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Effects of Dietary Capsaicin and *Yucca schidigera* Extracts as Feed Additives on Rumen Fermentation and Microflora of Beef Cattle Fed with a Moderate-Energy Diet

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Abstract: Capsaicin (CAP) and *Yucca schidigera* extract (YSE) are two types of plant extracts that can change rumen fermentation. This study was conducted to investigate whether supplementation of beef cattle diets with CAP and YSE for 90 days would affect rumen fermentation and microflora. Forty-five healthy Angus steers (initial body weight = 510.54 ± 41.27 kg) were divided into three groups: control (CON), CAP, and YSE. Ammonia nitrogen (NH_3-N) and total volatile fatty acid (TVFA) concentrations were significantly higher in the YSE group than in the CON group and significantly lower in the CAP group than in the CON group. At the phylum level, YSE increased the relative abundances of *Bacteroidota* and *Patescibacteria* and reduced that of *Bacillota*. At the genus level, CAP and YSE both increased the relative abundances of genera subordinate to *Bacteroidota* and decreased the relative abundances of genera subordinate to *Bacillota*. Our study shows that YSE and CAP have different effects on rumen fermentation and microflora after long-term supplementation.

Keywords: capsaicin; *Yucca schidigera* extract; beef cattle; rumen fermentation; microbial population

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1. Introduction

The transformation of nutrients ingested by beef cattle mainly depends on the activities of microbial populations in the rumen. A large number of microorganisms, such as bacteria, methanogenic archaea, anaerobic fungi, and protozoa are present in the rumen [1], of which bacteria may be the most important group. Because of the large number of enzymes secreted by bacteria, fats, proteins, and most carbohydrates are broken down gradually and changed into nutritious products, primarily volatile fatty acids (VFAs) and microbial protein that promote the growth of beef cattle [2]. Bacteria contribute significantly to the degradation of fibrous carbohydrates. Specifically, rumen microbiota begin to colonize the feed shortly after it enters the rumen, and bacteria are the first group to adhere to the feed [3,4]. Bacteria that form a dense biofilm on the feed surface play a core role in the degradation of fibrous carbohydrates such as neutral detergent fiber and acid detergent lignin [5]. However, the fermentation process is bound to cause energy loss. The reduction of energy loss during the rumen fermentation process and improvement of feed utilization efficiency are research hotspots in ruminant nutrition.

Plant extracts are bioactive substances obtained using physical, chemical, or biological methods [6,7]. Recently, plant extracts have been used as feed additives for livestock because of their multiple biological functions such as anti-oxidant, anti-inflammatory effects, and immune regulation [8–10]. Previous studies have shown that plant extracts can regulate rumen fermentation and improve energy utilization [11]. Capsaicin (CAP) is a type of highly pungent vanillin alkaloid produced by hot peppers [12]. It has antibacterial and anti-inflammatory functions [13,14]. *Yucca schidigera* extract (YSE) is rich in steroidal

saponins, resveratrol, and other polyphenols. YSE improves rumen fermentation and inhibits cellulolytic bacteria and fungi because of effects similar to those of ionophores [15,16]. Inconsistencies regarding the effects of these extracts on ruminal fermentation and microbial populations evaluated in previous studies could be attributable to the type of diet and duration of the evaluation [17–19].

To our knowledge, long-term evaluations of the effects of these extracts on ruminal fermentation and microbial populations have not yet been performed. We hypothesized that long-term supplementation of CAP and YSE can change the rumen bacterial community structure of cattle fed a moderate-energy diet, thereby affecting rumen fermentation.

2. Materials and Methods

2.1. Diets, Animals, and Experimental Design

Forty-five healthy Angus steers with similar body weights (initial body weight = 510.54 ± 41.27 kg) were selected and divided into 3 treatment groups (15 animals in each group) according to the single-factor completely randomized design. The 3 dietary treatments were as follows: CON group (basal diet), CAP group (basal diet + 1.50 g/day/animal CAP), and YSE group (basal diet + 2.40 g/day/animal YSE). All animal experiments were approved by the Animal Welfare and Ethical Committee of China Agricultural University (Permit No. DK18030608) and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (The State Science and Technology Commission of P.R. China, 1988). CAP used in this study was supplied by Leader Bio-Technology Co., Ltd. (Guangzhou, China), and the main bioactive compound of CAP was natural capsaicin (≥0.50%). YSE was a brown powder extracted from the whole plant of *Yucca schidigera* and contained 10% *Yucca* saponin, and it was provided by Zhongnong Xingyuan Biotechnology Co., Ltd. (Beijing, China). The dosages of CAP and YSE were recommended by the manufacturers. The daily additives used in the test group were premixed with 1 kg of concentrate and then mixed with total mixed ration (TMR) by using a paddle mixer. The experiment lasted for 104 days with 14 days for adaptation, and it was separated into 2 stages with 2 different concentrate-to-forage ratios to meet the nutrient levels recommended by NASEM [20]. Diet formulation and nutrient composition (DM basis) of the basal diets are shown in Table 1.

