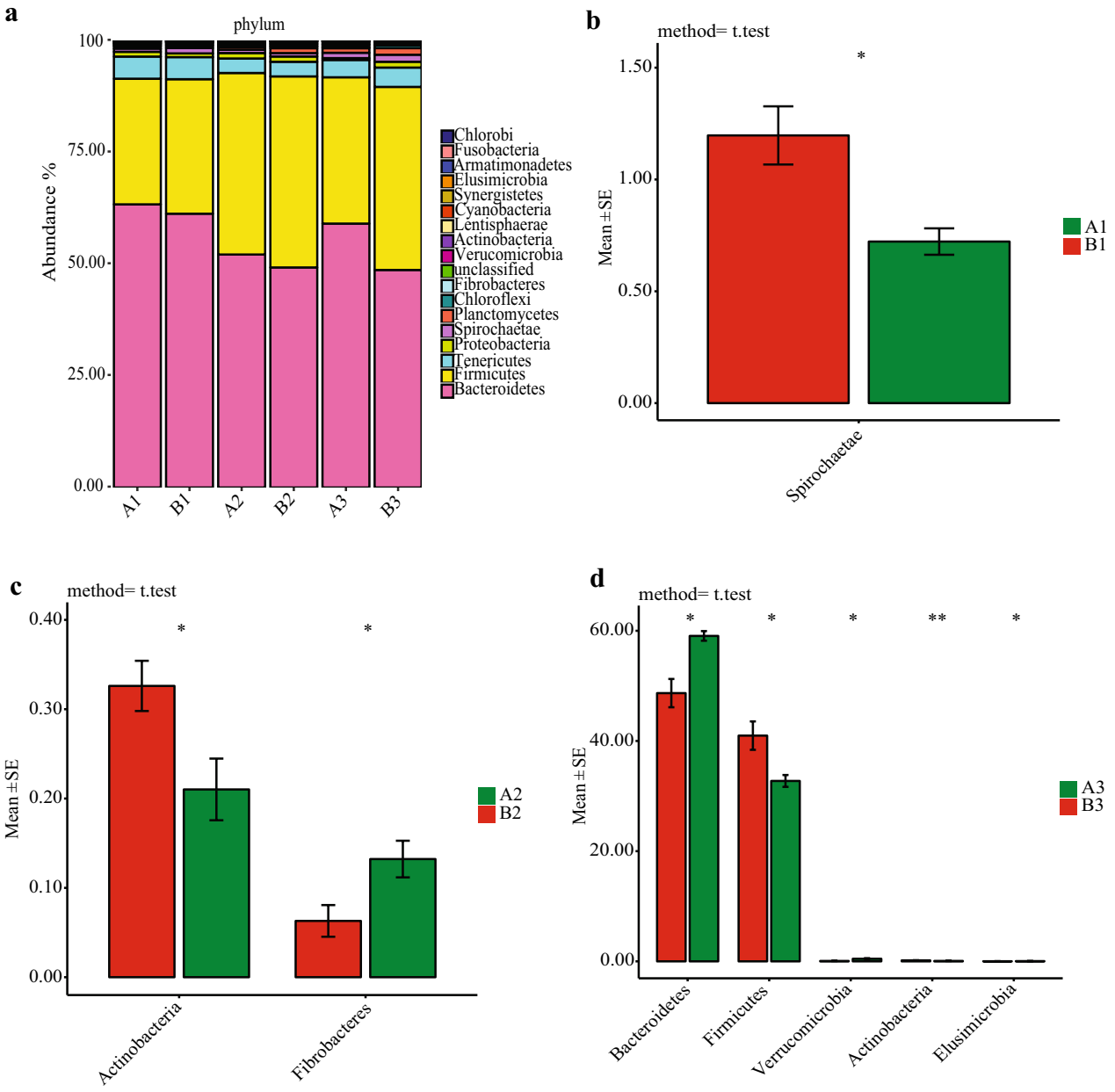

Figure 1. Changes of bacterial composition in fermented rice straw group and fresh corn Stover group. Bacterial composition at phylum level (**a**). The bacteria with significant difference in phylum level at d 30 (**b**), d 60 (**c**), d 90 (**d**) of feeding.

corn Stover. The metabolic pathway enrichment analysis in Table 5 shows that the metabolites identified in the rumen fluid of the cattle fed fermented rice straw and fresh corn Stover participate primarily in amino acid metabolism followed by carbohydrate metabolism, cofactors and vitamins metabolism, nucleotide metabolism and biosynthesis of other secondary metabolites.

Correlation analysis between differential bacteria and differential metabolites. Metabolites and bacteria significantly differing between treatment groups at d 30 and d 90 were used in a Spearman correlation analysis. At d 30 (Fig. 6a), the relative abundances of p-Spirochaetae and g-*Ruminiclostridium* were negatively correlated with levels of 2-methylglutaric acid ($P=0.029$ and $P=0.006$), 4-hydroxybenzoic acid ($P=0.013$ and $P=0.048$), *D*-arabitol ($P=0.011$ and $P=0.008$), phenylacetic acid ($P=0.016$ and $P=0.013$), and benzoic acid ($P=0.011$ and $P=0.029$), respectively. The relative abundance of p-Spirochaetae was negatively correlated with fumaric acid level ($P=0.038$). The relative abundance of g-*Ruminiclostridium* was positively correlated with threonine level ($P=0.048$). The relative abundance of g-*Ruminococcus* was positively correlated with levels of 2-methylglutaric acid ($P=0.008$), 4-hydroxybenzoic acid ($P=0.025$), adipic acid ($P=0.006$), salicylic acid ($P=0.043$), phenylacetic acid ($P=0.033$) and benzoic acid ($P=0.033$), respectively. At d 90 (Fig. 6b), the relative abundances of p-Actinobacteria, g-*Atopobium*, and g-*Olsenella* were negatively correlated with levels of 3-phosphoglycer-

Items	A1	B1	A2	B2	A3	B3
Firmicutes	28.12 ± 0.76	30.10 ± 0.25	40.63 ± 0.92	42.76 ± 1.57	32.75 ± 0.48	40.97 ± 1.15
Bacteroidetes	63.37 ± 0.55	61.28 ± 0.29	52.15 ± 0.77	49.25 ± 1.82	59.07 ± 0.39	48.69 ± 1.15
Planctomycetes	0.49 ± 0.02	0.48 ± 0.05	0.55 ± 0.03	1.09 ± 0.15	1.02 ± 0.07	1.47 ± 0.10
Tenericutes	4.96 ± 0.15	4.94 ± 0.11	3.24 ± 0.08	3.27 ± 0.09	3.88 ± 0.25	4.33 ± 0.15
Proteobacteria	1.07 ± 0.13	0.83 ± 0.03	1.22 ± 0.05	1.18 ± 0.06	0.41 ± 0.04	1.30 ± 0.19
Spirochaetae	0.72 ± 0.03	1.20 ± 0.06	0.73 ± 0.03	0.77 ± 0.03	1.20 ± 0.02	1.57 ± 0.13
Lentisphaerae	0.15 ± 0.02	0.10 ± 0.01	0.23 ± 0.01	0.16 ± 0.03	0.08 ± 0.01	0.06 ± 0.01
Unclassified	0.12 ± 0.01	0.12 ± 0.02	0.20 ± 0.02	0.40 ± 0.03	0.18 ± 0.02	0.45 ± 0.04
Fibrobacteres	0.26 ± 0.02	0.35 ± 0.03	0.13 ± 0.01	0.06 ± 0.01	0.50 ± 0.07	0.20 ± 0.02
Chloroflexi	0.21 ± 0.02	0.27 ± 0.04	0.21 ± 0.02	0.43 ± 0.05	0.24 ± 0.04	0.61 ± 0.12
Verrucomicrobia	0.14 ± 0.01	0.11 ± 0.01	0.25 ± 0.07	0.12 ± 0.02	0.47 ± 0.05	0.07 ± 0.00
Actinobacteria	0.05 ± 0.00	0.06 ± 0.01	0.21 ± 0.02	0.33 ± 0.01	0.07 ± 0.01	0.18 ± 0.01
Synergistetes	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
Cyanobacteria	0.23 ± 0.03	0.05 ± 0.01	0.16 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
Armatimonadetes	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Elusimicrobia	0.02 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.02 ± 0.00
Fusobacteria	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chlorobi	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3. The relative abundance of rumen fluid bacteria for the phyla level between two groups. A: rumen fluid sample collected from beef cattle fed fermented rice straw based diet. B: rumen fluid sample collected from beef cattle fed fresh corn Stover based diet. 1, 2, 3 means samples collected at d 30, d 60 and d 90, respectively. The data in the table are expressed as "Mean ± SE".

ate ($P = 0.009$, $P = 0.008$ and $P = 0.013$), xanthine ($P = 0.029$, $P = 0.029$ and $P = 0.022$) and adenosine ($P = 0.002$, $P = 0.006$ and $P = 0.001$), respectively. The relative abundances of p-Verrucomicrobia and g-*Pseudobutyrivibrio* were positively correlated with levels of 3-phosphoglycerate ($P = 0.001$ and $P = 0.002$), xanthine ($P = 0.006$ and $P = 0.002$) and adenosine ($P = 0.002$ and $P = 0.029$), respectively. The relative abundance of p-Bacteroidetes was positively correlated with levels of 3-phosphoglycerate ($P = 0.043$) and adenosine ($P = 0.048$), respectively. The relative abundance of p-Firmicutes was negatively correlated with levels of 3-phosphoglycerate ($P = 0.029$) and adenosine ($P = 0.013$), respectively.

