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Effects of Different Forage Types on Rumen Fermentation, Microflora, and Production Performance in Peak-Lactation Dairy Cows

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Abstract: Forages are vital in maintaining the dietary structures of ruminants, and reducing their costs is important for improving dairy production efficiency. Thus, this study investigated the effects of dietary forage types on dry matter intake, production, rumen fermentation, and the microbial profile in peak-lactating cows. Eight cows (600 ± 25 kg) with days in milk (60 ± 10 days) were assigned to four groups using a replicated 4 × 4 Latin square design: OG (oat hay + alfalfa hay + corn silage + concentrate), CW (*Leymus chinensis* + alfalfa hay + corn silage + concentrate), AS (alfalfa silage + oat hay + corn silage + concentrate), and AC (alkali-treated corn straw + alfalfa hay + corn silage + concentrate). The ruminal butyrate acid concentration was lower in the OG group than in the AS and AC groups post-feeding (12 h; $p < 0.05$). Ruminal NH3-N content was higher in the AS group than in the AC and CW groups post-feeding (9 h; $p < 0.05$). The percentage of ruminal *Oscillospira* and unknown microbes was higher in the CW group than in the other groups ($p < 0.05$). The total rumination time and rumination time per dry matter intake of AC were significantly higher than those of the other groups ($p < 0.05$). Milk lactose content in the AS group was highest among the groups ($p < 0.05$), and milk fat content was higher in the OG group than in the CW group (all $p < 0.05$). Overall, our results suggested that the butyrate acid content of forage from oat hay in rumen fermentation was significantly lower than that of calcium oxide and alfalfa silage sources when the dietary nutrition level was similar. The diet derived from *L. chinensis* increased the number of fibrillation helices related to fiber-decomposing bacteria and simultaneously increased unknown strains. Forage derived from alfalfa silage tended to increase milk protein levels. Alkali-treated corn straw could significantly increase the total rumination time and unit dry matter rumination time of dairy cows, which plays an important role in maintaining rumen health. The rational use of low-quality forage has broad prospects in China.

Keywords: rumen microflora; rumen fermentation; milk performance; forage types; dairy cows

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1. Introduction

The main nutritional function of forage is to meet the needs of ruminants for fiber. Owing to the special physiological structure of ruminants and in synergy with rumen microorganisms, forages are fermented and degraded into volatile fatty acids (VFA), which are absorbed by ruminants as nutrients [1,2]. Forages play an important role in the dietary health of ruminants. An excessive addition of concentrate leads to subacute ruminal acidosis [3]. Studies have shown that subacute ruminal acidosis in dairy cows can cause a decline in milk fat, and the proportion of forage in the diet directly affects the synthesis of

milk fat [4]. Therefore, optimizing the feed formula, especially forage component, is key to improving dairy production efficiency [5].

Seeking a local economy-friendly forage can benefit the cow and dairy industry. Oat grass is favored for its high palatability and suitable fiber content and is widely used as a dairy feed. *Leymus chinensis* is a unique source of forage in China [6], which has high fiber content and is widely used in dairy cow diets to maintain ruminant function and rumen health. Alfalfa silage and calcium oxide straw have emerged as coarse feeds for pit storage. Each has different characteristics, but both have high palatability and suitable moisture content. Although the alfalfa silage dry matter content was significantly lower than that of alfalfa hay, the leaf part containing a lot of protein was preserved; that is, protein loss was prevented in order to reduce feed cost [7]. A previous study showed that replacing chopped alfalfa hay with alfalfa silage of lactating dairy cows could increase milk fat concentration, milk fat yield, and rumen pH, and has no significant effect on milk yield, milk protein concentration, dry matter intake (DMI), and rumen ammonia concentration [8].

China's grassland resources and stocking capacity are inadequate. China needs to import large amounts of alfalfa hay to compensate for the shortage of high-quality forage. Considering the cost factor, alfalfa silage may be more suitable for producstion and use in some parts of China than alfalfa hay [9]. Although high-quality feed is essential for the production and maintenance of high-yielding dairy cows, the development and utilization of low- and medium-quality forage cannot be disregarded because low- and middle-yielding dairy cows, dry cows, reserve cattle, and other herbivores do not have a particularly high demand for nutrients. If low-quality feed is used rationally after treatment, feed cost can be greatly reduced without reducing production performance. Low-quality forage can find new feed sources for cows and reduce feed costs without affecting milk production. Studies have shown that rational use and scientific processing of forage can reduce concentrate use and feeding costs, conducive to prolonging animal production and reproductive life [10]. Therefore, optimizing the feed composition, especially forage component, is the key to improve dairy production efficiently and economically in the long run. This study aimed to formulate equal nitrogen and energy diets with similar nutritional levels from forage grass and new pit storage forage widely used in China, and to compare the effects of total mixed rations (TMR) with different forage sources and compositions on dairy cow physiology and production. This study provides a reference for promoting new forage and selecting forage raw materials according to local conditions.

2. Materials and Methods

2.1. Experimental Materials

2.1.1. Experimental Animals

According to the principle of similar parity, lactation days, and milk yield, 8 Holstein cows with 60 ± 10 d in milk (DIM), 2 parity, and 600 ± 25 kg body weight (BW) (cows with rumen cannula) were selected. The average milk yield of dairy cows last month was 30 ± 3.25 kg.

2.1.2. Experimental Feed

The test group was divided into four groups: OG (oat hay + alfalfa hay+ corn silage + concentrate), CW (*Leymus chinensis* + alfalfa hay+ corn silage + concentrate), AS (alfalfa silage + oat hay + corn silage + concentrate), AC (alkali-treated corn straw + alfalfa hay+ corn silage + concentrate). According to the milk production of the selected cows and Nutrient Requirements of Dairy Cattle (NRC) [11], diets are formulated according to the nutritional needs of Holstein cows with a body weight of 600 kg, milk yield 30 kg/d and 4.0% milk fat (Table 1).

Table 1. Ingredient and nutrient compositions (% of DM unless noted) of experimental diets [1].

Item	Dietary Treatment			
	OG	**CW**	**AS**	**AC**
Feed composition (%)				
Corn silage	55	55	50.5	55
Alfalfa silage	-	-	13.9	-
Alfalfa hay	3.9	4.2	-	5.3
Calcium oxide straw	-	-	-	4.1
Leymus chinensis	-	5.2		-
Oat hay	5.5	-	0.1	-
Corn	5.5	5.5	5.5	5.5
Steam-flaked corn	9.8	9.8	9.8	9.8
Expanded soybean	0.9	0.9	0.9	0.9
Expanded soybean meal	6.4	6.4	6.4	6.4
Rapeseed meal	0.9	0.9	0.9	0.9
Apple meal	1.3	1.3	1.3	1.3
Beet residue	2.4	2.4	2.4	2.4
Cottonseed	5.0	5.0	5.0	5.0
BERGAFAT T-3001	0.9	0.9	0.9	0.9
Premix	2.5	2.5	2.5	2.5
Nutrition level (%DM)				
Forage to concentrate ratio	62:38	60:40	62:38	61:39
DM (%)	48.9	49.2	45.5	52.8
NE (MJ/kg)	7.4	7.4	7.5	7.4
CP	16.58	16.58	16.58	16.58
NDF	29.29	29.49	29.58	29.66
ADF	18.85	18.92	19.27	19.19
NFC	32.08	31.38	31.10	31.35
NDF/NFC	0.91	0.94	0.95	0.95
Starch	31.5	31.3	31.6	31.8
EE	6.63	6.63	6.73	6.56
Ca	0.80	0.81	0.83	0.87
p	0.35	0.35	0.35	0.34

[1] DM is dry matter; NE is net energy of milk production; CP is crude protein; NDF is neutral detergent fiber; ADF is acid detergent fiber; NFC is non fiber carbohydrate; EE is ether extract; BERGAFAT T-300 is rumen protected fat, the main component is palm fat powder, and the crude fat content is 99.5%. Premix per kg contains: vitamin A 440000 IU, vitamin D3 110000 IU, vitamin E 4000 IU, copper 750 mg, manganese 1140 mg, zinc 2970 mg, iodine 30 mg, selenium 36 mg; NE was calculated value, the rest were measured values.