Table 1. Diet formulation and nutrient composition (DM basis) of the basal diets.

Item	1–59 Days	60–90 Days
Ingredient (% of DM)		
Ground corn	31.90	37.70
Soybean meal	9.35	11.05
Jujube powder	8.25	9.75
Whole-plant corn silage	24.75	19.25
Corn stalker	20.25	15.75
Salt	1.10	1.30
Premix [1]	2.20	2.60
Calcium hydrophosphate	1.10	1.30
Sodium bicarbonate	1.10	1.30
Chemical composition		
CP, % of DM	11.33	11.94
NDF, % of DM	41.75	33.84
Ca, % of DM	0.50	0.52
P, % of DM	0.43	0.48
ME [2] MJ/kg DM	10.42	10.59

[1] Premix (per kg of DM) contains 150,000–450,000 IU vitamin A acetate, 40,000–120,000 IU vitamin D3, 400 mg DL-α-tocopherol acetate, 250–750 mg copper, 1000–5000 mg iron, 1000–3000 mg manganese, 1500–3700 mg of zinc, 10–25% calcium, 0.3% total phosphorus, and 15–30% sodium Chloride. [2] ME (metabolizable energy) was calculated and other components were determined by NASEM (2016) [20].

2.2. Feeding Management

This study was conducted at the beef cattle experimental base of China Agricultural University (116.09° E, 39.65° N). Before the experiment, the beef cattle were dewormed, weighed, and ear-labeled using the Hi-Hog squeeze chute (1556; Hi-Hog Farm & Ranch Equipment, Ltd., Calgary, AB, Canada). The feed intake of the beef cattle was recorded every day by using the automatic feed intake recording system. All steers were fed twice a day (09:00 and 16:00), allowed to feed *ad libitum*, and provided with clean water throughout the experimental period.

2.3. Ruminal Fluid Sample Collection and Measurement

An oral stomach tube was used to collect rumen fluid before morning feeding on the last day of the experiment. The initial 200 mL of rumen fluid was discarded. Then, the rumen fluid was filtered through 4 layers of cheesecloth, and the pH was recorded with a pH meter (PHSJ-4F; Shanghai Yidian Scientific Instrument Co., Ltd., Shanghai, China). The collected rumen fluid was sub-packed into 10 mL cryopreservation tubes and stored in a −80 °C refrigerator for subsequent analysis. The filtered rumen fluid was centrifuged at 8000× *g* and 4 °C for 15 min. The supernatant was used to determine the concentrations of volatile fatty acids (VFAs) and ammonia nitrogen (NH_3-N). The VFA profile was determined with GC 8600 gas chromatography, as reported by Erwin [21]; NH_3-N concentration was colorimetrically measured using the method described by Broderick and Kang [22].

2.4. DNA Extraction, PCR Amplification, and Sequencing

Total DNA was extracted from the rumen fluid samples of 45 Angus steers (15 for each treatment) by using the FastDNA® SPIN for Soil kit (MP Biomedicals, Solon, OH, USA), according to the manufacturer's protocol. The quality of all DNA samples was checked, and the concentration was quantified using NanoDrop 2000 spectrophotometers (Thermo Fisher Scientific, Wilmington, DE, USA). Bacterial 16S rRNA gene fragments (V3-V4) were amplified from the extracted DNA by using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and the following PCR conditions: 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C for 27 cycles. PCRs were performed with 4 µL of 5× TransStart FastPfu buffer, 2 µL of 2.5 mM deoxynucleoside triphosphates, 0.8 µL of each primer (5 µM), 0.4 µL of TransStart FastPfu DNA Polymerase, 10 ng of the extracted DNA, and ddH2O to make up a final volume of 20 µL. Agarose gel electrophoresis was performed to verify the size of amplicons, which were subjected to paired-end sequencing on the Illumina MiSeq sequencing platform by using PE300 at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

After demultiplexing, the resulting sequences were merged with FLASH (v1.2.7) [23] and filtered with fastp (0.19.6) [24]. The high-quality sequences were de-noised using DADA2 [25] plugin in the Qiime2 [26] (version 2020.2) pipeline with recommended parameters, resulting in single nucleotide resolution based on error profiles within samples. DADA2-denoised sequences are usually called amplicon sequence variants (ASVs). To minimize the effects of sequencing depth on alpha and beta diversity measurements, the number of sequences from each sample was rarefied to 5780, which yielded an average Good's coverage of 99.10%. Taxonomic assignment of ASVs was performed using the Naive Bayes consensus taxonomy classifier implemented in Qiime2 and SILVA 16S rRNA database (v. 138). Analyses of the 16S rRNA microbiome sequencing data were performed using the free online Majorbio Cloud Platform (cloud.majorbio.com, accessed on 22 March 2022).

2.5. Statistical Analysis

The data were preliminarily sorted using Excel 2010, and a one-way analysis of variance was performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA) with the following model:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the j observation (j = 1–15) in treatment i (i = control, CAP, and YSE), μ is the overall mean, τ_i is the effect of the treatment (denoted as an unknown parameter), and ε_{ij} is the random error with a mean of 0 and variance of σ^2. A p-value < 0.05 indicated a significant difference, $p < 0.01$ indicated an extremely significant difference, and $0.05 < p < 0.10$ indicated an increasing or decreasing trend.