Discussion

The rumen is a complex microecosystem that processes complex dietary carbohydrates into VFAs such as acetate, propionate, and butate[19], which are the main energy sources for ruminants[20]. Data indicated that fermentation efficiency may be improved by increasing VFA and decreasing methane (CH_4) production[21] and increasing rumen VFA levels is a concern when ruminants are fed basal diets comprising cereal residues[22]. Rumen VFA production is contingent upon the chemical properties of cereal residues and the microbial communities in the rumen[23] and treatment of cereal residues with various additives may degrade the lignocellulose complexes into simpler, readily fermentable materials[24]. Many studies demonstrated that treatment of wheat straw with enzymes enhanced neutral detergent fiber (NDF) digestion and fermentation[25], addition of probiotics and fibrolytic enzymes to paddy straw diet significantly improved nutrient degradability and total rumen VFA[26] and use of fibrolytic enzymes improved the ruminal fermentation characteristics of rice straw[27] and increased the apparent digestibility of neutral and acid detergent fiber of rice straw based total mixed ration[28]. It is reported that the cuticle wax silica layer and lignin impede microbial and enzymatic cellulose and hemicellulose degradation[10] and the cuticle wax silica layer must be removed before enzymatic digestion and microbial attachment can proceed[29]. Data showed that the cuticle wax silica layer on straw may be removed by fermentation with *Streptomyces griseorubens* C-5[30] and lignocellulose can be hydrolyzed by acetic acid treatment[31]. Rice straw has higher lignin content and thicker cuticle wax silica layers than fresh corn Stover[12], this results in a lower palatability and digestibility compared rice straw to corn Stover[32], some studies reported that ruminants had higher dry matter intake and nutrients digestibility when fed treated rice straw compared to untreated rice straw[33,34]. Data in this study indicated that relative to beef cattle fed a diet based on untreated fresh corn Stover, cattle fed a diet based on fermented rice straw with probiotics and enzymes had numerically higher dry matter intake ($P = 0.433$) and significantly higher levels of acetic acid and propionate in rumen liquid at d 60 ($P = 0.022$, $P = 0.043$) and d 90 ($P = 0.023$, $P = 0.014$), respectively. This increased levels of acetic acid and propionate should be related to the increase in dry matter intake and fermentable sugars of fermented rice straw, some experiments found that rice straw can release more fermentable surgars after a biotreatment[35], solid-state fermentation of fibrous materials by *Bacillus subtilis* can lower cellulose, hemicellulose and lignin levels and raise arabinose, xylose and glucose content[36], Bacilli can also synthesize pectin lyase to break down pectin[37].

Dietary composition influences both rumen VFA level and microbial community structure[38–40]. Previous studies showed that Firmicutes and Bacteroidetes predominated at the phylum level and there were relatively more Bacteroidetes than Firmicutes in the rumen fluid of animals fed high-forage or high-concentrate diets[41–46]. These

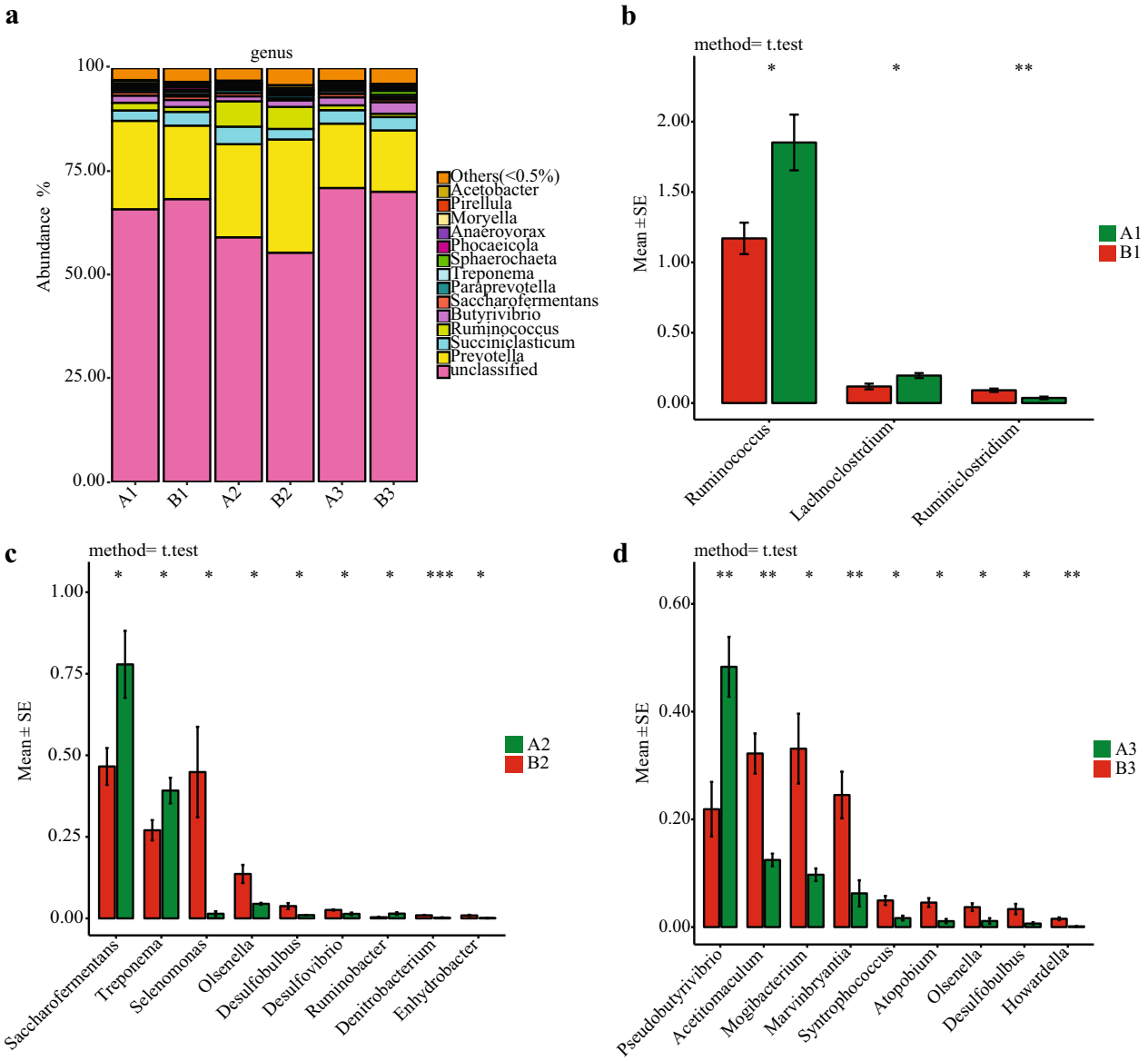

Figure 2. Changes of bacterial composition in fermented rice straw group and fresh corn Stover group. Bacterial composition at genus level (**a**). The bacteria with significant difference in genus level at d 30 (**b**), d 60 (**c**), d 90 (**d**) of feeding.

observations are consistent with our findings. The present study indicated that Firmicutes and Bacteroidetes comprised > 90% of all bacterial populations in rumen fluid. Cattle fed fermented rice straw had higher proportions of Bacteroidetes and lower proportions of Firmicutes in their rumen fluid than those fed fresh corn Stover. One possible explanation is that cattle fed fermented rice straw has a higher dry matter intake than those fed fresh corn Stover, because the proportions of Bacteroidetes in buffalo rumen fluid increased with the increased fibous materials intake[47]. Firmicutes participate in carbohydrate digestion and fiber component (cellulose and hemicellulose) utilization[41,48]. However, it is mainly the Bacteroidetes that degrade complex polysaccharides (cellulose, hemicellulose, pectin, and xylan) as they bear more genes encoding glycoside hydrolases and polysaccharide lyases than the Firmicutes and other bacterial phyla[49–51]. Bacteroidetes also degrade oligosaccharides[52] and proteins[48] by secreting carbohydrate-digesting enzymes[50] and dipeptidyl peptidases[53]. Comparatively more xylan was released by the hemicellulose breakdown during rice straw fermentation and it accelerated Bacteroidetes growth[54]. For this reason, cattle fed fermented rice straw had higher Bacteroidetes levels in their rumen fluid than those fed fresh corn Stover. Bacteroidetes ferment complex carbohydrates into acetic acid and propionate[55]. Thus, there was greater acetic acid and propionate production in the rumen fluid of fermented rice straw-fed cattle than in that of fresh corn Stover-fed cattle as the former had relatively higher numbers of Bacteroidetes and Fibrobacteres. With increasing Bacteroidetes relative abundances, the use of H_2 increases, resulting propionate levels increase and methane levels decrease[2]. Maximizing the flow of metabolic hydrogen away from methane and towards propionate might increase the efficiency of feed energy conversion in the rumen[56]. The rumen fluid of fermented rice straw-fed cattle had more Bacteroidetes than that of fresh corn Stover-fed cattle, thus,