2.2. Animal Feeding and Experimental Design

2.2.1. Feeding and Management

The cows were fed twice at 7:30 and 15:30, and also had free access to water. Cows were milked mechanically three times per day at 7:00, 14:00, and 20:30. According to Table 1, four groups of different feeds were mixed evenly. Then, feed of each group was added to the automatic feeding trough. The automatic feeding system could recognize the electronic ear tags of cows. According to the electronic ear tags of cows, experimental cows could use the automatic feeding system to freely access their own experimental group feed, but the feed of other test groups could not be accessed. All cows shared two open troughs.

2.2.2. Experimental Design

A repeated 4 × 4 Latin design was used and conducted in 4 periods (20 days per-period). The feeding sequence was random. Every two cows received the same feed at the same time. All four diets were tested in parallel. In each period, cows were pre-fed for 17 days. Cows were sampled in the last 3 days of each period. The milk, rumen fluid and feed samples were collected during the pre-test period. Feed intake and rumination were monitored daily.

2.3. Sample Collection and Index Determination

2.3.1. Collection, Preservation and Pretreatment of Rumen Fluid

Rumen fluid was collected from dairy cows. On the 20th day of each period, 100 mL of rumen fluid of fistula bovine was collected at 5 time points: before feeding in the afternoon (0 h), and after feeding in the afternoon (3 h, 6 h, 9 h and 12 h). pH was determined at the end of collection (pHS-3E, INESA Scientific Instrument Co., Ltd., Shanghai, China), then filtered with 4 layers of gauze immediately, and centrifuged 15 min at the rotational speed of 4000 r/min (r/min is the unit of speed, and r/min represents the number of revolutions per minute). The supernatant was collected into the sampling bottle to determine the concentration of NH3-N, VFA and microflora in rumen fluid. All samples were stored below −20 °C. The concentration of ammonia nitrogen was determined by spectrophotometer colorimetry described by Broderick and Kang [12], and the concentration of VFA was determined by gas chromatography (6890 N; Agilent Technologies, Avondale, PA, USA), as described by Zhang and Yang [13].

The rumen fluid samples were stored below −80 °C for testing the rumen microflora. The rumen fluid samples were sent to Beijing Computing Center for determination of rumen microbiota. After DNA extraction, primer design (forward primer F: GATCCTACGGGAGGCAGCA; reverse primer R: GCTTACCGCGGCTGCTGGC, the pair was designed to bind 16S rDNA V3.) and PCR amplification, the samples were sequenced by Hiseq2500 sequencing platform. The analysis process is shown in Table 2.

Table 2. PCR reaction system (Kapa DNA polymerase) and reaction conditions.

Reaction System PCR		**Reaction Conditions**		
Substrate	Consumption	Temperature	Band	
ddH_2O	x	95 °C	5′	
Buffer (10X Taq A with Mg)	3 μL			
dNTP	1 μL	95 °C	1′	25 cycles
DNA	100~200 ng	50 °C	1′	
Primer F Mix	1 μL	72 °C	1′	
Primer R Mix	1 μL			
Enzym	0.5 μL	72 °C	7′	
Total	30 μL	4 °C		

2.3.2. Collection and Preservation of Feed, Milk and Fecal Samples, and Recording of Feed Intake and Rumination Data

The daily feed intake and feeding time of each cow were recorded in the automatic feeding trough, and the average food intake, feeding time and intake velocity were calculated. The rumination times were measured by the ruminant counter every day (SCR collar, SCR corporation, Netanya, Israel).

All feed samples were dried in an oven at 65 °C for 48 h to prepare air-dried samples, crushed and preserved for testing [14]. The dry matter (DM), organic matter (OM), crude protein (CP), crude fat (EE), calcium (Ca) and phosphorus (P) in feed were determined according to the method described by AOAC [15], the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Van Soest [16], and starch was determined by colorimetry [17]. The content of acid insoluble ash (AIA) in TMR and residue was determined according to the method of Van Keulen and Young [18].

On day 18 of each period of the experiment, according to the proportion at each milking relative to total daily milk yield, the milk was collected once in the morning, at noon and in the evening (morning: noon: evening = 4:3:3). One aliquot was preserved with potassium dichromate for the determination of milk routine (milk fat, lactose, milk protein) (Milko Scipe jule, Beijing Lanbosi Technology Co., Ltd., Beijing, China). The other aliquot was stored at −20 °C for the determination of urea nitrogen in milk. Urea nitrogen was determined by the urea nitrogen kit (Nanjing Jiancheng Co., Ltd., Nanjing, China).

During the 18–20 days of each trial, fecal samples (300–500 g) were collected successively for 12 times (4:00, 09:00, 14:00 and 19:00 on the day 18, 5:00, 10:00, 15:00 and 20:00 on the 19th day, and 6:00, 11:00, 17:00 and 22:00 on the 20th day, respectively). The feces of each cow were evenly mixed and approximately 400 g was sampled, and 10% tartaric acid was added according to the weight of each cow. It was mixed well, dried and regained moisture at 65 °C. After crushing, it was preserved to be tested. The analysis and calculation method of nutrient composition was the same as that of feed samples. The apparent digestibility of nutrients was calculated by model (1) according to the method described by Zhong [19].

$$\text{Apparent digestibility} = (1 - (\text{Ad} \times \text{Nf})/(\text{Af} \times \text{Nd})) \times 100. \quad (1)$$

where Ad (g/kg) and Af (g/kg) refer to the content of AIA in diet and feces, and Nd (g/kg) and Nf (g/kg) refer to the corresponding nutrients in diet and feces, respectively.

2.4. Data Processing

2.4.1. Data Acquisition and Processing of Rumen Microflora

The original data were controlled by the software FastQC (v 0.11.3, Babraham Institute, UK). The complete target sequence was obtained by merging the double-ended strips with the FLASH v1.2.7 software (v1.2.7, Johns Hopkins University, Baltimore, MD, USA) for subsequent analysis. The split_libraries_fastq.py filter sequence in the QIIME 1.8 software (v 1.8, Knight Lab at the University of Colorado at Boulder, CO, USA) was used. The Chimera generated in the process of PCR amplification was removed by the USEARCH v6.1 software (Robert C. Edgar, Tiburon, CA, USA) to produce the filtered sequence. The OTUs of each sample were calculated by uclust v1.2.22 software (Robert C. Edgar, Tiburon, CA, USA), and the sequence number and OTU number of samples were obtained. The species structure was analyzed by RDP classifier software, and the data results were plotted by R software 4.1.3 (Lucent Technologies, Murray Hill, NJ, USA).

2.4.2. Data Significance Analysis

The data were analyzed by using the generalized linear model (GLM) of repeated 4 × 4 Latin square design in SAS 9.4(SAS Institute Inc., Cary, NC, USA) for ANOA analysis, and multiple Tukey comparisons were made for the significance test. A comparison with a p value of < 0.05 was considered significantly different. The residual plot was used to check the randomness of the error.

The linear model (2) of statistical variables is:

$$\text{Yijk} = \mu + \text{Ti} + \text{Pj} + \text{Sk} + \text{Eijk}. \quad (2)$$

Yijk is the observed value; μ is the overall mean; Ti is the treatment effect; Pj is effect during the experiment; Sk is the animal effect; Eijk is the random error.

3. Results

3.1. Effects of TMR with Different Forage Types on Intake, Rumination, Milk Production Performance, and Apparent Digestibility

3.1.1. Effects of TMR with Different Forage Sources on Feed Intake and Rumination

Table 3 shows the effects of TMR from different forage sources on feed intake, feeding time and rumination in dairy cows. TMR with different forage sources had no significant effects on dry matter intake and intake time but had significant effects on ruminant behavior. The rumination time of the AC group was significantly higher than that of the other groups, whereas the rumination time of unit dry matter intake was significantly lower in the AS group than in the other groups.

Table 3. Effects of dietary treatments on DMI, feed intake and rumination time [2].