The microbial data were analyzed on the free online Majorbio Cloud Platform (cloud.majorbio.com, accessed on 22 March 2022). Beta diversity distance measurements were performed using the weighted and unweighted UniFrac distance matrix in Qiime and visualized with principal coordinate analysis (PCoA). Alpha diversity (Sobs, Shannon, Ace, Chao, and Coverage) was measured using the Mothur software (version v.1.30, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity, accessed on 24 March 2022). The effects of CAP and YSE on rumen bacteria at the phylum and genus levels were evaluated using a non-parametric test (Kruskal–Wallis test). Each Angus steer was considered an experimental unit.

3. Results

3.1. Feed Intake and Rumen Fermentation Parameters

The effects of CAP and YSE on feed intake and rumen fermentation parameters of the Angus steers are shown in Table 2. No differences in feed intake were observed in the three treatment groups. The addition of CAP had significant effects on the rumen fermentation parameters, include NH_3-N concentration, TVFA concentration, isobutyric acid percentage, and isovaleric acid percentage (which decreased significantly; $p < 0.01$), when compared with the CON group. Meanwhile, significant differences were found in most of the parameters, except propionic acid percentage, valeric acid percentage, and Ac:Pr, when the YSE group was compared with the CON group. Specifically, pH and acetic acid percentage, isobutyric acid percentage, and isovaleric acid percentage were significantly lower in the YSE group than in the CON group ($p < 0.01$), whereas NH_3-N concentration, TVFA concentration, and butyric acid percentage were significantly higher in the YSE group than in the CON group ($p < 0.01$).

Table 2. Feed intake and rumen fermentation parameters of Angus steers fed with different diets.

Item	CON	CAP	YSE	SEM	*p*-Value
Feed intake					
Dry matter intake, kg/day	12.12	12.18	12.10	0.17	0.98
Fermentation characteristics					
pH	6.98 ^{a}	7.01 ^{a}	6.80 ^{b}	0.02	<0.01
NH_3-N, mg/100 mL	5.16 ^{b}	4.30 ^{c}	5.68 ^{a}	0.11	<0.01
TVFA, mmol/L	51.75 ^{b}	46.47 ^{c}	70.91 ^{a}	2.06	<0.01
Individual VFA, % of total VFA					
Acetic acid	70.42 ^{a}	71.11 ^{a}	69.19 ^{b}	0.25	<0.01
Propionic acid	15.81	15.83	15.96	0.11	0.83
Butyric acid	9.54 ^{b}	9.18 ^{b}	11.21 ^{a}	0.22	<0.01
Isobutyric acid	1.53 ^{a}	1.40 ^{b}	1.19 ^{c}	0.03	<0.01
Valeric acid	0.65 ^{ab}	0.60 ^{b}	0.68 ^{a}	0.01	0.04
Isovaleric acid	2.06 ^{a}	1.88 ^{b}	1.77 ^{b}	0.04	<0.01
Ac:Pr	4.46	4.50	4.34	0.04	0.21

Note: CON: control group, CAP: capsaicin group, YSE: *Yucca schidigera* extract group. NH_3-N: ammoniacal nitrogen, TVFA: total volatile fatty acid, Ac:Pr: acetic acid to propionic acid ratio. SEM: standard error of the mean. ^{abc} Mean values in the same row (corresponding to the same variable) with different letters differ significantly ($p < 0.05$).

3.2. Bacterial Abundance and Diversity in the Rumen

A total of 4371 ASVs were obtained after 16S rRNA high-throughput sequencing of 45 rumen fluid samples from Angus steers in the three treatment groups. The ASVs in each group were used to generate rarefaction curves, which were used to assess whether

the sequencing depth was sufficient (Supplementary Figure S1). As the number of sample reads increased, the identification rate of ASVs gradually decreased and then plateaued, indicating that the sequencing depth was sufficient to assess the major members of the rumen bacterial community.

The alpha diversity indexes of the rumen bacterial community of the Angus steers are presented in Table 3. The Sobs, Shannon, and ACE indexes of the CAP and YSE groups were all significantly higher than those of the CON group ($p < 0.01$). The coverage index was 1.00, indicating that the sequencing results can truly reflect the rumen microbial community.

Table 3. Alpha diversity indices of ruminal bacteria in Angus steers fed with different diets.

Item	Treatment			SEM	*p*-Value
	CON	CAP	YSE		
Sobs	323.47 ^{c}	538.60 ^{a}	428.00 ^{b}	15.32	<0.01
Shannon	5.37 ^{c}	5.89 ^{a}	5.63 ^{b}	0.04	<0.01
Ace	324.58 ^{c}	549.75 ^{a}	434.59 ^{b}	16.09	<0.01
Coverage	0.9942	0.9992	0.9965	0.0004	<0.01

Note: CON: control group, CAP: capsaicin group, YSE: *Yucca schidigera* extract group. Sobs: a number of ASVs; Shannon: to reflect the diversity and evenness of community species; Ace: to estimate the total number of species in the sample. Coverage: sequencing depth. SEM: standard error of mean. ^{abc} Mean values in the same row (corresponding to the same variable) with different letter differ significantly ($p < 0.05$).