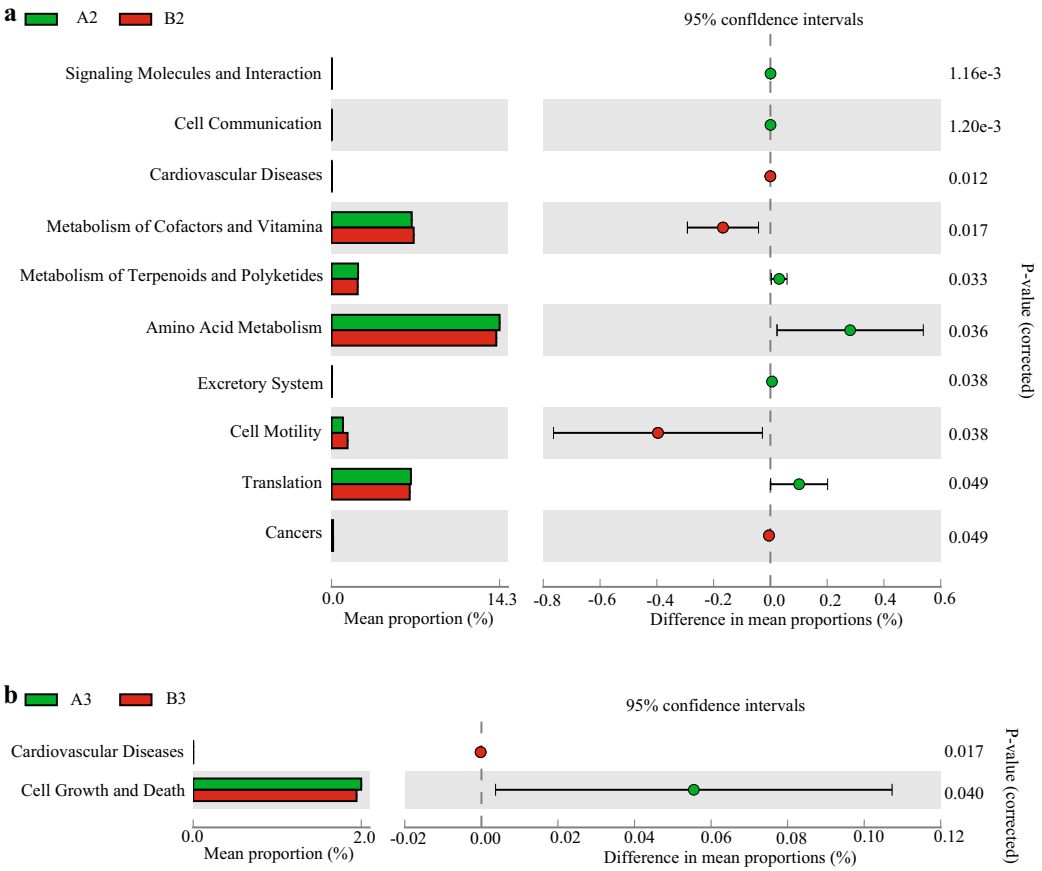

Figure 3. Bacteria function prediction in the rumen fluid of fermented rice straw group and fresh corn Stover group. The second level of KEGG pathway were showed in extended error bar at d 60 (**a**) and d 90 (**b**) of feeding.

fermented rice straw may more effectively improve the efficiency of feed energy conversion in the rumen than fresh corn Stover. Moreover, cattle fed fermented rice straw had higher proportions of Fibrobacteres ($P=0.035$) and lower proportions of Spirochaetae ($P=0.018$) and Actinobacteria ($P=0.033$ and $P=0.001$) than those fed fresh corn Stover. The Fibrobacteres comprise highly efficient cellulolytic bacteria that break down cellulose, xylan, and cellobiose and ferment their degradation products to acetic acid[57,58]. The proportions of short chain fatty acids (SCFAs) produced increase with the number of Fibrobacteres[59]. Spirochaetae and Actinobacteria produce several enzymes that break down plant biomass into simple sugars or fatty acids[60,61]. Nevertheless, these phyla had only minor impacts on the total VFA level in the rumen fluid as their total abundance for both treatment groups was < 1.6%.

Here, *Prevotella* were the dominant bacteria in rumen fluid. However, there was no significant difference in *Prevotella* abundance between treatments ($P=0.293$, $P=0.386$ and $P=0.783$). Cattle fed fermented rice straw with lower fiber (NDF and ADF) content had higher relative abundances of *Prevotella* than those fed fresh corn Stover with higher fiber (NDF and ADF) content. These findings aligned with an earlier report stating that high concentrate levels in the ruminant diet promoted *Prevotella* growth[62]. In contrast, goats fed high-fiber diets had higher relative *Prevotella* abundance in their rumens than those fed low-fiber diets[63]. *Prevotella* secrete hemicellulolytic and proteolytic enzymes[64] and degrade polysaccharides such as xylan, pectin, and starch[65]. *Prevotella* activity is positively associated with butyrate level[13]. Therefore, cattle fed fermented rice straw had higher butyric acid levels in their rumen fluid than those fed fresh corn Stover at d 60 ($P=0.108$) and d 90 ($P=0.255$), respectively.

Cattle fed fermented rice straw also had significantly higher numbers of *Ruminococcus* ($P=0.023$), *Saccharofermentans* ($P=0.036$), *Treponema* ($P=0.043$), *Lachnoclostridium* ($P=0.020$), *Ruminobacter* ($P=0.043$), and *Pseudobutyrivibrio* ($P=0.008$) in their rumen fluid than those fed fresh corn Stover. *Ruminococcus, Saccharofermentans,* and *Pseudobutyrivibrio* are anaerobic cellulolytic bacteria that ferment cellulose, hemicelluloses, and other polysaccharides into acetate and succinate[66–69]. Certain *Ruminococcus* and *Saccharofermentans* species can shift from succinate to acetate and propionate[67,69], *Ruminococcus* and *Prevotella* secrete enzymes that convert fumaric acid into succinic acid[70,71]. Here, it was determined that the *Ruminococcus* level was positively correlated with the fumaric acid content. Fumaric acid inhibits rumen methane production[72]. Cattle fed fermented rice straw had higher fumaric acid levels in their rumen than those fed fresh corn Stover. Thus, fermented rice straw may more effectively lower methane generation and improve feed energy conversion efficiency than fresh corn Stover. Methane production decreases with pH and is completely inhibited at pH < 6.0[73]. In the present study,