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
DMI, kg/d	20.04	20.98	22.08	19.50	0.998	0.49
Feeding time						
Daily intake time, min	193.84	189.94	196.81	192.15	12.121	0.98
Feeding time per kg of DMI, min	10.72	10.25	10.85	10.95	0.246	0.22
Rumination time, min	315.39 ^{b}	303.73 ^{b}	316.86 ^{b}	337.68 ^{a}	10.196	0.18
Rumination time per unit DMI, min	24.28 ^{a}	22.59 ^{a}	18.12 ^{b}	24.33 ^{a}	0.682	0.002

[2] DMI is dry matter intake. Means within the same row with different superscripts are significantly different ($p < 0.05$). The same below.

3.1.2. Effects of TMR with Different Forage Types on Feed Apparent Digestibility

As shown in Table 4, there was no significant difference in the apparent digestibility of dry matter, crude protein, crude fat, neutral detergent fiber and acid detergent fiber among the different forage sources.

Table 4. Apparent digestibility of different forage types of TMR. %.

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
DM	71.38	73.93	76.51	72.06	3.403	0.68
CP	69.71	75.83	75.49	73.90	3.471	0.53
NDF	54.07	56.51	59.23	53.55	3.207	0.55
ADF	59.59	59.81	63.61	50.55	2.755	0.13
EE	80.07	83.30	84.88	80.76	2.154	0.38

3.1.3. Effects of TMR with Different Forage Sources on Milk Yield, Milk Composition, and Conversion Efficiency

As shown in Table 5, there were no significant differences in milk yield, 4% corrected milk, somatic cell count, and feed conversion efficiency among the treatment groups. The lactose content in the AS group was higher than that in the other groups. The milk fat content of the OG group was higher than that of the other groups. The milk protein content of the AC group was significantly higher than that of the CW group. There was no significant difference in the daily milk fat and milk protein yield among the four groups, but the daily lactose yield in the AS group was significantly higher than that in the OG group. The urea nitrogen of the CW group was the lowest.

Table 5. Effects of TMR with different forage sources on milk yield and milk composition.

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
Milk yield, kg/d	31.38	32.28	32.20	32.30	0.560	0.61
4% standard milk, kg/d	35.58	32.34	32.90	34.03	1.614	0.52
Lactose, %	4.93 ^{b}	4.98 ^{b}	5.09 ^{a}	4.99 ^{b}	0.033	0..01
Milk fat, %	4.44 ^{a}	4.04 ^{b}	4.18 ^{ab}	4.40 ^{a}	0.132	0.03
Milk protein, %	3.02	2.95	3.07	3.05	0.054	0.26
Somatic cell count, 1000/mL	67.06	40.38	36.13	37.25	13.902	0.41
Lactose yield, kg/d	1.53	1.61	1.64	1.61	0.026	0.19
Milk fat yield, kg/d	1.53	1.30	1.33	1.41	0.102	0.38
Milk protein yield, kg/d	0.93	0.93	0.99	0.97	0.033	0.26
MUN, mmol/L	4.70	4.14	4.47	4.75	0.368	0.62
FE	1.75	1.54	1.45	1.74	0.167	0.94

Means within the same row with different superscripts are significantly different ($p < 0.05$).

3.2. Effects of TMR with Different Forage Types on Rumen Fermentation Indices

3.2.1. Effects of Diets with Different Forage Types on Rumen Fermentation Index in 12 h

Table 6 shows the effects of TMR from different forage types on various rumen fermentation indices after feeding 12 h in the afternoon. There were no significant differences in pH value, ammonia nitrogen, total VFA, acetic acid, and propionic acid among the groups. The butyric acid concentration in rumen fluid of the AS and AC groups was significantly higher than that of the OG group. The molar ratio of isobutyric acid in the OG group was significantly higher than that in the AC group.

Table 6. Effects of total mixed diets with different forage type on 12 h rumen fermentation [3].

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
pH	6.84	6.73	6.66	6.62	0.062	0.16
NH_3-N mg/dL	12.58	13.57	16.30	14.74	0.767	0.31
Total VFA, mmol/L	127.47	123.24	127.21	126.54	13.589	0.46
Acetic acid, mmol/L	71.04	73.34	75.14	76.21	2.860	0.62
Propionic acid, mmol/L	29.48	29.41	31.84	31.49	1.963	0.74
Butyric acid, mmol/L	11.21 ^{b}	12.78 ^{ab}	14.55 ^{a}	14.08 ^{a}	0.651	0.04
Isobutyric acid, mmol/L	1.04	1.05	1.07	1.00	0.069	0.90
Pentanoic acid, mmol/L	1.82	1.82	2.05	1.99	0.173	0.75
Isovaleric acid, mmol/L	2.13	2.31	2.59	2.14	0.227	0.48
Ratio of mole						
Acetic acid, %	61.47	60.22	59.36	60.44	0.924	0.51
Propionic acid, %	23.59	24.97	23.93	24.50	0.552	0.50
Butyric acid, %	10.28	10.05	10.55	11.20	0.471	0.38
Isobutyric acid, %	10.60 ^{a}	9.84 ^{a}	8.51 ^{a}	7.52 ^{b}	0.764	0.11
Pentanoic acid, %	1.41	1.64	1.76	1.46	0.126	0.60
Isovaleric acid, %	2.02	1.95	2.05	1.74	0.224	0.69

[3] The rumen collection time was before morning feeding (0 h) and 3 h, 6 h, 9 h, and 12 h after feeding; the above indexes were the average values of five time points in a day. Means within the same row with different superscripts are significantly different ($p < 0.05$).

3.2.2. Effects of Diets with Different Forage Types on Rumen Fermentation at Different Time Points

The effects of different forage sources on rumen fermentation indices at 0, 3, 6, 9, and 12 h are shown in Table 7. The NH3-N concentration in the different treatment groups was significantly different after the afternoon feeding (9 h). The NH3-N concentration was significantly higher in the AS group than in the CW and AC groups. Moreover, there were also significant differences in VFA; the propionic and butyric acid concentrations were significantly higher in the AC group than in the CW group after feeding (0 h). Butyric acid concentration was significantly higher in the AS group than in the OG group after feeding (6 h).

Table 7. Effects of dietary treatment on pH, NH_3-N and VFA concentration at five time points in lactating cows [4].

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
pH						
0 h	7.31	7.35	7.43	7.05	0.743	0.05
3 h	6.88	6.97	6.67	6.68	0.065	0.03
6 h	6.84	6.54	6.44	6.42	0.131	0.20
9 h	6.75	6.54	6.53	6.80	0.109	0.28
12 h	6.40	6.22	6.22	6.17	0.110	0.52
Mean	6.84	6.73	6.66	6.62	0.063	0.16