The beta diversity of the rumen bacterial community was used to study the degree of similarity in the composition of the sampled communities. The contribution rates of PC1 and PC2 were 11.83% and 6.75%, respectively. The PCoA showed a clear distinction between the CON group and CAP and YSE groups (Figure 1).

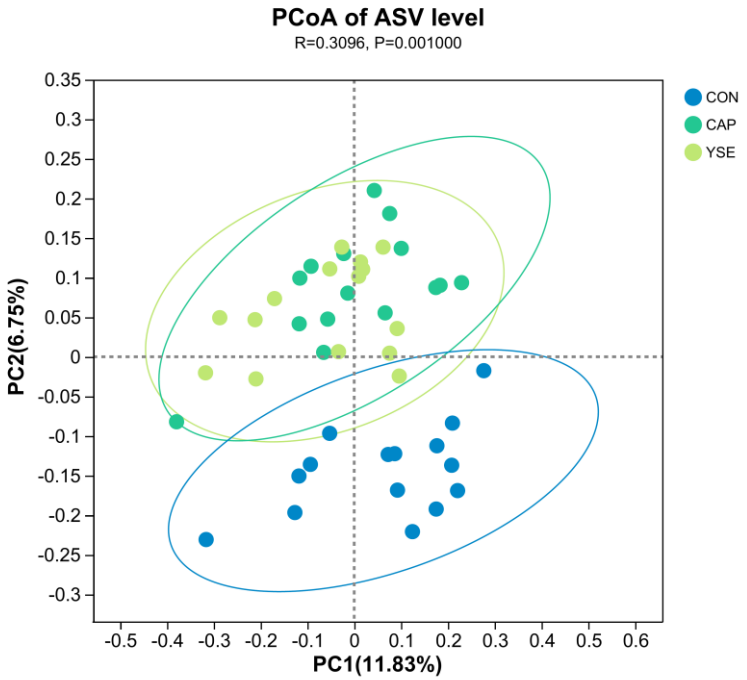
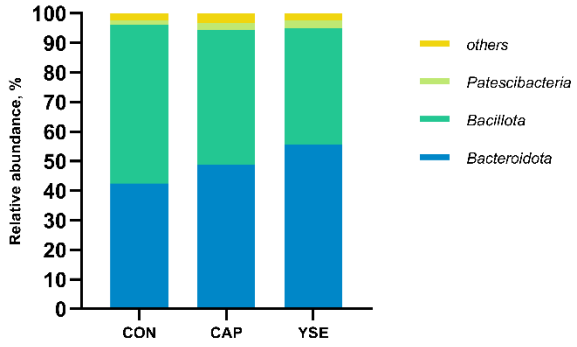


Figure 1. Plot for the principal coordinate analysis. A shorter distance between the sample points denotes greater similarity of the bacteria. CON: control group; CAP: capsaicin group; YSE: *Yucca schidigera* extract group.

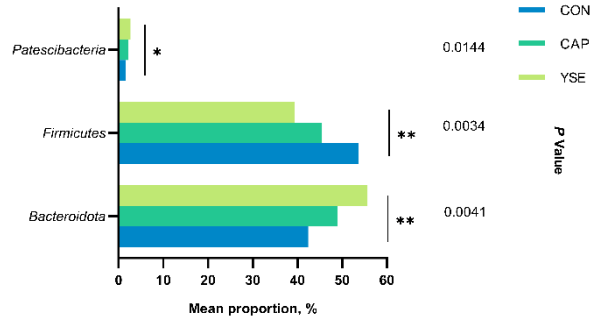
3.3. Rumen Bacterial Community Composition and Species Differences

Figure 2 depicts the composition of rumen bacterial communities and details of the intergroup differences in bacterial phyla and genera in terms of abundance. At the phylum