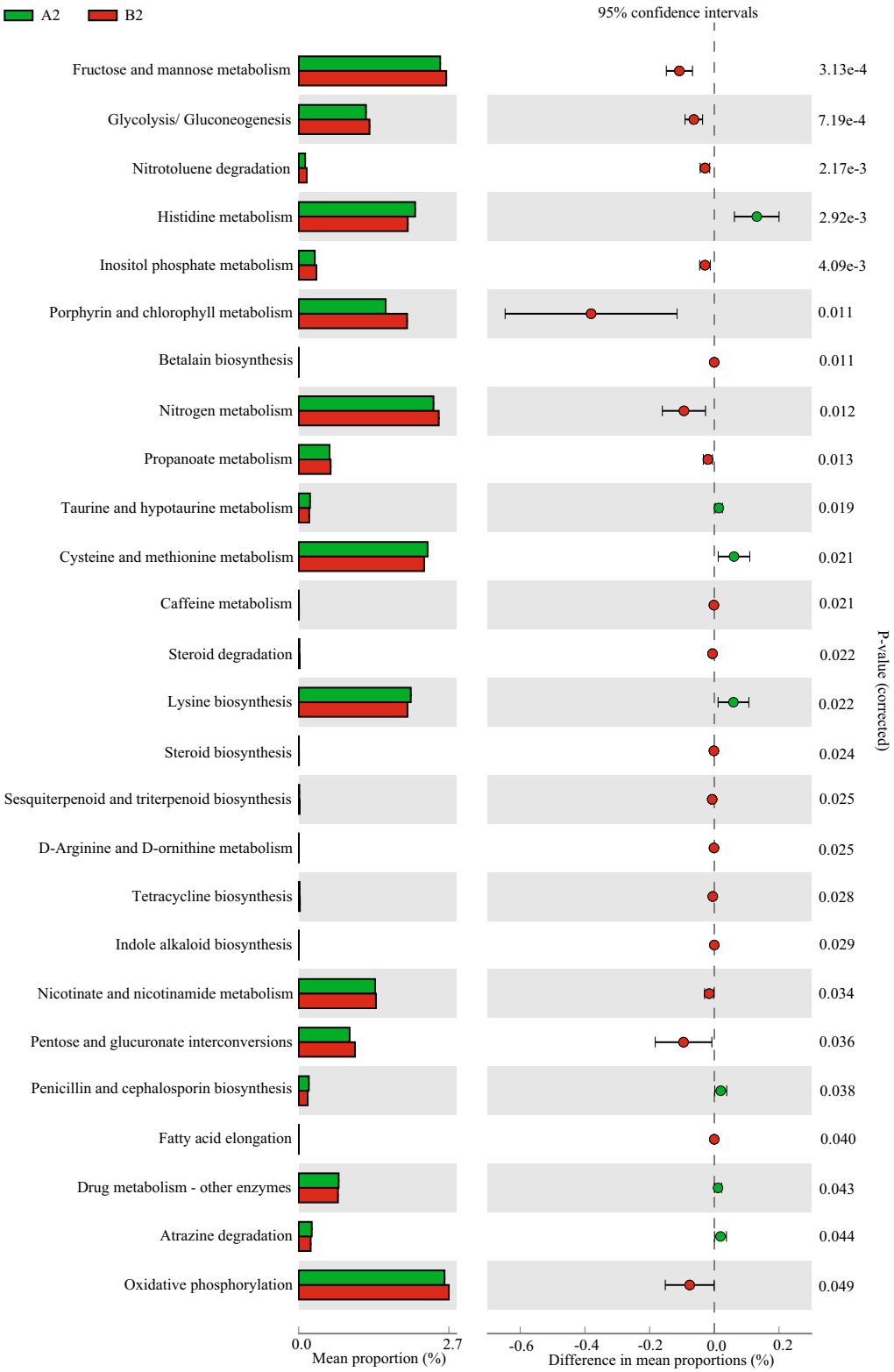

Figure 4. Bacteria function prediction in the rumen fluid of fermented rice straw group and fresh corn Stover group. The third level of KEGG pathway were showed in extended error bar at d 60 of feeding.

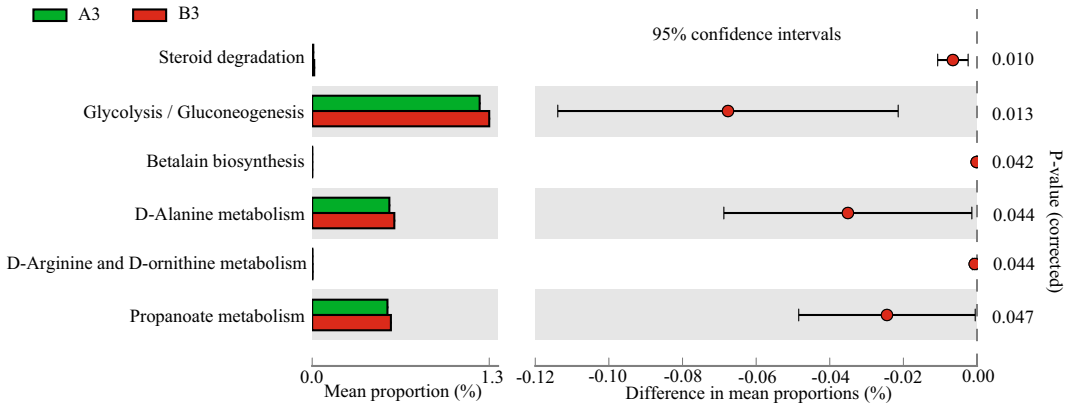

Figure 5. Bacteria function prediction in the rumen fluid of fermented rice straw group and fresh corn Stover group. The third level of KEGG pathway were showed in extended error bar at d 90 of feeding.

Sampling time	Differential metabolites	Fermented rice straw group (Group A)	Fresh corn Stover group (Group B)	Fold change	*P*
d 30 of feeding	Benzoic acid	1.94 ± 0.37	0.70 ± 0.08	2.77	0.040
	Hydrocinnamic acid	100.45 ± 19.49	40.55 ± 8.90	2.48	0.037
	Azelaic acid	5.17 ± 1.07	1.82 ± 0.21	2.84	0.047
	Phenylacetic acid	17.66 ± 4.33	3.50 ± 0.83	5.05	0.042
	D-arabitol	0.53 ± 0.10	0.18 ± 0.02	2.94	0.035
	Fumaric acid	0.87 ± 0.17	0.34 ± 0.05	2.56	0.049
	Threonine	0.04 ± 0.02	0.19 ± 0.05	0.21	0.045
	Salicylic acid	0.19 ± 0.03	0.08 ± 0.01	2.38	0.029
	Adipic acid	0.31 ± 0.06	0.10 ± 0.02	3.10	0.034
	Glutamic	0.02 ± 0.02	0.18 ± 0.05	0.11	0.037
	Hydroxybenzoic acid	0.07 ± 0.01	0.02 ± 0.00	3.50	0.047
	Methylglutaric acid	0.03 ± 0.01	0.01 ± 0.00	3.00	0.039
	Pimelic acid	0.20 ± 0.04	0.06 ± 0.01	3.33	0.033
d 90 of feeding	Adenosine	0.16 ± 0.04	0.03 ± 0.02	5.33	0.037
	Xanthine	1.67 ± 0.40	0.45 ± 0.17	3.71	0.036
	Phosphoglycerate	0.04 ± 0.01	0.01 ± 0.01	4.00	0.017

Table.4. Differential metabolites identified in rumen fluid.

Metabolism pathway	Differential metabolites
Amino acid metabolism	Benzoic acid, Phenylacetic acid, fumaric acid, hydrocinnamic acid, salicylic acid, 3-Hydroxybenzoic acid, glutamic, threonine
Carbohydrate metabolism	Fumaric acid, glutamic, D-arabitol
Cofactors and vitamins metabolism	Fumaric acid, glutamic, pimelic acid
Nucleotide metabolism	Adenosine, xanthine
Biosynthesis of other secondary metabolites	Xanthine

Table.5. Enrichment analysis of KEGG pathway of differential metabolites.

the rumen fluid of the fermented rice straw-fed cattle had a lower pH than that of the fresh corn Stover-fed cattle. *Pseudobutyrivibrio* degrade hemicellulose and ferment carbohydrates to butyric acid[74]. The proportion of *Pseudobutyrivibrio* was higher in the rumen fluid of the fermented rice straw-fed cattle than in that of the fresh corn Stover-fed cattle. For this and other reasons, the butyric acid content was higher in the rumen fluid of fermented rice straw-fed cattle than in that of fresh corn Stover-fed cattle.

Treponema degrade pectin, xylan, and fructan and hydrolyze cellobiose, xylose, arabinose, and galacturonic acid[75–77]. Pectin fermentation by pectinolytic bacteria such as *Treponema* yields acetate[78]. *Treponema* and *Prevotella* grow faster on high-pectin than low-pectin media[77,79]. The pectin content in rice straw is higher than that in