Table 7. *Cont.*

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
NH_3-N, mg/dL						
0 h	16.03	16.85	18.43	16.18	0.606	0.41
3 h	11.95	19.57	19.74	16.76	1.583	0.24
6 h	8.18	8.36	11.80	11.46	1.292	0.54
9 h	14.96 [ab]	11.61 [b]	17.65 [a]	13.55 [b]	0.599	0.03
12 h	11.82	11.46	13.87	15.78	1.010	0.34
Mean	12.58	13.57	16.30	14.74	0.771	0.31
Total VFA, mmol/L						
0 h	87.57	66.71	77.83	97.35	11.373	0.38
3 h	121.78	120.77	132.89	130.07	6.818	0.56
6 h	126.58	144.04	150.05	135.10	11.688	0.52
9 h	123.45	132.26	138.18	129.63	14.753	0.92
12 h	137.92	154.06	135.68	140.54	6.767	0.31
Mean	172.47	123.24	127.21	126.54	23.590	0.46
Acetic acid, mmol/L						
0 h	49.65	40.50	47.73	59.95	6.063	0.25
3 h	78.83	73.83	79.08	78.08	3.991	0.67
6 h	77.26	80.83	88.41	81.21	6.765	0.71
9 h	73.84	81.01	83.64	79.62	7.646	0.83
12 h	80.62	90.53	76.81	81.48	4.079	0.21
Mean	71.04	73.34	75.14	76.21	2.862	0.62
Propionic acid, mmol/L						
0 h	17.05 [ab]	12.52 [b]	17.06 [ab]	22.34 [a]	2.347	0.12
3 h	30.93	28.30	32.75	32.26	2.336	0.57
6 h	32.36	32.06	36.56	34.03	3.589	0.81
9 h	29.29	34.63	36.50	31.60	4.654	0.69
12 h	37.74	40.48	36.32	37.24	2.365	0.66
Mean	29.48	29.41	31.84	31.49	1.961	0.74
Butyric acid, mmol/L						
0 h	7.76 [ab]	6.80 [b]	8.65 [ab]	10.77 [a]	0.955	0.10
3 h	9.27	13.25	16.82	14.88	0.922	0.05
6 h	11.78 [b]	14.43 [ab]	16.82 [a]	14.88 [ab]	0.970	0.05
9 h	12.91	13.30	15.51	14.31	1.576	0.67
12 h	14.35	17.14	16.96	16.52	1.295	0.46
Mean	11.21 [b]	12.78 [ab]	14.55 [a]	14.08 [a]	0.644	0.04
Isobutyric acid, mmol/L						
0 h	1.19	1.12	1.19	1.16	0.103	0.95
3 h	1.22	1.14	1.16	1.10	0.084	0.72
6 h	1.04	1.15	1.14	0.95	0.097	0.95
9 h	0.92	0.91	0.99	095	0.099	0.95
12 h	0.83	0.95	0.88	0.80	0.062	0.44
Mean	1.04	1.05	1.07	1.00	0.071	0.90
Valeric acid, mmol/L						
0 h	1.03	1.08	1.26	1.38	0.323	0.81
3 h	1.92	1.66	2.17	2.00	0.308	0.72
6 h	1.95	1.93	2.25	1.87	0.245	0.70
9 h	2.01	2.04	2.13	1.67	0.189	0.51
12 h	2.18	2.36	2.16	2.27	0.178	0.86
Mean	1.82	1.82	2.05	1.88	0.174	0.75
Isovaleric acid, mmol/L						
0 h	1.92	1.92	2.18	2.00	0.275	0.90
3 h	2.35	2.58	2.65	2.21	0.330	0.72
6 h	2.20	2.36	3.10	2.14	0.334	0.24
9 h	1.97	2.11	2.45	2.11	0.261	0.61
12 h	2.19	2.60	2.55	2.22	0.218	0.46
Mean	2.13	2.31	2.59	2.14	0.223	0.48
Acetic acid/propionic acid						
0 h	3.43	3.23	3.04	3.00	0.202	0.44
3 h	2.51	2.63	2.45	2.53	0.103	0.67
6 h	2.47	2.54	2.44	2.43	0.145	0.95
9 h	2.64	2.55	2.27	2.53	0.179	0.57
12 h	2.17	2.31	2.13	2.19	0.124	0.74
Mean	2.64	2.65	2.47	2.53	0.122	0.66

[4] Rumen collection time was 3 h, 6 h, 9 h and 12 h before morning feeding (0 h) and 3 h, 6 h, 9 h and 12 h after feeding. Means within the same row with different superscripts are significantly different ($p < 0.05$).

3.3. Effects of Different Diets on Rumen Microflora

3.3.1. OTU of Rumen Samples in Different Treatment Groups

OTU statistics are shown in Table 8. Before feeding (0 h), the number of OTU in the AC group was the highest, and that in the CW group was the lowest. In contrast, the number of OTU in the OG group was the highest, and that in the AC group was the lowest at 3 h after feeding.

Table 8. Reads and OTU number of samples on threshold of 97% [5].

Group	SampleID	SeqsNum	OTUsNum	EvenSeqsNum	EvenOTUsNum	Mean
Pre-feeding (0 h)						
AC	AC1.0	260,361	6990	16,000	5726	6119.25
	AC2.0	244,209	7665	160,000	6444	
	AC3.0	245,685	7475	160,000	6258	
	AC4.0	166,804	6142	160,000	6049	
AS	AS1.0	229,687	7114	160,000	6106	5979.75
	AS2.0	215,149	7084	160,000	6207	
	AS3.0	208,640	6516	160,000	5857	
	AS4.0	207,145	6420	160,000	5749	
CW	CW1.0	229,720	6432	160,000	5557	5847.75
	CW2.0	266,161	7738	160,000	6201	
	CW3.0	206,982	6241	160,000	5650	
	CW4.0	234,814	6950	160,000	5983	
OG.	OG1.0	241,574	6456	160,000	5478	6044.25
	OG2.0	215,472	8068	160,000	7086	
	OG3.0	230,100	6714	160,000	5815	
	OG4.0	209,751	6472	160,000	5798	
Total		3,612,254	25241	2,560,000	19,993	5997.75
Post-feeding (3 h)						
AC	AC1.3	227,604	6826	160,000	5898	5486.50
	AC2.3	212,160	6995	160,000	6267	
	AC3.3	194,961	6289	160,000	5778	
	AC4.3	596,813	6590	160,000	4003	
AS	AS1.3	238,845	7306	160,000	6172	5678.50
	AS2.3	230,521	6894	160,000	5899	
	AS3.3	178,867	5858	160,000	5589	
	AS4.3	211,399	5682	160,000	5054	
CW	CW1.3	225,105	7168	160,000	6210	6078.50
	CW2.3	218,291	6921	160,000	6114	
	CW3.3	235,790	6921	160,000	5888	
	CW4.3	308,894	7964	160,000	6102	
OG.	OG1.3	232,579	6633	160,000	5634	6132.50
	OG2.3	241,113	7919	160,000	6695	
	OG3.3	224,659	6374	160,000	5601	
	OG4.3	315,506	8783	160,000	6600	
Total		4,093,107	25,241	2,560,000	20,348	5844

[5] OTU operation sequence unit sequence is arranged from large to small.

3.3.2. Single Sample Sequencing Depth Analysis (Rarefaction Curve)

From the observed species curve (Figure 1A,B), the number of species sequenced reached a maximum of 800 and tended to be flat. From the goods coverage curve (Figure 1C,D), the coverage is close to saturation 1, indicating that the test covers most of the microorganisms in the sample.