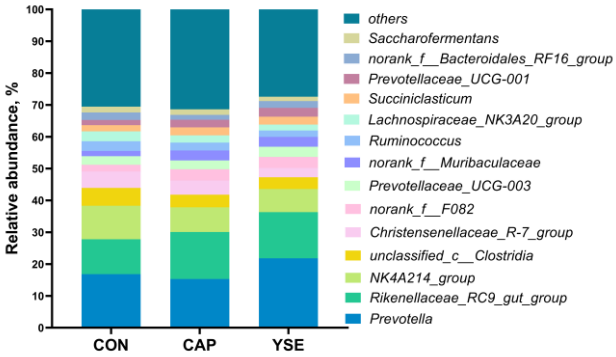
level, *Bacillota*, *Bacteroidota,* and *Patescibacteria* were the dominant phyla detected in the rumen bacterial community (Figure 2a). However, the relative abundances of *Bacillota*, *Bacteroidota,* and *Patescibacteria* were significantly different ($p < 0.05$) between the CON and YSE groups (Figure 2b). At the genus level, *Prevotella*, *Rikenellaceae_ RC9_ gut*_group, and *NK4A214_group* accounted for the highest proportion (Figure 2c). The bacterial structures of the CAP and YSE groups were similar, which could also be inferred from the PCoA. Specifically, the relative abundances of *NK4A214_group*, *unclassified_c__Clostridia*, and *norank_f__F082* were all different from the CON group ($p < 0.05$). However, YSE had a greater effect on the bacteria (Figure 2d), and the relative abundances of *Christensenellaceae_R-7_group*, *Ruminococcus*, *Lachnospiraceae_NK3A20_group*, and *Saccharofermentans* were all lower in the YSE group than in the CON group; CAP affected the relative abundance of only *Rikenellaceae_RC9_gut_group* ($p < 0.05$). Further, *Ruminococcus* was significantly enriched in the CON group and had a significant impact on the differences among the 3 groups (Figure 2e,f).



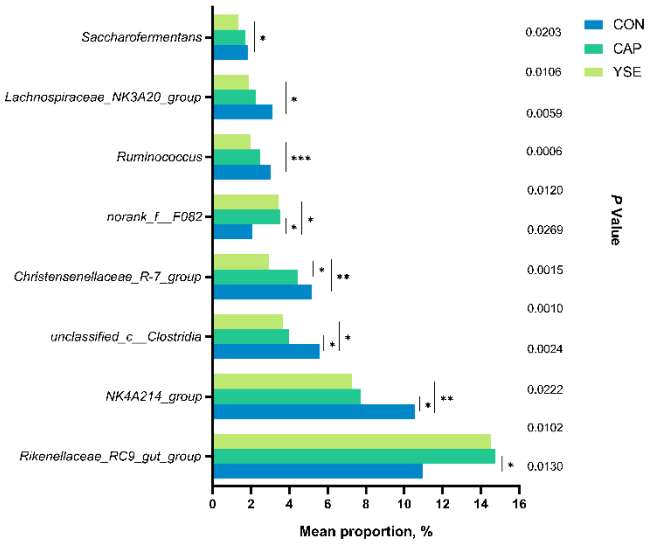
(**a**)



(**b**)

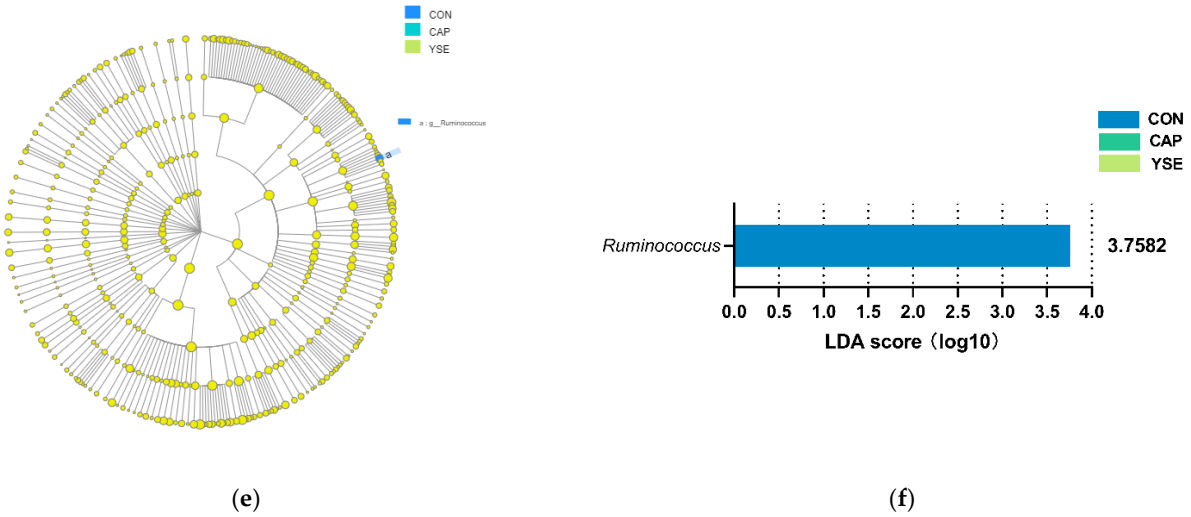


(**c**)



(**d**)

Figure 2. *Cont.*



(**e**) (**f**)

Figure 2. Relative abundance of rumen bacteria at (**a**) phylum and (**c**) genus levels in the 3 groups. Histogram of group difference test for rumen bacteria at (**b**) phylum and (**d**) genus levels. (**e**) LEfSe multilevel species hierarchy tree diagram; LDA > 3.5. (**f**) LDA discriminant result graph LDA > 3.5. CON: control group; CAP: capsaicin group; YSE: *Yucca schidigera* extract group. * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $p \leq 0.001$.