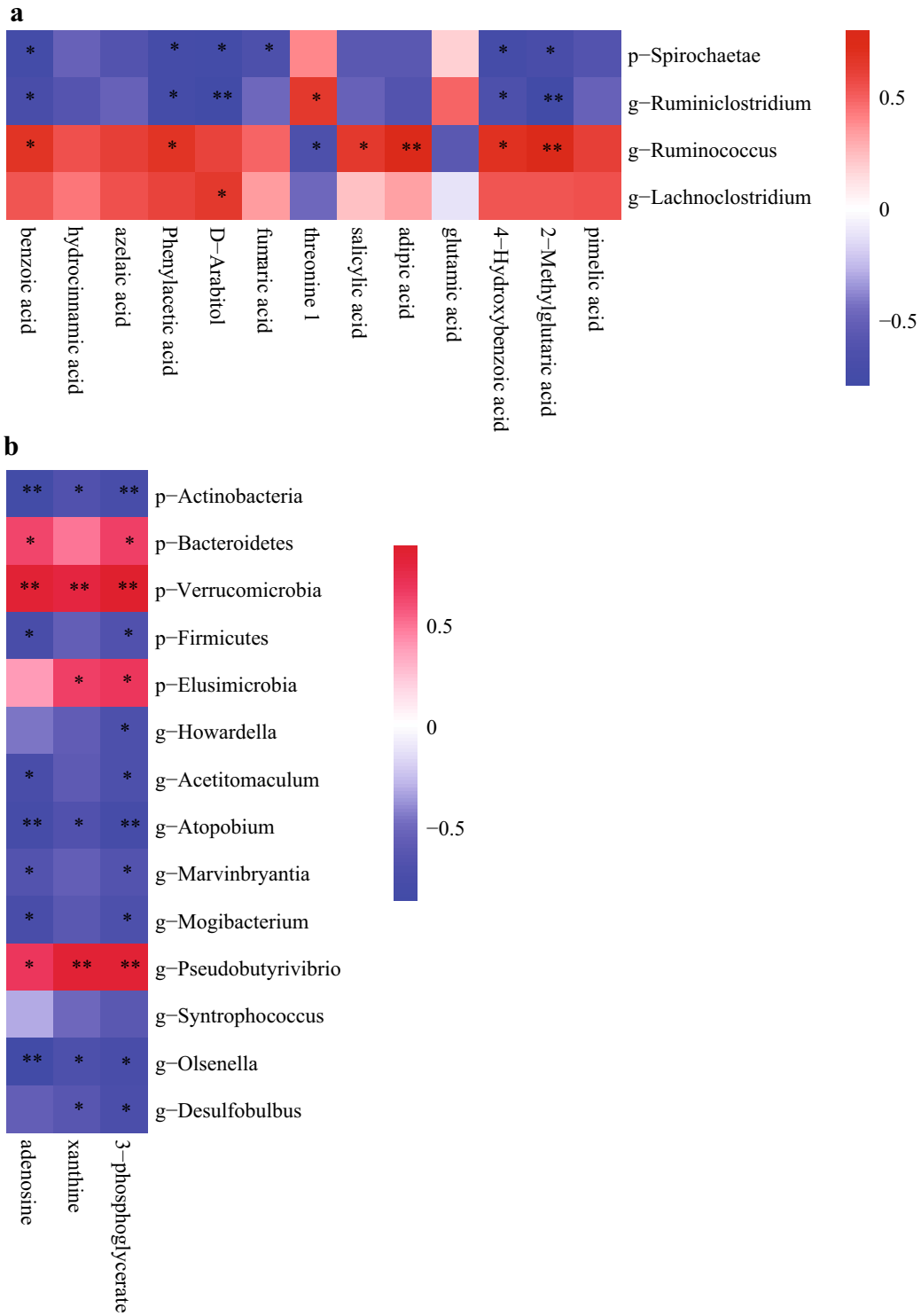

Figure 6. Correlation between difference bacteria and difference metabolite in the rumen fluid of fermented rice straw group and fresh corn Stover group at 30 (**a**) and 90 (**b**) days of feeding. The color was according to the Spearman correlation coefficient distribution. Asterisks indicate significant difference ($P < 0.05$).

corn Stover. Moreover, fermentation releases free pectin from the carbohydrate polymer. Thus, there was a higher level of free pectin in fermented rice straw than fresh corn Stover. Consequently, cattle fed fermented rice straw had higher numbers of *Treponema* and *Prevotella* in their rumen fluid than cattle fed fresh corn Stover. *Lachnoclostridium* are anaerobic bacteria that degrade cellulose and related plant cell wall polysaccharides to simple sugars that can be used as substrates for microbial growth and fermentation[80]. *Ruminobacter* are amylolytic bacteria and their growth is promoted in the rumens of cattle fed diets high in starch and fermentable sugars and low in fiber[81].

To avoid lowering the pH below 5.5, *Ruminobacter* ferment carbohydrates into formate, acetate, propionate, and succinate instead of lactic acid[82,83]. Rice straw fermented with *Bacillus subtilis, Enterococcus faecalis*, cellulase, and xylanase generated higher levels of fermentable sugars than fresh corn Stover. Thus, fermented rice straw more effectively enhanced *Ruminobacter* growth and maintained rumen pH balance than fresh corn Stover.

Studies demonstrated that phenylacetic acid can enhance cellulose degradation and growth of several strains of *Ruminococcus albus*[84,85], fumaric acid can increase the production of propionate[86,87] and benzoic acid not only can increase the production of propionate and butyric acid but also can improve the digestibility of energy and neutral detergent fibre[88]. Data in this experiment showed that beef cattle fed a diet based on fermented rice straw had significantly higher levels of phenylacetic acid, hydrocinnamic acid, azelaic acid, D-arabitol, fumaric acid, salicylic acid, benzoic acid, adipic acid, 4-hydroxybenzoic acid, 2-methylglutaric acid, pimelic acid, adenosine, xanthine and 3-phosphoglycerate compared beef cattle fed a diet based on fresh corn Stover. The increased levels of phenylacetic acid, fumaric acid and benzoic acid didn't significantly increase the propionate production ($P = 0.809$) but the increased levels of phenylacetic acid and benzoic acid significantly elevated the relative abundance of g-*Ruminococcus* ($P = 0.033$ and $P = 0.033$) at d 30 in the rumen liquid of beef cattle fed fermented rice straw based diet compared with beef cattle fed fresh corn Stover based diet.

Conclusions

Feeding rice straw co-fermented with probiotics and enzymes, instead of fresh corn Stover to beef cattle altered the ruminal bacterial community toward increased relative abundance of p-Fibrobacteres, p-Bacteroidetes, g-*Ruminococcus*, g-*Lachnoclostridium*, g-*Pseudobutyrivibrio*, g-*Saccharofermentans*, g-*Treponema* and g-*Ruminobacter*, elevated the rumen metabolites toward *Ruminococcus* growth and propionate production, changed the rumen fermentation pattern from acetate to propionate.

Methods

Preparation of fermented rice straw and fresh corn Stover. A starter culture was prepared and it consisted of *Bacillus subtilis* (viable cell count 1.0×10^{11} CFU g^{-1}, Jiangxi Xinwei Biotechnology Co., Ltd, China), *Enterococcus faecalis* (viable cell count 1.0×10^{11} CFU g^{-1}, Jiangxi Xinwei Biotechnology Co., Ltd, China), cellulase (activity 1.0×10^{4} U g^{-1}, Shandong Xindeli biology Co., Ltd, China), and xylanase (activity 5.0×10^{4} U g^{-1}, Shandong Xindeli biology Co., Ltd, China) at a 1:1:10:30 ratio. Then 6.2 g starter culture, 75 g brown sugar, and 15 g sodium chloride (NaCl) were dissolved in 1.3 kg water to ferment 1 kg rice straw at ~ 13% moisture content. Rice straw was chopped into segments 3–5 cm long, placed in polypropylene bags[89], and weighed, then the fermentation fluid was sprayed uniformly on the rice straw at a 1:1.3 rice straw:fluid ratio. The air was evacuated from the bags and they were sealed with plastic cable ties. The sealed bags were stored for 14 d before their contents were fed to the cattle. Fresh corn Stover was chopped into segments 3–5 cm long then fed as is to the cattle.

Animals and experimental design. All experimental procedures involving animal care and sampling were approved by the Ethics Committee for Animal Experimentation of Jiangxi Agricultural University and all methods were performed in accordance with the relevant guidelines and regulations. Twenty Simmental hybrid bulls at 13–14 months of age were randomly divided into two groups based on the initial average body weight (369.60 ± 18.53 kg vs. 371.50 ± 15.84 kg, $P = 0.939$) and fed ad libitum, each group had ten bulls. One group was fed rice straw co-fermented with probiotics and enzymes as roughage while the other group received fresh corn Stover as roughage. The trial ran for 90 days. Composition and nutrient levels of the basal diet are as follows (Table 6).

Sample collection. On the day of 30, 60 and 90 in experimental period, about 100 mL rumen fluid was collected at 8:00 am with an esophageal tube vacuum pump sampling device (Anscitech, Wuhan Kelibo Equipment co. Ltd., Wuhan, China) from each animal and delivered into a 500-mL beaker. The rumen fluid was separated into solid and liquid fractions by pressing it through four layers of muslin cloth. The pH of rumen liquid was immediately measured, 8-mL liquid aliquots were placed in seven 10-mL sterile centrifuge tubes and all tubes with rumen liquid samples were immediately frozen in liquid nitrogen and stored at −80 °C until subsequent 16S rRNA sequencing and metabolomics analysis.

Chemical analysis. Rumen liquid portion pH was measured with a digital pH meter (Leici PHB-4; Shanghai INESA Scientific Instrument Co. Ltd., Shanghai, China) precalibrated with standard pH buffers. VFAs were analyzed by gas chromatography (GC-2014; Shimadzu Corp., Kyoto, Japan) according to a previously published method[90]. Lactic acid concentration was determined with an assay kit according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Sequencing of rumen microbiota. Genomic DNA was isolated from rumen liquid portion according to a previously published method[91]. PCR amplification was performed with the universal primers 515F and 926R and targeted the V4–V5 regions of bacterial 16S rRNA[92]. The PCR products were collected with an AxyPrep-DNA gel recovery kit (Aisijin Biotechnology Co. Ltd., Hangzhou, China) and sequenced on Miseq 2×300-bp platform (Illumina, San Diego, CA, USA).