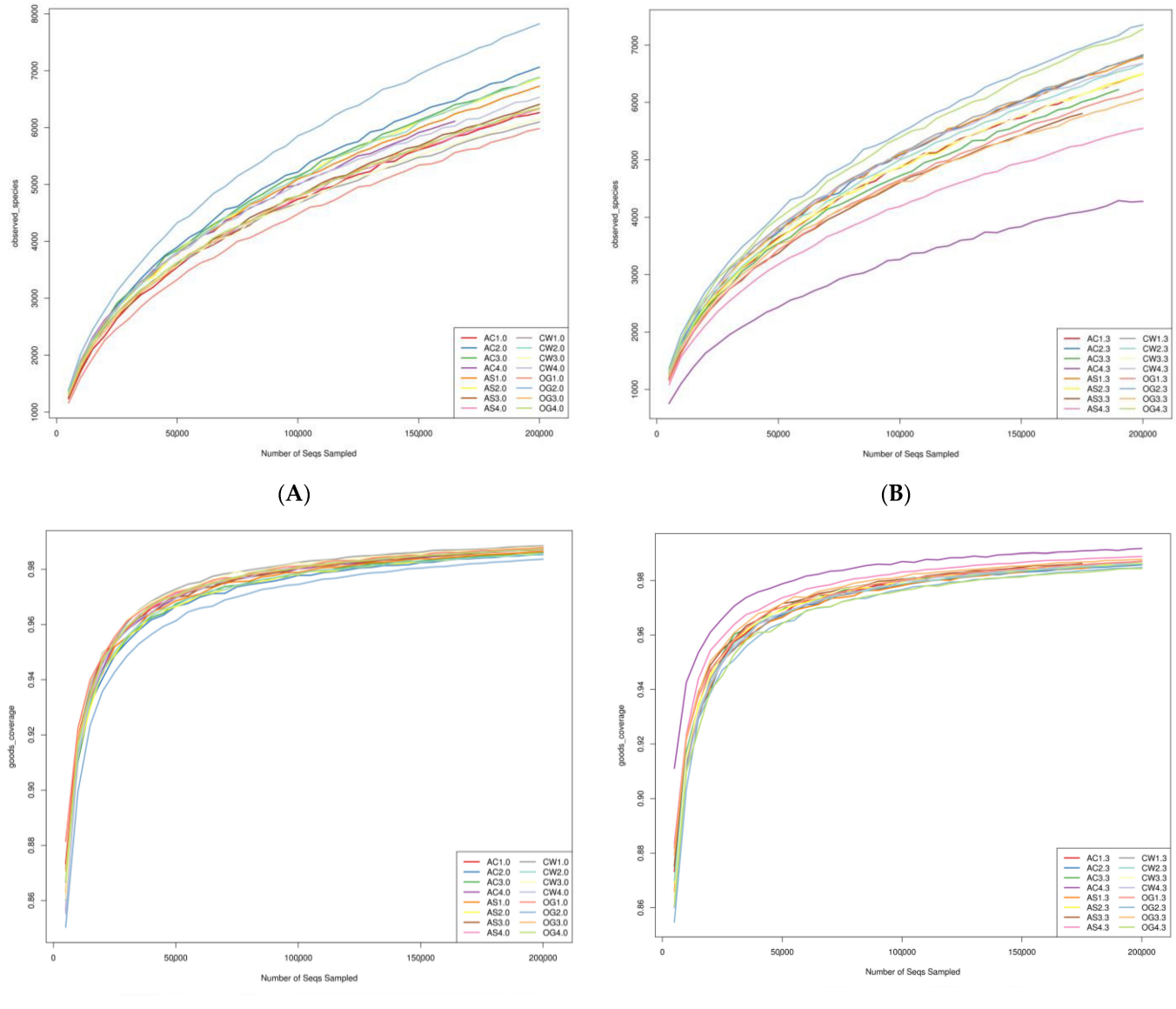

Figure 1. Rarefaction curves of microbes in different samples. ((**A**,**C**) are 0 h; (**B**,**D**) are 3 h).

3.3.3. Microbial Community Structure Map

The distribution of rumen microorganisms in each group did not differ before and after feeding (Figure 2).

Table 5 is taxonomy profile of rumen bacteria on genus level under different dietary treatments. As observed in Figure 3, the percentage of unknown microbes was higher in the CW group than in the other groups, and the other species distribution among the groups was relatively consistent at the genus level.

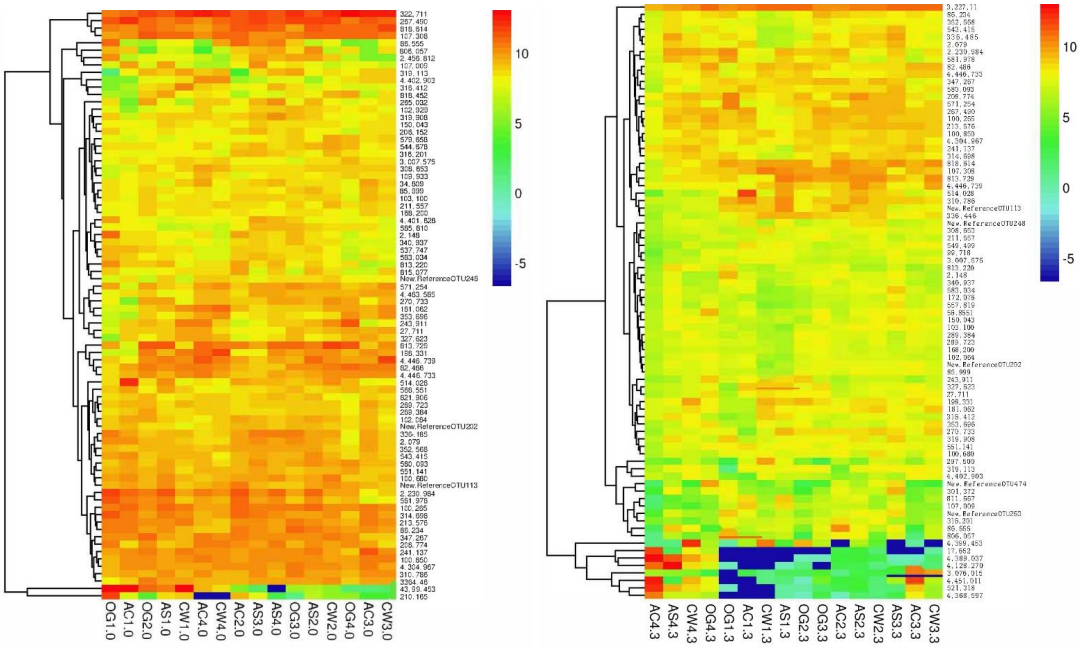

Figure 2. The heat map of different ruminal microbes on genus. (Select the OTU drawing with the top 80 reads; each column represents a sample, each row represents an OTU and its corresponding species information, color depth represents the number of reads contained in the OTU, red represents high, blue represents low).

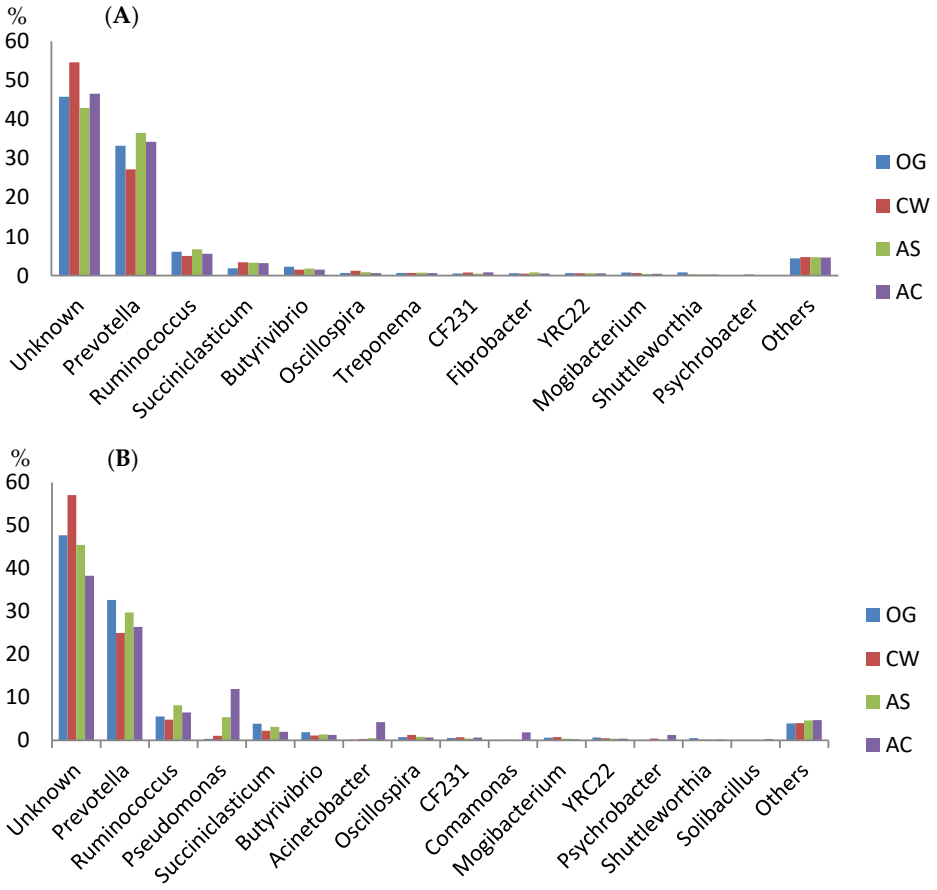

Figure 3. Taxonomy profile of rumen bacteria on genus level under different dietary treatments. ((**A**) is 0 h, (**B**) is 3 h).

3.3.4. Principal Component Analysis of 16S rRNA Gene of Rumen Microorganisms

According to the specific species and species diversity of the samples, the unweighted-unifrac and weighted-unifrac distance matrix calculation methods were used for principal coordinate analysis (PCoA). As shown in Figure 4A, the microorganisms in the AS and CW groups were clustered and separated before feeding (0 h), and from Figure 4B, the CW, AS, and OG groups clustered and coincided after feeding (3 h). As shown in Figure 4C, the AS and CW groups clustered and separated from each other before feeding (0 h), whereas the other two groups were relatively dispersed. However, as observed in Figure 4D, the relative abundance of species in each group changed after feeding (3 h) compared to that before feeding (0 h) and gathered together; however, the distribution of the AC group was still scattered.