3.4. Correlation between Rumen Bacteria and Rumen Fermentation Parameters

On the basis of the Spearman correlation coefficients, genera with significant differences in the top 14 abundance rankings were correlated with the rumen fermentation parameters (Figure 3). We found that *Christensenellaceae_R-7_group* and *Saccharofermentans* were similarly correlated with the rumen fermentation parameters, except for NH_3-N concentration. Concurrently, *NK4A214_group*, *Christensenellaceae_R-7_group*, and *Lachnospiraceae_NK3A20_group* were negatively correlated with NH_3-N concentration, whereas *Prevotella* was positively correlated with NH_3-N concentration ($p < 0.05$). Moreover, similar to *Christensenellaceae_R-7_group* and *Saccharofermentans*, *NK4A214_ group* and *unclassified_c__Clostridia* was positively correlated with pH value ($p < 0.05$).

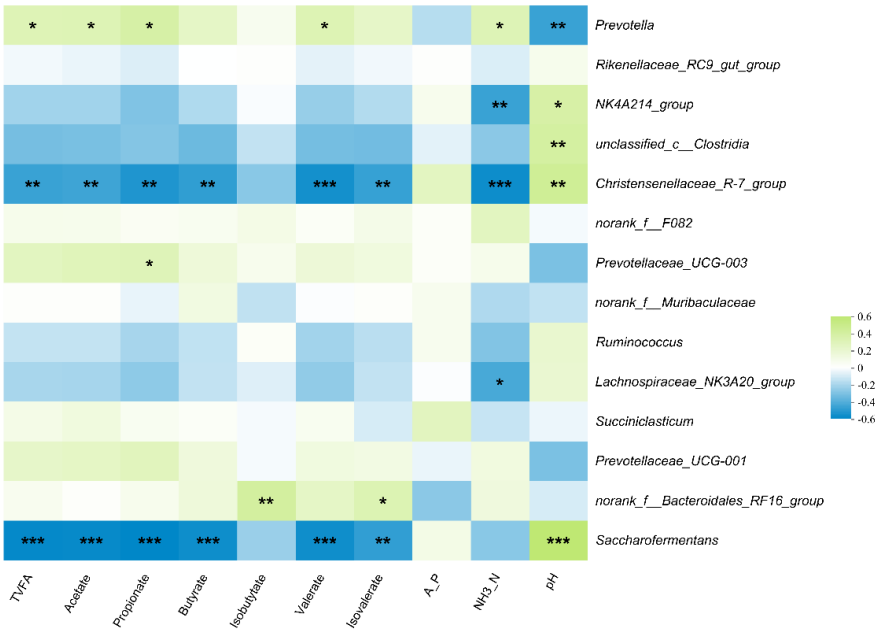


Figure 3. Heat map of correlations between rumen bacteria and rumen fermentation parameters. * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $p \leq 0.001$.

3.5. Correlation of Rumen Bacteria at the Genus Level

At the genus level, we used single factor correlation analysis to further study the relationship between rumen bacteria (Figure 4). The network showed a positive correlation among *NK4A214_group*, *unclassified_c__Clostridia*, *Christensenellaceae_R-7_group*, *Ruminococcus*, *Lachnospiraceae_NK3A20_group*, *Saccharofermentans*, *Acetitomaculum*, and *Family_XIII_AD3011_group*.

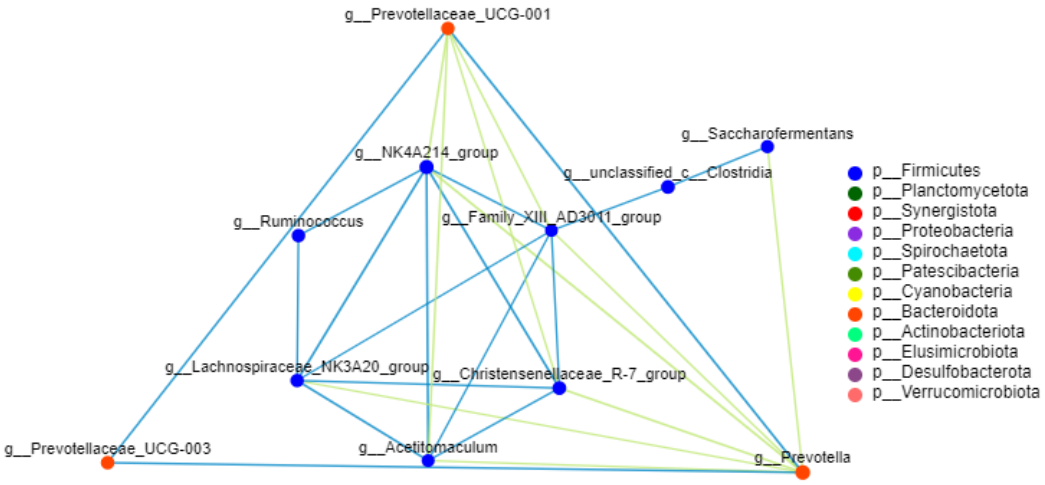


Figure 4. Correlation of rumen bacteria at the genus level. The correlation network is based on species at the genus level of rumen bacteria. Spearman rank and other correlation coefficients were calculated to reflect the correlation among species. By default, this figure shows the species information whose absolute value of correlation coefficient data is greater than or equal to 0.6 ($p < 0.05$). The size of the nodes in the figure represents the size of species abundance. The colors of species (such as phyla) at the same taxonomic level are consistent; The color of the line indicates positive and negative correlations: blue indicates a positive correlation and green indicates a negative correlation. The thickness of the line indicates the size of the correlation coefficient; the thicker the line, the higher the correlation between species. More the number of lines, the closer the relationship between the species and other species.