GC–TOF/MS analysis. Metabolites in samples of the rumen liquid portion from the animals in both treatment groups were compared by GC–TOF/MS according to a previously published method[93]. Brief procedures are as follows: Firstly, sample was mixed with methanol-chloroform and ribitol in EP tube by vortexing and

	Fermented rice straw group (Group A)	Fresh corn Stover group (Group B)
Ingredients		
Corn	16	16
Soybean	2.8	2.8
Premix[1]	0.8	0.8
$NaHCO_3$	0.2	0.2
Nacl	0.2	0.2
Brewer's grains	40	40
Fermented rice straw	40	0
Fresh corn Stover	0	40
Nutrient levels[2]		
DM	42.23	37.79
NEmf (MJ/kg)	4.92	5.18
CP	10.03	11.25
NDF	33.18	34.81
ADF	15.67	17.43

Table.6. Composition and nutrient levels of the basal diet (air-dry basis) % [1]Per kg of premix included the following: VitA 200 000 IU, VitD3 25 000 IU, VitE 4 000 IU, Fe 3,500 mg, Mn 2000 mg, Zn 1,500 mg, Cu 550 mg, I 30 mg, Se 15 mg, Co 15 mg, Ca 150 g, P 60 g. [2] NEmf were calculated values, while others were measured values.

extracted, then EP tube was centrifuged and supernatant was removed from EP tube and pipetted into a 2 mL glass vial. Secondly, glass vial with supernatant was dried and incubated with methoxymethyl amine salt, another incubation followed with Bis-(trimethylsilyl)-trifluoroacetamide, then the glass vial was mixed with fatty acid methyl esters for GC–TOF–MS analysis. The GC–TOF–MS analysis was performed using an Agilent 7,890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer according to the manufacture's instruction.

Statistical analysis. *Rumen fluid analysis.* Differences in the mean pH, VFA, and lactic acid concentrations between groups were analyzed by *t*-test in SPSS v. 17.0 (IBM Corp., Armonk, NY, USA). Data are means ± SEM. $P < 0.05$ was considered statistically significant.

16S rRNA sequence analysis. Data were filtering according to a previously published method[94]. High-quality sequences were subjected to OTU (operational taxonomic unit) analysis in Usearch v. 7.1[95]. OTUs with > 97% similarity were clustered[96]. The OTU clusters were compared against the Silva 128 database to identify the microbial classifications of the representative OTU sequences. Significant differences between treatment groups in terms of their microorganism profiles were identified by *t*-tests. $P < 0.05$ was considered statistically significant. Based on the precalculated Silva 128 database, Tax4FUN v. 0.3.1 (https://tax4fun.gobics.de/)[97] was run on the abundance predictions of the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs and bacterial pathways[98,99]. A two-sided Welch's *t*-test was used for two-group bacterial functional prediction analyses. Functional differences between treatment groups were compared by Statistical Analysis of Metagenomic Profiles (STAMP)[100].

GC–MS data acquisition and analysis. Chroma TOF v. 4.3X software (https://www.lecosoftware.com/chromatof) and the LECO-Fiehn Rtx5 database (LECO Corporation, St. Joseph, MI, USA) were used to identify raw peaks, filter and calibrate data baselines, align and identify the peaks, integrate their areas, and perform a deconvolution analysis[101]. Mass spectrum and retention index matching were considered in metabolite identification. Peaks detected in < 50% and RSD in > 30% of the QC samples were removed[102]. Differential metabolite screening was conducted using previously published methods[93] and peaks with similarity greater than 700, variable importance projection (VIP) exceeding 1.0 and $P < 0.05$ by *t*-test were selected as the reliable differentially expressed metabolites. KEGG database was used to search for differential metabolite pathways[103,104].

Correlation analyses of microbiomes and metabolomes. The cor.test function in R v. 3.5.1 was used to calculate the Spearman correlation coefficients among differential bacteria and differential metabolites in the rumen fluid of the treatment groups at d 30 and d 90, statistical significance was set at $P < 0.05$. The pheatmap package v. 1.0.12 (https://CRAN.R-project.org/package=pheatmap) was used to plot the correlation heatmap among the different bacteria and metabolites.

Received: 9 October 2019; Accepted: 10 June 2020
Published online: 01 July 2020

References

1. Cottle, D. J., Nolan, J. V. & Wiedemann, S. G. Ruminant enteric methane mitigation: a review. *Anim. Prod. Sci.* **51**, 491 (2011).
2. Huang, X. D. *et al.* Methanogen diversity in indigenous and introduced ruminant species on the Tibetan Plateau. *Archaea*. 1–10 (2016).
3. Martinez-Fernandez, G. *et al.* Methane inhibition alters the microbial community, hydrogen flow, and fermentation response in the rumen of cattle. *Front. Microbiol.* **7**, 1122 (2016).
4. Denman, S. E., Martinez-Fernandez, G., Shinkai, T., Mitsumori, M. & McSweeney, C. S. Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog. *Front. Microbiol.* **6**, 1087 (2015).
5. Spanghero, M., Zanfi, C., Fabbro, E., Scicutella, N. & Camellini, C. Effects of a blend of essential oils on some end products of in vitro rumen fermentation. *Anim. Feed Sci. Technol.* **145**, 364–374 (2008).
6. Owens, D., McGee, M., Boland, T. & O'Kiely, P. Rumen fermentation, microbial protein synthesis, and nutrient flow to the omasum in cattle offered corn silage, grass silage, or whole-crop wheat 1. *J. Anim. Sci.* **87**, 658–668 (2009).
7. Boadi, D., Benchaar, C., Chiquette, J. & Massé, D. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Can. J. Anim. Sci.* **84**, 319–335 (2004).
8. Hash, C. T. *et al.* Opportunities for marker-assisted selection (mas) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crop Res.* **84**, 88 (2003).
9. Barana, D., Salanti, A., Orlandi, M., Ali, D. S. & Zoia, L. Biorefinery process for the simultaneous recovery of lignin, hemicelluloses, cellulose nanocrystals and silica from rice husk and Arundo Donax. *Ind. Crop. Prod.* **86**, 31–39 (2016).
10. Mosier, N. *et al.* Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **96**, 673–686 (2005).
11. Wang, J. K., Liu, J. X., Li, J. Y., Wu, Y. M. & Ye, J. A. Histological and rumen degradation changes of rice straw stem epidermis as influenced by chemical pretreatment. *Anim. Feed Sci. Technol.* **136**, 51–62 (2007).
12. Shawky, B. T., Mahmoud, M. G., Ghazy, E. A., Asker, M. M. S. & Ibrahim, G. S. Enzymatic hydrolysis of rice straw and corn stalks for monosugars production. *J. Gen. Eng. Biotechnol.* **9**, 59–63 (2011).
13. Li, Z. P. *et al.* Bacterial community composition and fermentation patterns in the rumen of sika deer (*Cervus nippon*) fed three different diets. *Microb. Ecol.* **69**, 307–318 (2015).
14. Hughes, S. A., Shewry, P. R., Gibson, G. R., Mccleary, B. V. & Rastall, R. A. In vitro fermentation of oat and barley derived β-glucans by human faecal microbiota. *FEMS Microbiol. Ecol.* **64**, 482–493 (2008).
15. Wu, Y. M., Liu, J. X., Liu, D. & Lu, J. M. Effects of addition of cellulase-xylanase based enzyme and/or wheat bran on the quality of corn Stover and rice straw silages and on their digestibility by sheep. *Chin. J. Vet.* **24**, 298–303 (2004).
16. Wadhwa, M., Kaur, K. & Bakshi, M. P. Effect of naturally fermented rice straw based diet on the performance of buffalo calves. *Indian J. Anim. Sci.* **80**, 249–252 (2010).
17. Han, R. *et al.* Milk fatty acid profiles in Holstein dairy cows fed diets based on corn Stover or mixed forage. *Arch. Anim. Nutr.* **68**, 63–71 (2014).
18. Chumpawadee, S., Sommart, K., Vongpralub, T. & Pattarajinda, V. Nutritional evaluation of crop residues and selected roughages for ruminants using in vitro gas production technique. *Chiang. Mai. J. Sci.* **33**, 371–380 (2006).
19. Bainbridge, M. L., Cersosimo, L. M., Wright, A. D. G. & Kraft, J. Rumen bacterial communities shift across a lactation in Holstein, Jersey, and Holstein x Jersey dairy cows and correlate to rumen function, bacterial fatty acid composition, and production parameters. *FEMS Microbiol. Ecol.* **92**, fiw059 (2016).
20. Chaput, J. P., Thivierge, M. C. & Tremblay, A. Propionate: hypophagic effects observed in animal models might be transposed to the human obesity management. *Curr. Nutr. Food Sci.* **2**, 375–379 (2006).
21. Tan, H. Y. *et al.* Effects of condensed tannins from leucaena on methane production, rumen fermentation and populations of methanogens and protozoa in vitro. *Anim. Feed Sci. Technol.* **169**, 193 (2011).
22. Wanapat, M. & Rowlinson, P. Nutrition and feeding of swamp buffalo: feed resources and rumen approach. *Italian J. Anim. Sci.* **6**, 67–73 (2007).
23. Mohammed, R. *et al.* Bacterial communities in the rumen of Holstein heifers differ when fed orchardgrass as pasture vs. hay. *Front. Microbiol.* **5**, 689 (2014).
24. Abd El-Tawab, M. M., Youssef, I. M. I., Bakr, H. A., Fthenakis, G. C. & Giadinis, N. D. Role of probiotics in nutrition and health of small ruminants. *Polish. J. Vet. Sci.* **19**, 893–906 (2016).
25. Rodrigues, M. *et al.* Effect of enzyme extracts isolated from white-rot fungi on chemical composition and in vitro digestibility of wheat straw. *Anim. Feed Sci. Technol.* **141**, 326–338 (2008).
26. Sheikh, G. G., Ganai, A. M., Ishfaq, A., Afzai, Y. & Ahmad, H. A. In vitro effect of probiotic mix and fibrolytic enzyme mixture on digestibility of paddy straw. *Adv. Anim. Vet. Sci.* **5**, 260–266 (2017).
27. Sujani, S., Piyasena, T., Seresinhe, T., Pathirana, I. & Gajaweera, C. Supplementation of rice straw (*Oryza sativa*) with exogenous fibrolyticenzymes improves in vitro rumen fermentation characteristics. *Turk. J. Vet. Anim. Sci.* **41**, 25–29 (2017).
28. Yuangklang, C. *et al.* Growth performance and macronutrient digestion in goats fed a rice straw based ration supplemented with fibrolytic enzymes. *Small Ruminant Res.* **154**, S0921448817301682 (2017).
29. Abraham, A., Mathew, A. K., Sindhu, R., Pandey, A. & Binod, P. Potential of rice straw for bio-refining: an overview. *Bioresour. Technol.* **215**, 29–36 (2016).
30. Xu, J. & Yang, Q. Isolation and characterization of rice straw degrading streptomyces griseorubens C-5. *Biodegradation* **21**, 107 (2010).
31. Krishania, M., Kumar, V. & Sangwan, R. Integrated approach for extraction of xylose, cellulose, lignin and silica from rice straw. *Bioresour. Technol. Rep.* **1**, 89–93 (2018).
32. Omer, H. A. A. *et al.* Nutritional impact of partial or complete replacement of clover hay by untreated or biologically treated rice straw and corn stalks on: 1. Growth performance and economic evaluation of growing New Zealand (NZW) White rabbits. *Bull. Natl. Res. Centrol* **43**, 192 (2019).
33. Wanapat, M., Kang, S., Hankla, N. & Phesatcha, K. Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. *Afr. J. Agr. Res.* **8**, 1677–1687 (2013).
34. Elmenofy, E. K. *et al.* Improving the nutritive value of ensiled green rice straw 2—in vitro gas production. *Nat. Sci.* **10**, 86–91 (2012).
35. Fonseca, B. G., Mateo, S., López, A. J. M. & Roberto, I. Biotreatment optimization of rice straw hydrolyzates for ethanolic fermentation with scheffersomyces stipitis. *Biomass. Bioenergy* **112**, 19–28 (2018).
36. Liu, P. *et al.* Dietary corn bran fermented by bacillus subtilis MA139 decreased gut cellulolytic bacteria and microbiota diversity in finishing pigs. *Front. Cell Infect. Mi.* **7**, 526 (2017).
37. Raj, K. D., Kumar, S. S. & Tewari, R. Enhanced production of pectinase by bacillus Sp. DT7 using solid state fermentation. *Bioresour. Technol.* **88**, 251–254 (2003).
38. Pitta, D. W. *et al.* Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microb. Ecol.* **59**, 511–522 (2010).