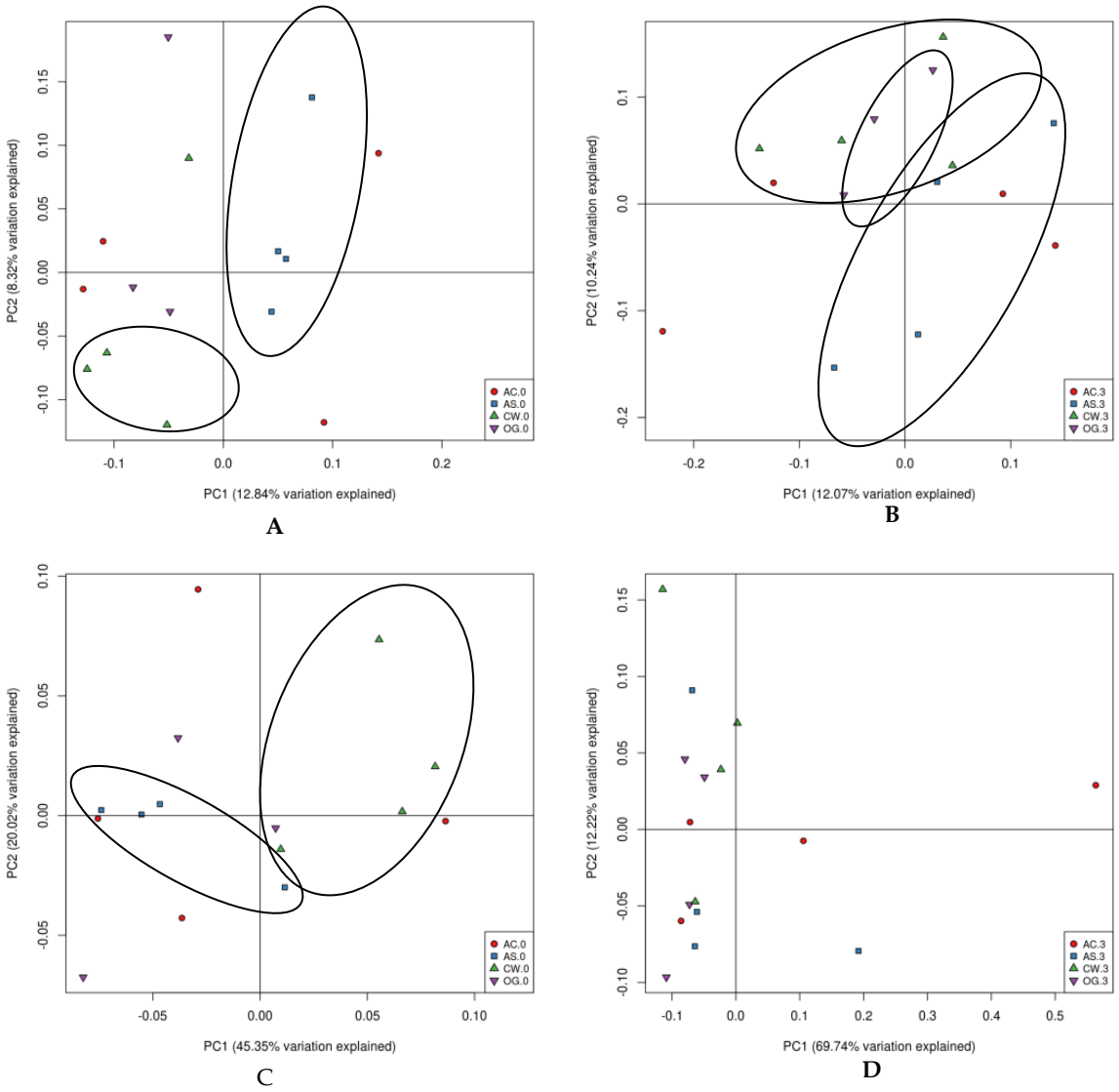

Figure 4. Principal coordinates analysis of rumen microbes with different treatment. ((**A**) 0 h unweighted-unifrac-PCoA; (**B**) 3 h unweighted-unifrac PCoA; (**C**) 0 h weighted-unifrac PCoA; (**D**) 3 h unweighted_unifrac PCoA); The source and combination of the forage are the influencing factors, which serve as the abscissa and ordinate of PCoA analysis.

3.3.5. Effects of Diets from Different Forages on the Percentage of Total Bacteria in Different Genera

Table 9 shows the percentage of bacterial genera in the rumen of each group before and after feeding among the total measured bacterial genera. The rumen microorganisms

changed before and after feeding, becoming more abundant after feeding. After feeding, *Pseudomonas*, *Acinetobacter*, *Comamonas*, and *Solibacillus* were found in the rumen, and their abundance increased. In addition, the abundance of *Oscillospira* was significantly different before and after feeding, and the CW group was significantly higher than the other groups ($p < 0.05$). The abundance of *Succiniclastium* was significantly different before feeding, and AS was lower than in the other groups. Concurrently, many unknown strains appeared in the CW group, which was significantly higher than in the other groups ($p < 0.05$).

Table 9. Varied genus account for the whole testing rumen microbes pre-feeding and post-feeding. %.

Item	Feed Treatment				SEM	*p*
	OG	CW	AS	AC		
Pre-feeding (0 h)						
Unknown	45.79 ^{b}	54.63 ^{a}	42.93 ^{b}	46.58 ^{ab}	0.022	0.06
Prevotella	33.24	27.21	36.52	34.26	0.032	0.21
Ruminococcus	6.11	5.02	6.72	5.59	0.010	0.75
Succiniclasticum	1.86 ^{a}	3.41 ^{b}	3.31 ^{ab}	3.17 ^{ab}	0.004	0.11
Butyrivibrio	2.26	1.51	1.77	1.54	0.005	0.66
Oscillospira	0.68 ^{b}	1.24 ^{a}	0.85 ^{ab}	0.64 ^{b}	0.001	0.08
Treponema	0.68	0.69	0.77	0.66	0.001	0.08
CF231	0.52	0.78	0.51	0.84	0.001	0.08
Fibrobacter	0.62	0.49	0.81	0.54	0.001	0.18
YRC22	0.64	0.60	0.61	0.57	0.006	0.97
Mogibacterium	0.79	0.65	0.38	0.44	0.002	0.62
Shuttleworthia	0.82	0.29	0.31	0.29	0.003	0.55
Psychrobacter	0.04	0.28	0.04	0.04	0.001	0.39
Others	4.42	4.74	4.70	4.62	0.002	0.54
Post-feeding (3 h)						
Unknown	47.71 ^{ab}	57.06 ^{a}	45.45 ^{b}	38.30 ^{b}	0.027	0.02
Prevotella	32.64	24.99	29.74	26.40	0.048	0.66
Ruminococcus	5.54	4.79	8.14	6.47	0.011	0.46
Pseudomonas	0.36	1.04	5.36	11.93	0.043	0.31
Succiniclasticum	3.87	2.21	3.14	2.00	0.009	0.32
Butyrivibrio	1.90	1.10	1.39	1.25	0.004	0.61
Acinetobacter	0.14	0.30	0.52	4.23	0.023	0.32
Oscillospira	0.75 ^{b}	1.24 ^{a}	0.83 ^{b}	0.64 ^{b}	0.001	0.02
CF231	0.54 ^{ab}	0.73 ^{a}	0.42 ^{b}	0.66 ^{ab}	0.001	0.10
Comamonas	0.04	0.12	0.00	1.86	0.013	0.45
Mogibacterium	0.61	0.75	0.37	0.25	0.002	0.47
YRC22	0.64	0.48	0.39	0.39	0.001	0.48
Psychrobacter	0.02	0.41	0.11	1.26	0.005	0.42
Shuttleworthia	0.51	0.14	0.13	0.17	0.002	0.51
Solibacillus	0.001	0.02	0.00	0.27	0.001	0.45
Others	3.93 ^{a}	4.00 ^{a}	4.64 ^{b}	4.71 ^{b}	0.001	0.05

Means within the same row with different superscripts are significantly different ($p < 0.05$).