4. Discussion

For beef cattle, dry matter intake is a key indicator of measuring nutrient intake. Factors such as feed, environment, and management jointly regulate animal appetite and metabolism [27]. Moreover, VFA patterns are strongly affected by the level of dry matter intake [28]. However, in this study, no differences in dry matter intake were found among the 3 treatment groups, which is consistent with the results of previous studies [29,30]. Rumen fermentation parameters reflect the digestion and metabolism of nutrients by rumen microorganisms, which is extremely important for ruminants. NH_3-N is an important product of nitrogen metabolism in rumen. Most nitrogen compounds in the feed are used by rumen microorganisms to synthesize microbial proteins, and the other compounds are absorbed by the rumen wall or enter the reticulum. The concentration of NH_3-N in the rumen is the result of its production and utilization balance, which reflects the utilization rate of protein in the rumen. Previous studies reported that ammonia levels and protozoal counts were reduced by *Yucca* saponins [31–35], which is inconsistent with the results of this study. Francis et al. [36] reported that saponins inhibited the activity of protozoa in the rumen thus reducing the concentration of NH_3-N. Protozoa are net producers of ammonia because they continuously degrade dietary protein to produce NH_3-N and cannot use NH_3-N to synthesize protein. *Yucca* saponins act as surfactants; this enables them to combine with cholesterol and lipid on the protozoan cell membrane to form irreversible complexes that are discharged from the body, thus forming cavities on the cell surface. Small molecules in protozoa continue to flow outward so that the protozoa lose proliferative

function and gradually die [37]. However, some studies have reported that *Yucca* extracts had no effect on both NH_3-N concentration and protozoal counts in the rumen [30,38]. On the basis of these studies, we attributed the differences to different experimental conditions such as dosage [39,40], dietary composition [31], and in vivo or in vitro. [32]. Besides, the concentration of NH_3-N in the rumen is affected by many factors, among which the activity of bacteria is an important factor. TVFA concentration in the rumen of the YSE group was 37.02% higher than that in the rumen of the CON group, indicating that the rumen fermentation rate of the YSE group was much higher than that of the CON group; in addition, more substrates, including nitrogen compounds, were degraded, so more NH_3-N was produced. Liu et al. [41] showed that saponins increased TVFA concentration in the rumen and digestibility of organic matter and fiber. Goodall [42] found that YSE can improve the digestibility of dry matter in vitro, which is consistent with the changes in TVFA concentration in the rumen in this study. VFAs in the rumen are the main energy sources of ruminants. VFAs produced in the rumen can account for 50–75% of the energy required by the body [43,44], and the content and proportion can reflect rumen digestion and metabolism activities. Acetic acid, propionic acid, and butyric acid are the highest concentrations of volatile acids in the rumen, and they are also the3 most important VFAs in ruminants. Acetic acid can be converted into acetyl coenzyme A and directly enter the tricarboxylic acid cycle to participate in fat synthesis. Previous studies have reported that branched-chain VFAs act as specific nutrients for the ruminal cellulolytic bacteria and they are used for the synthesis of branched-chain amino acids, branched-chain fatty acids, and aldehydes, and probably for other cellular constituents possessing branched-carbon chains [45,46]. As rumen metabolites, NH_3-N and VFAs concentrations are closely related to the rumen microflora [47]. Therefore, we conducted high-throughput sequencing of rumen bacteria.