39. Almeida, P. N. M. *et al.* Aerobic fungi in the rumen fluid from dairy cattle fed different sources of forage. *Rev. Braz. Zoote.* **41**, 2336–2342 (2012).
40. Akinbode, R. M., Isah, O. A., Oni, A. O., Adewumi, O. O. & Omoniyi, L. A. Effect of different tropical roughages on nutrient digestibility and rumen fermentation parameters of West African dwarf sheep during dry season. *Indian J. Anim. Sci.* **84**, 1105–1108 (2014).
41. Brulc, J. M. *et al.* Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. *Proc. Natl. Acad. Sci. USA* **106**, 1948–1953 (2009).
42. Fernando, S. C. *et al.* Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl. Environ. Microb.* **76**, 7482–7490 (2010).
43. Petri, R. M. *et al.* Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. *PLoS ONE* **8**, e83424 (2013).
44. Pitta, D. W. *et al.* Bacterial diversity associated with feeding dry forage at different dietary concentrations in the rumen contents of Mehshana Buffalo (Bubalus Bubalis) Using 16S Pyrotags. *Anaerobe* **25**, 31–41 (2014).
45. McCann, J. C. *et al.* Relationship between the rumen microbiome and residual feed intake-efficiency of brahman bulls stocked on bermudagrass pastures. *PLoS ONE* **9**, e91864 (2014).
46. Myer, P., Smith, T., Wells, J., Kuehn, L. & Freetly, H. Rumen microbiome from steers differing in feed efficiency. *PLoS ONE* **10**, e129174 (2015).
47. Pitta, D. W. *et al.* Bacterial diversity dynamics associated with different diets and different primer Pairs in the Rumen of Kankrej Cattle. *PLoS ONE* **9**, e111710 (2014).
48. Huo, W., Zhu, W. & Mao, S. Impact of subacute ruminal acidosis on the diversity of liquid and solid-associated bacteria in the rumen of goats. *World J. Microbiol. Biotechnol.* **30**, 669–680 (2014).
49. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
50. Thomas, F., Hehemann, J., Rebuffet, E., Czjzek, M. & Michel, G. Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* **2**, 93 (2011).
51. Walker, A. W. *et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISEM J.* **5**, 220–230 (2011).
52. Leser, T. D. *et al.* Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl. Environ. Microb.* **68**, 673–690 (2002).
53. Walker, J. A., Kilroy, G. E., Xing, J., Shewale, J. & Batzer, M. A. Human DNA quantitation using Alu element-based polymerase chain reaction. *Anal. Biochem.* **315**, 122–128 (2003).
54. Carlos, C., Fan, H. & Currie, C. R. Substrate shift reveals roles for members of bacterial consortia in degradation of plant cell wall polymers. *Front. Microbiol.* **9**, 364 (2018).
55. Ozbayram, E., Kleinsteuber, S., Nikolausz, M., Ince, B. & Ince, O. Enrichment of lignocellulose-degrading microbial communities from natural and engineered methanogenic environments. *Appl. Microbiol. Biot.* **102**, 1035–1043 (2018).
56. Ungerfeld, E. M. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. *Front. Microbiol.* **6**, 37 (2015).
57. Joshua, M. & Daniel, B. G. Digestion of cell-wall monosaccharides of ryegrass and alfalfa hays by the ruminal bacteria fibrobacter succinogenes and butyrivibrio fibrisolvens. *Can. J. Microbiol.* **39**, 780–786 (1993).
58. Emma, R. J., Jones, D. L., McCarthy, A. J. & McDonald, J. E. The fibrobacteres: an important phylum of cellulose-degrading bacteria. *Microb. Ecol.* **63**, 267–281 (2012).
59. Deng, Y. F. *et al.* Influence of dairy by-product waste milk on the microbiomes of different gastrointestinal tract components in pre-weaned dairy calves. *Sci Rep.* **7**, 42689 (2017).
60. Shivlata, L. & Tulasi, S. Thermophilic and alkaliphilic actinobacteria: biology and potential applications. *Front. Microbiol.* **6**, 1014 (2015).
61. Nuli, R., Cai, J., Kadeer, A., Zhang, Y. & Mohemaiti, P. Integrative analysis toward different glucose tolerance-related gut microbiota and diet. *Front Endocrinol.* **10**, 295 (2019).
62. Luo, D. *et al.* Niacin alters the ruminal microbial composition of cattle under high-concentrate condition. *Chin. J. Anim. Nutr.* **3**, 180–185 (2017).
63. Grilli, D. J. *et al.* Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. *Anaerobe* **42**, 17–26 (2016).
64. Matsui, H. *et al.* Phenotypic characterization of polysaccharidases produced by four prevotella type strains. *Curr. Microbiol.* **41**, 45–49 (2000).
65. Kabel, M. A. *et al.* Biochemical characterization and relative expression levels of multiple carbohydrate esterases of the xylanolytic rumen bacterium prevotella ruminicola 23 grown on an ester-enriched substrate. *Appl. Environ. Microb.* **77**, 5671–5681 (2011).
66. Chassard, C., Delmas, E., Robert, C., Lawson, P. A. & Bernalier, D. A. *Ruminococcus champanellensis* Sp. Nov., a cellulose-degrading bacterium from human gut microbiota. *Int. J. Syst. Evol. Microbiol.* **62**, 138 (2012).
67. Li, R. W., Connor, E. E., Li, C., Baldwin, R. L. & Sparks, M. E. Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environ. Microbiol.* **14**, 129–139 (2012).
68. Wegmann, U. *et al.* Complete genome of a new firmicutes species belonging to the dominant human colonic microbiota ('Ruminococcus Bicirculans') reveals two chromosomes and a selective capacity to utilize plant glucans. *Environ. Microbiol.* **16**, 2879 (2014).
69. La Reau, A. J. & Suen, G. The Ruminococci: key symbionts of the gut ecosystem. *J. Microbiol.* **56**, 199–208 (2018).
70. Hopgood, F. M. & Walker, J. D. Succinic acid production by rumen bacteria. III. Enzymic studies on the formation of succinate by Ruminococcus Flavefaciens. *Aust. J. Biol. Sci.* **22**, 1413–1424 (1969).
71. Song, H. & Lee, S. Y. Production of succinic acid by bacterial fermentation. *Enzyme Microb. Technol.* **39**, 352–361 (2006).
72. Yuan, Z. P. *et al.* Inhibition of methanogenesis by tea Saponin and tea Saponin plus disodium fumarate in sheep. *J. Anim. Feed Sci.* **16**, 560–565 (2007).
73. Russell, J. B. The importance of ph in the regulation of ruminal acetate to propionate ratio and methane production in vitro. *J. Dairy Sci.* **81**, 3222 (1998).
74. Kopec˘ny, J., Zorec, M., Zek, J. M., Kobayashi, Y. & Marins˘ek-Logar, R. Butyrivibrio Hungatei Sp. Nov. And Pseudobutyrivibrio Xylanivorans Sp. Nov., butyrate-producing bacteria from the Rumen. *Int. J. Syst. Evol. Microbiol.* **53**, 201–209 (2003).
75. Piknova, M. *et al.* Treponema Zioleckii Sp. Nov., A Novel Fructan-utilizing species of rumen treponemes. *FEMS Microbiol. Lett.* **289**, 166–172 (2010).
76. Bekele, A. Z., Koike, S. & Kobayashi, Y. Phylogenetic diversity and dietary association of rumen treponema revealed using group-Speci¢c16S rRNA gene-based analysis. *FEMS Microbiol. Lett.* **316**, 51–60 (2011).
77. Svartström, O. *et al.* Ninety-nine de novo assembled genomes from the moose (Alces Alces) rumen microbiome provide new insights into microbial plant biomass degradation. *ISME J.* **11**, 2538–3255 (2017).
78. Dusková, D. & Marounek, M. Fermentation of Pectin and Glucose, and Activity of Pectin-Degrading Enzymes in the Rumen Bacterium Lachnospira Multiparus. *Lett. Appl. Microbiol.* **33**, 159–163 (2010).