4. Discussion

4.1. Feeding Effects of Different Forage Types

Dietary Intake and Rumination

Dietary energy determines DMI, and there was no significant difference in DMI, as shown in Table 3, because of the same energy in the diets. Notably, the intake time of DMI per kg in the AC group was longer than that of the other groups, and the corresponding rumination time and DMI rumination time per kg of DMI were also longer than those of the other groups. The total rumination time of the AC group was significantly higher than that of other groups. The reason for the results above may be that calcium oxide straw contains more physically effective fiber, which a study has shown can promote intake, chewing, and rumination time [20]. In addition, the results also showed a positive correlation between DMI intake time per kilogram and rumination time and DMI rumination time

per kilogram. Rumination helps to digest nutrients and maintain the balance of the rumen environment [21] while also causing a waste of energy; thus, calcium oxide straw sources should be reasonably used to configure diets. However, the apparent fiber digestibility of calcium oxide straw was low. Therefore, when using forage with calcium oxide straw, it is necessary to supplement energy and protein feeds, such as alfalfa and corn silage.

There was no significant difference in the apparent digestibility of DM, CP, and EE among different forage combinations, but the apparent digestibility of ADF and NDF in the AS group was higher than that in the other groups. Several studies have shown that the forage part composed of corn silage and AS helps maintain the balance between rumen degradable protein and rumen undegradable protein, thus, improving the apparent digestibility of proteins and carbohydrates. Alfalfa silage contains high CP and rumen degradable protein, but low rumen undegradable protein content [22,23]. Alfalfa silage alone converts a large amount of rumen degradable protein into NH_3-N in the rumen [22,24], while corn silage provides enough energy to be utilized in the process of alfalfa silage degradation, thus, improving the protein utilization efficiency [25]. Therefore, the digestibility of the AS group was higher than that of the other groups. In addition, the apparent digestibility of ADF in the AC group was 45.44%, which was significantly lower than that of the other groups. Ghasemi reported that the apparent digestibility of ADF in the straw diet treated with 5% $Ca(OH)_2$ was 46.5% [26]. This may be due to the high degree of cellulose lignification in the straw and the uneven composition of nutrients; therefore, low-quality forage should not be used excessively.

The results showed no significant difference in milk yield among the four groups. A study reported a direct correlation between milk yield and dietary energy level [27]. There was no significant difference in DMI, corresponding to the milk yield results. The results showed that when the dietary energy levels were similar, the source and composition of forage had no significant effect on the milk yield of dairy cows.

In addition, some studies have shown that alfalfa silage can significantly increase milk and milk protein yields in dairy cows [28]. Dhiman studied the effects of different alfalfa and corn silages ratios on dairy cow performance and found that ideal milk production performance could be obtained when the dry matter ratio of corn silage to alfalfa silage was 1:1 [22]. However, the dry matter ratio of corn silage to alfalfa silage was approximately 1:3, lower than the 1:2–2:1 ratio reported by Dhiman [22]. Therefore, the AS group did not exert the maximum production advantage, which may be one of the reasons why the milk protein yield of AS showed no significant advantage compared to other diets in this experiment.

4.2. Effects of TMR with Different Forage Types on Rumen Fermentation of Dairy Cows

4.2.1. Rumen pH and NH3-N

There is a close relationship between the rumen pH and dietary type. Many early studies have shown that rumen pH varies from 5.6–6.4 [29–32]. In these studies, low rumen pH values were followed by high organic matter intake, high organic digestibility, high VFA, low milk fat rate, low forage NDF, and small feed size [33]. Eating high-concentrate diets or feed pellets that are too small directly affect feeding behavior and reduce the number of chews, resulting in low rumen pH [34]. Simultaneously, high-quality forage grass causes a decrease in rumen pH but has a higher organic digestibility [35]. The rumen pH values of the AS and AC groups were lower than those of the other groups because the AC group used higher concentrate ratios and reduced rumen pH value in order to maintain the same dietary energy and nitrogen level as the other groups. The lower pH of the AS group was due to the lower pH of the alfalfa silage; thus, there was a tendency to reduce the pH value of the rumen, which was consistent with previous reports.

NH3-N is the product of the intake of feed protein decomposition. Therefore, the rate of NH3-N released from feed degradation should be balanced by the rate of rumen microorganisms using NH3-N. Generally, protein utilization in the rumen is explored by measuring the NH3-N concentration in rumen fluid. Normally, the most suitable NH3-N

concentration for rumen microorganisms is 6.3–27.5 mg/dL [36]. Beyond this range, this indicates that the decomposition and utilization of proteins in the rumen are imbalanced. The NH3-N in this experiment was within the normal range, and there was no significant difference in the average value of NH3-N at 12 h between the groups. Nine hours after morning feeding, the NH3-N of the AS group was significantly higher than that of the CW and AC groups, which may be due to the high content of rumen-degradable protein in alfalfa silage, which NH3-N rapidly released during rumen degradation.

4.2.2. Volatile Fatty Acids

All groups showed significant differences in acetic and isobutyric acids in the 12 h rumen fermentation. The butyric acid content in the AS and AC groups was significantly higher than that in the OG group, but there was no significant difference in acetic acid content. As butyric acid is produced by acetic acid through the β-oxidation pathway [37], the results showed that high-quality forage (alfalfa silage) might be easier to promote the conversion of acetic acid to butyric acid.

There was no significant difference between acetic and propionic acids and their ratio because the ratio of acetic acid to propionic acid was affected by the type of diet; crude fiber fermentation produced acetic and butyric acids, while a high-concentrate diet produced a large amount of propionic acid [38]. The ratio of concentrate to forage varied from 50:50 to 55:46, and the difference was not significant; therefore, there was no significant difference between acetic, propionic, and acetic/propionic acids. The results showed that the VFA in rumen fermentation did not change, which may be due to the similar VFA composition caused by the similar dietary structure.

This study showed that the lactose content and yield of the AS group were higher, whereas the milk fat content of the CW group was lower. The rumen fermentation index results (Table 4) showed that acetic acid/propionic acid in the AS group was 2.47, which was lower than that in the other groups, resulting in higher lactose content. In contrast, the ratio of acetic acid to propionic acid in the OG group was 2.64, which was higher than that in the AS and AC groups. Therefore, the milk fat content was higher in the OG group than in the other groups. However, the acetic acid/propionic acid ratio of the CW group was 2.65, but it had the lowest milk fat content, which may be because *L. chinensis* belongs to low-quality forage, and the nutritional composition of low-quality forage is often unbalanced, leading to the decrease in acetic acid utilization efficiency.

4.3. Effects of TMR with Different Forage Types on Rumen Microflora of Dairy Cows

4.3.1. Changes in Rumen Microbial Diversity

OTU is an indicator of microbial diversity, and it increases with an increase in rumen microbial species. Saro found that the concentration of DNA fragments in sheep rumen fluid decreased after feeding [39], consistent with the results of this experiment. The average corrected OTU of each group was 5997 before feeding (0 h) and 5844 after feeding (3 h), which may be due to the dilution of rumen fluid by a large amount of saliva secreted after feeding. Concurrently, Saro et al. also concluded that feeding low-quality forage could increase rumen microbial richness [39]. The results of this study confirmed this view. In this study, the average corrected OTU in the rumen fluid of the AC group was the highest on an empty stomach. Saro considered this an adaptive response to digest more cellulose and fibers with more complex structures. However, after feeding (3 h), the average corrected OTU of rumen fluid was higher in the OG group and lowest in the AC group, indicating that the oat diet contributed to the enrichment of rumen microorganisms. In contrast, calcium oxide straw had the opposite effect, which may be because the nutrient composition of calcium oxide straw was relatively simple and early.

The microbial communities of the AS and AC groups gathered and separated specifically before and after feeding, while PCoA analysis was based on the source and tissue structure of forage. The results of the PCoA analysis showed that the fiber structure of alfalfa silage and calcium oxide straw might be very different from that of forage in other

groups, so the specific species of the AS and AC groups were aggregated and separated from other groups.