The alpha diversity index can reflect the species abundance of a microbial community to a certain extent. Specifically, the Sobs index is an actual observation value of richness, the Shannon index is used to reflect the diversity and evenness of community species, and the Ace index reflects community richness [48]. The sequencing results showed that *Bacteroides* and *Bacillota* were the dominant bacteria at the phylum level, which was consistent with the finding of previous studies [49]. *Bacteroides* and *Bacillota* have formed a symbiotic relationship of mutual promotion during the long-term evolution process. *Bacteroides* and *Bacillota* jointly participate in the succinate pathway in the rumen [50]. After cellulose is decomposed by cellulase produced by *Bacillota*, *Bacteroides* can effectively use xylan and fructose as they have more genes that encode glycoside hydrolases and polysaccharide lyases [51,52]. *Bacteroides* and *Bacillota* together jointly promote the host to absorb or store energy, which is crucial for the normal life activities of ruminants. In this study, the bacterial sequencing results showed significant differences at the phylum level. *Bacteroidota* ferment complex carbohydrates into acetic acid and propionate [53]. According to a recent study, beef cattle with a higher relative abundance of *Bacteroides* and a lower abundance of *Bacillota* in the rumen had a higher feed conversion ratio [54]. The phylum *Bacteroidota* is mainly involved in the degradation of non-fibrous material, whereas *Bacillota* is mainly involved in the catabolism of fibrous material [51,55]. In combination with the structural characteristics of bacteria, we speculated that the regulation of rumen microorganisms by YSE may be attributed to its rich saponin content. YSE has an inhibitory effect on gram-positive bacteria, and most *Bacillota* are gram-positive bacteria [15,34]. Recent studies showed that saponins reduced *Bacillota*:*Bacteroidota* in mice intestines, which is similar to our results [56–58]. *Patescibacteria* is a supergroup established recently. Brown named it a candidate phyla radiation (CPR) for the first time in 2015, and Park called CPR *Patescibacteria* in 2018 [59]. Previous studies have shown that *Patescibacteria* lost the genes of major metabolic pathways, such as those associated with de novo biosynthesis of amino acids, nucleotides, fatty acids, and cofactors; thus, they have to rely on other microorganisms for their survival [60]. We speculated that acceleration of the rumen fermentation rate leads to an increase in various substances related to de novo biosynthesis,

which promotes an increase in *Patescibacteria*. The pili-like structures of *Patescibacteria* enable them to attach themselves to other microorganisms, which may be strong evidence that they rely on other microorganisms [61]. These structures may act as tunnels for the exchange of metabolites, thereby facilitating the direct import of metabolites from their syntrophic partner.

At the genus level, *Rikenellaceae_RC9_gut_group* is known to produce propionate, succinate, butyrate, and acetate, which serve as important energy sources for ruminal epithelial cells and help in regulating rumen function [62]. *NK4A214_group* is believed to participate in fiber degradation in the rumen because it is rich in endo-1, 4-beta-xylanase, and cellulase genes [55,63]. It is worth mentioning that microorganisms with different taxonomic characteristics may have the same function; similarly, microorganisms with the same taxonomic characteristics may have different functions [50]. For example, *NK4A214_group* and *Christensenellaceae_R-7_group* do not belong to the same family, but they may promote rumen biohydrogenation alone or cooperate with each other [64]. Moreover, according to the correlation analysis, *Saccharofermentans* and *Christensenellaceae_R-7_group* showed almost the same correlation with the rumen fermentation parameters. *Saccharofermentans* belongs to the phylum *Bacteroidota* and has 116 genes that encode glycosyl hydrolases associated with hemicellulose, pectin, arabinogalactan, and starch [65]. *Ruminococcus* are major cellulolytic bacteria and play an important role in fiber degradation because they are rich in genes that encode cellulase and hemicellulose [66,67]. Moreover, *Ruminococcus* were significantly enriched in the CON group, which showed that the CON group had a stronger ability to degrade cellulose and produce more acetic acid. Similar to *Ruminococcus*, both *Christensenellaceae_R-7_group* and *Lachnospiraceae_NK3A20_group* are cellulolytic bacteria. *Christensenellaceae* is a family that belongs to the phylum *Bacillota* [68] and mainly decomposes fiber in the rumen [69,70], and *Lachnospiraceae_NK3A20_group* mainly degrades cellulose and hemicellulose in the rumen [71,72]. However, the effects of CAP on bacterial flora were not reflected in the fermentation parameters when compared with the CON group. Like bacteria, protozoa are an indispensable part of the rumen microbiota. A previous study showed that the elimination of protozoa from the rumen significantly decreased organic matter digestibility and particularly NDF digestibility in the rumen; lower rumen digestibility may result in a shift towards more energy-efficient reactions in the rumen and less requirement for metabolic energy to eliminate excess urea because of lower bacterial protein breakdown and ammonia levels in the rumen [73]. Although rumen pH was unaffected, lower TVFA and butyrate concentrations were observed in defaunated ruminants [73]. The changes in NH_3-N, TVFA and butyrate concentrations in defaunated ruminants were similar to those in our CAP group. Nevertheless, we did not measure the number of rumen protozoa in this study. Some studies have shown that CAP can be considered a potent and selective anti-*Trypanosoma cruzi* agent because it is active in nanomolar concentrations, and is more potent than benznidazole [74,75]. Moreover, CAP performed even better than resveratrol with respect to trypanocidal activity [74,76]. Thus, it is necessary to investigate the effects of CAP on rumen protozoa and its trypanocidal activity in the future.

5. Conclusions

In this study, two plant extracts, CAP and YSE, had different effects on rumen fermentation. On one hand, YSE increased TVFA and NH_3-N concentrations and decreased the pH and acetic acid percentage, but it had no effect on the Ac:Pr ratio. On the other hand, CAP reduced NH_3-N and TVFA concentrations. YSE increased the relative abundance of *Bacteroidota* and decreased the relative abundance of *Bacillota*. CAP decreased the relative abundance of *NK4A214_group* and *unclassified_c__Clostridia* and increased the relative abundance of *norank_f__F082.*

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9010030/s1, Figure S1: Rarefaction curves of the groups.

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