79. Liu, J., Pu, Y., Xie, Q., Wang, J. & Liu, J. Pectin induces an in vitro rumen microbial population shift attributed to the pectinolytic treponema group. *Curr. Microbiol.* **70**, 67 (2015).
80. Ravachol, J. *et al.* Combining free and aggregated cellulolytic systems in the cellulosome-producing bacterium Ruminiclostridium Cellulolyticum. *Biotechnol. Biofuels* **8**, 114 (2015).
81. Nurmeiliasari, N., Priyanto, R., Astuti, D. A., Salundik, & Takahashi, J. Utilization of rumen mechanical stimulator as pseudo fiber in ruminant to minimize metabolic problem. *Indonesian Bull. Anim. Vet. Sci.* **27**, 67–80 (2017).
82. Cotta, M. A. Interaction of Ruminal bacteria in the production and utilization of Maltooligosaccharides from starch. *Appl. Environ. Microb.* **58**, 48–54 (1992).
83. González, A. C., Barraza, M. B., Viveros, J. D. & Martínezb, A. C. Rumen microorganisms and fermentation. *Arch. Med. Vet.* **46**, 349–361 (2014).
84. Stack, R. J., Hungate, R. E. & Opsahl, W. P. Phenylacetic acid stimulation of cellulose digestion by Ruminococcus albus 8. *Appl. Environ. Microbiol.* **46**, 539–544 (1983).
85. Stack, R. J. & Cotta, M. A. Effect of 3-phenylpropanoic acid on growth of, and cellulose utilization by, cellulolytic ruminal bacteria. *Appl. Environ. Microbiol.* **52**, 209–210 (1986).
86. Castro-Montoya, J., Campeneere, S. D., Ranst, G. V. & Fievez, V. Interactions between methane mitigation additives and basal substrates on in vitro methane and vfa production. *Anim. Feed. Sci. Technol.* **176**, 47–60 (2012).
87. Li, Z. J. *et al.* Effects of fumaric acid supplementation on methane production and rumen fermentation in goats fed diets varying in forage and concentrate particle size. *J. Anim. Sci. Biotechnol.* **9**, 21 (2018).
88. Bühler, K., Bucher, B., Wenk, C. & Broz, J. Influence of benzoic acid in high fibre diets on nutrient digestibility and VFA production in growing/finishing pigs. *Arch. Anim. Nutr.* **63**, 127–136 (2009).
89. Ogunade, I. M. *et al.* Bacterial diversity and composition of alfalfa silage as analyzed by illumina miseq sequencing: effects of, escherichia coli, o157:h7 and silage additives. *J Dairy Sci.* **101**, 1–12 (2017).
90. Li, L. Z. *et al.* Effects of Recombinant Swollenin on the enzymatic hydrolysis, Rumen fermentation, and rumen Microbiota during in vitro incubation of agricultural straws. *Int. J. Biol. Macromol.* **122**, 348–358 (2019).
91. Lima, F. S. *et al.* Prepartum and postpartum rumen fluid microbiomes: characterization and correlation with production traits in dairy cows. *Appl. Environ. Microb.* **81**, 1327–1337 (2015).
92. Kumar, A. *et al.* Impact of nutrition and rotavirus infection on the infant gut microbiota in a humanized pig model. *BMC Gastroenterol.* **18**, 93 (2018).
93. He, Y. Y. *et al.* Identification of differential metabolites in liquid diet fermented with bacillus subtilis using gas chromatography time of flight mass spectrometry. *Chin. J. Anim. Nutr.* **2**, 351–356 (2016).
94. Zhang, J., Kobert, K., Flouri, T. & Stamatakis, A. PEAR: a fast and accurate Illumina paired-end read merger. *Bioinformatics* **30**, 614 (2014).
95. Krumbeck, J. A. *et al.* In vivor selection to identify bacterial strains with enhanced ecological performance in synbiotic applications. *Appl. Environ. Microb.* **81**, 2455–2465 (2015).
96. Li, Y. *et al.* Intestinal Microbiome–metabolome responses to essential oils in piglets. *Front. Microbiol.* **9**, 1988 (2018).
97. Aßhauer, K. P., Wemheuer, B., Daniel, R. & Meinicke, P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* **31**, 2882–2884 (2015).
98. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucl. Acids Res.* **28**, 27–30 (2000).
99. Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucl. Acids Res.* **45**, D353–D361 (2017).
100. Parks, D. H., Tyson, G. W., Hugenholtz, P. & Beiko, R. G. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* **30**, 3123 (2014).
101. Kind, T. *et al.* FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal. Chem.* **81**, 10038–10048 (2009).
102. Dunn, W. B. *et al.* Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat. Protoc.* **6**, 1060–1083 (2011).
103. Kanehisa, M. *et al.* Data, information, knowledge and principle: back to metabolism in KEGG. *Nucl. Acids Res.* **42**, D199–D205 (2014).
104. Xia, J., Psychogios, N., Young, N. & Wishart, D. S. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucl. Acids Res.* **37**, W652–W660 (2009).

Acknowledgments

This research was funded by Jiangxi Mordern Agricultural Scientific Reaseach Cooperative Innovation Project (No. JXXTCX2016003-02).

Author contributions

Conceived and designed the experiments: W.L., Y.Y.H. Performed the experiments: Y.Q.H., Y.Y.H., S.G., Z.L., L.L., H.Z., Q.C., T.L., H.G., W.L. Analyzed the data: Y.Q.H., W.L. Wrote the paper: W.L, Y.Y.H., Y.Q.H.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to W.L.