4.3.2. Changes in Rumen Cellulolytic Bacteria

Ruminococcus flavefaciens, *Ruminococcus albus*, *Butyrivibro fibrisolvens*, *Fibrobacter succinogenes*, and *Clostridium* are the main cellulolytic bacteria in the rumen. *Ruminococcus* is an important rumen cellulolytic bacterium, and Flint and Bayer reported the presence of Ruminococcus (mainly *R. flavefaciens* and *R. albus*) from 3 d after birth in calves [40]. However, no significant difference in Ruminococcus was observed before and after feeding (Table 4), which may be related to the diet having similar levels of NDF.

Clostridium is also an important cellulolytic bacterium, but its abundance in the rumen is very low. *Succiniclastium* in the rumen belongs to the *Clostridium* genus. Tajima used macrogenomic sequencing to find that the Selenamonas-Succiniclastium-Megasphaera flora, which was fed a high-concentrate diet, was dominant in *Clostridium*; therefore, in the case of high-concentrate feed, *Succiniclastium* can be used to explain the change in *Clostridium* [25]. A high-concentrate diet was used in this study. The results showed a significant difference in *Succiniclastium* among the groups before feeding (0 h). The lowest *Succiniclastium* in the OG group before feeding was 1.86%, indicating that oats had an inhibitory effect on *Succiniclastium*; that is, oats may have an inhibitory effect on *Clostridium*.

In addition, *Ruminococcus* was dominant in the CW, whereas *Succiniclastium* was dominant in the AC. The results showed that feeding calcium oxide straw would make *Succiniclastium* in rumen cellulolytic bacteria more abundant when the dietary nutrition level was similar. *Succiniclastium* was the main producer of succinic acid, and it was also one of the main bacteria causing subacute ruminal acidosis [41]. Therefore, the focus should be on the ratio of concentrate to forage and the reasonable NDF content when using calcium oxide straw to configure the diet. In addition, *L. chinensis* can enrich *Ruminococcus* in rumen cellulolytic bacteria, and possibly the fiber of *L. chinensis* is not treated with alkali; thus, it is more difficult to degrade than calcium oxide straw. *Ruminococcus* is the most important cellulolytic bacteria in the rumen, and the number of *Ruminococcus* can be increased to adapt to this diet.

4.3.3. Changes in Rumen Amylolytic Bacteria

Prevotella is the main amylolytic bacteria in the rumen [42], and concentrates are the main source of dietary starch. The results showed that the content of *Prevotella* in the AS group was less than that in the other groups, indicating that alfalfa silage could reduce the number of rumen amylolytic bacteria. This may be due to the reduction in starch content in the concentrate caused by reducing the proportion of concentrate in the diet. Due to the rapid degradation of starch in the concentrate, the release of a large amount of acid causes rumen acidosis, while alfalfa silage reduces the use of concentrate to reduce the risk of rumen acidosis [43,44]. As shown in Figure 2, the rumen pH changes in the AS group before and after feeding were slower than those of the other groups, proving that alfalfa silage could maintain the stability of the rumen environment.

Prevotella is widespread in the rumen and contains the largest number of bacteria [45]. In this study, *Prevotella* accounted for a large proportion of the bacterial community, and there were no significant differences before and after feeding. Pitta reported that *Prevotella* was 33% in the rumen microflora of the group fed with the Bermudagrass diet and 56% fed with the winter wheat diet, resulting in rumen acidosis [35]. The results of the Pitta test showed that *Prevotella* could indicate rumen health status, thus, reflecting whether the diet configuration is reasonable. Furthermore, this experiment showed that the genus *Prevotella* was not abundant, indicating that the diet configuration in this experiment was reasonable.

4.3.4. Changes in Rumen Other Bacteria

Oscillospira is a common microorganism in the rumen closely related to rumen cellulolytic bacteria and may be involved in the proliferation of cellulolytic bacteria. The quiver helix content in the CW group is significantly higher than that in other groups, which may be because *L. chinensis* contains a lot of fiber, while the fiber content in other groups is relatively low.

In addition, the bacteria also changed before and after feeding. *Treponema* and *Fibrobacter* appeared before feeding but were not detected after feeding. New bacteria such as *Pseudomonas*, *Acinetobacter*, *Comamonas*, and *Solibacillus* appeared in the rumen after feeding. This may be related to changes in the rumen environment before and after feeding, including changes in the pH and temperature, nutrient intake, and metabolite accumulation.

Notably, the unknown bacteria in the CW group were significantly higher than those in other groups, probably due to the more complex fiber composition of *L. chinensis* and the need for more species of bacteria to decompose, resulting in more specific bacteria. From the PCoA analysis -, the microbial community of the CW group was clustered, and the factor affecting PCoA clustering was the forage source. This indicated that L. chinensis had a unique fiber composition structure, which was very different from oat, alfalfa silage, and calcium oxide straw, probably due to the different connection modes of chemical bonds between cellulose. Oat hay and alfalfa silage had lower cellulose, and the cellulose of calcium oxide straw was degraded greatly due to alkalization, while the cellulose of *L. chinensis* was higher than that of other groups and without any treatment; thus, the diet of the CW group was clustered.

4.3.5. Possible Factors Affecting Rumen Microflora

The microflora and total number of cellulolytic, amylolytic, and proteolytic bacteria in the rumen were approximately 3.3×10^9, 1.1×10^8, 1.0×10^8, 5.1×10^{10} CFU/mL, respectively [28,29]. There was no specific distribution of rumen microflora in Figures 3 and 4, which may be due to the similar levels of energy, protein, and fiber content in the four diets.

The nutritional levels of the two diets used by Pitta in the experiment were very different [35]. The protein levels (% DM) of the Bermuda grass and winter wheat groups were 11.2% and 20.8%, respectively, and the NDF was 67.9% and 39.7%, respectively. Therefore, the microbial composition of the rumen is very different. The *Prevotella* content in the winter wheat group was significantly higher than that in the Bermudagrass group ($p < 0.05$). The composition of rumen microorganisms was very different, and the content of Prevotella in the winter wheat group was significantly higher than that in the Bermuda grass group ($p < 0.05$). The protein content in the experimental diet was 16.58%. Protein levels of NDF were 29.29% and 29.66%, respectively. As observed in Table 4, the genus *Prevotella* varied from 27.21% to 36.52%, but the difference was not significant, which may be due to the different forage structures of the diets. In addition, Tukey's multiple comparisons revealed no significant difference in the percentage of bacteria among different groups. However, there was a significant difference among Pitta groups [35], indicating that forage structure was the main factor affecting rumen microflora to a certain extent.

Saro provided sheep diets based on hay and alfalfa hay (concentrate/forage ratio of 30:70), and PCR results showed that more *F. succinogenes* and *R. flavefaciens* were found in the rumen of sheep fed a hay-based diet ($p < 0.001$–0.05) [39]. This finding is consistent with the results of the present study. In this study, the rumen microorganisms of dairy cows fed two low-quality diets (*L. chinensis* and calcium oxide straw) were lower than those in the other two groups. In addition, there were no significant differences between the two groups, which may be due to the similar dietary nutrition levels in each group in this experiment.

Rumen microflora changes with different diets [44,46,47]. High-concentrate diets reduce the proportion of cellulolytic bacteria, while low-concentrate diets increase the proportion of fiber-decomposing bacteria [48,49]. The diets used in this experiment were all high-concentrate diets for lactating cows, and the ratio of concentrate to forage was

similar, which may be an important reason for the insignificant difference in the proportion of cellulolytic bacteria.

Wanapat et al. studied the effects of cassava chip and corncob groups on buffalo rumen microflora, and found that the differences in dietary NDF and ADF contents of the corncob and cassava group were 13.30–14.20% and 38.40–39.20%, respectively, which were close to the differences in NDF and ADF contents of the OG, CW, AS, and AC groups in this experiment [50]. Simultaneously, the results of this test also showed that the difference in fiber-decomposing bacteria was not significant (Figure 4), corresponding to the results of Wanapat's test. Therefore, the insignificant difference between NDF and ADF is another important reason for the small difference in the rumen microflora in this experiment.

5. Conclusions

In conclusion, the four forage combinations OG, CW, AS, and AC had no significant effect on dairy cow production and health. The rational use of low-quality forage can reduce the cost of dairy cow feed and has broad prospects in China.

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