

Article

Not peer-reviewed version

Effects of Adding Sodium Diacetate and *Aspergillus oryzae* to TMR Diets on Lactating Camel Production Performance, Milk Quality, and Fecal Microbiota

[Ziting Wang](#), Jingjing Wu, Dehang Song, Qiyuan Deng, Ali Har, [Zhijun Zhang](#)^{*}, [Wenxin Zheng](#)^{*}

Posted Date: 8 December 2025

doi: 10.20944/preprints202512.0635.v1

Keywords: Xinjiang Bactrian camel; milk quality; TMR; sodium diacetate; *Aspergillus oryzae*



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Effects of Adding Sodium Diacetate and *Aspergillus oryzae* to TMR Diets on Lactating Camel Production Performance, Milk Quality, and Fecal Microbiota

Ziting Wang ^{1,2,4}, Jingjing Wu ^{1,4}, Dehang Song ^{1,3}, Qiyuan Deng ^{1,3,4}, Ali Har ^{1,4}
and Zhijun Zhang ^{1,*}, Wenxin Zheng ^{3,*}

- ¹ Institute of Feed Research, Xinjiang Academy of Animal Science, Urumqi 830011, China
- ² Fujian Agriculture and Forestry University, Fuzhou 350000, China
- ³ Xinjiang Agricultural University, Urumqi 830011, China
- ⁴ Xinjiang Key Laboratory of Herbivorous Livestock Feed Biotechnology, Urumqi 830011, China
- * Correspondence: zzj850916@sina.com (Z.Z.); 303004083@qq.com (W.Z.)

Simple Summary

Low milk production and inconsistent milk quality in lactating camels are significant challenges in the camel breeding industry that require urgent attention. The use of feed additives to enhance both milk yield and quality represents an efficient and cost-effective approach. This study assessed the effects of two feed additives—sodium diacetate and *Aspergillus oryzae*—on lactating camels. The results suggest that these additives have the potential to improve the bioactive content in camel milk, offering a promising strategy for enhancing milk quality. These findings provide valuable insights for the future development of feed additives tailored for lactating camels, guiding the industry toward improved productivity and milk quality.

Abstract

Camel milk is highly favored by consumers for its high nutritional value and health benefits in preventing various diseases. However, camel farming is still mainly extensive, making it difficult to control the quality of camel milk. This experiment investigated the effects of adding sodium diacetate and kojimabase, feed additives, on the quality of camel milk from lactating Bactrian camels. The results of this experiment show that adding sodium diacetate can effectively increase the content of vitamin E and vitamin C in camel milk and can also effectively increase the content of unsaturated fatty acids in camel milk, thus effectively improving the nutritional quality of camel milk. The fecal microbial analysis results showed that there was no significant impact on the microbial community among the groups, but the addition of SDA significantly increased the number of *Melanocarpus* fungi. This demonstrates that adding 1000 mg of sodium diacetate per kilogram to the camel diet can effectively improve the quality of camel milk, providing a technical reference and demonstration for stabilizing camel milk quality through nutritional regulation.

Keywords: Xinjiang Bactrian camel; milk quality; TMR; sodium diacetate; *Aspergillus oryzae*

1. Introduction

Camel milk, with its similar composition to human milk, is a good source of nutrition and health products for residents of arid or semi-arid regions [1]. Its unique nutritional composition also gives it a wide range of medicinal uses, such as treating autism, controlling diabetes, preventing cirrhosis, and replacing cow's milk to avoid allergic reactions in infants [2]. In recent years, the demand for camel milk has been growing, but the production of camel milk products is still insufficient. Because camel farming is still carried out in a traditional extensive farming method, it is difficult to achieve large-scale production, and the quality of dairy products is difficult to meet uniform standards [3].

Currently, lactating camel farming in China is mainly distributed in arid desert grassland regions such as Inner Mongolia, Gansu, Xinjiang, Qinghai, and Ningxia. Among them, the camels in Xinjiang are mainly Bactrian camels, and the milk production of camels is generally low. The main reasons include internal factors such as genetics and physiology, and external factors such as feeding management, environment, and diet nutrition. The influence of feed additives cannot be ignored. Current nutritional studies of ruminants show that adding feed additives to the diet can effectively improve the production performance, rumen fermentation and milk production of ruminants, reduce the use of antibiotics, and has a significant antioxidant effect [4].

Sallam [5] significantly improved milk production and dry matter digestibility in female camels and significantly reduced the number of rumen protozoa after adding a mixture of brewer's yeast and rumen cocci to the camel diet. Adding date seed-derived biochar to camel diets by Ghazzawy [6] can significantly reduce CH₄ emissions in camels and improve nutrient digestibility. Studies have shown that the main mechanism of action of sodium diacetate (SDA) is that sodium diacetate is easily ionized to form acetic acid, which interferes with the interaction process between enzymes in the cell wall of fungal tissues, reduces the activity of harmful microorganisms [7], and when used as a feed additive, it can improve the milk fat conversion efficiency of dairy animals[8]. *Aspergillus oryzae* (AO), as a prebiotic additive, can improve feed intake, dry matter and fiber digestibility in ruminants, and increase rumen fiber-decomposing bacteria. It has been widely used in ruminant feed [9]. Studies by Ekin [10] and Sallam [11] showed that adding koji mold extract to the diets of Holstein dairy cows significantly increased milk production and feed intake without affecting the health of the cows. In summary, current research on the nutrition of lactating camels shows that commonly used feed additives are regionally specific, and there is no single feed additive that is suitable for use in multiple regions. Furthermore, SOD and AO, which are commonly used feed additives for dairy cows, have not been studied in camel production.

In summary, this experiment investigated whether different types of feed additives affect milk yield and quality in lactating camels by selecting sodium diacetate and *Aspergillus oryzae* as feed additives, thereby clarifying a camel feed additive that can be used on a large scale without regional specificity.

2. Materials and Methods

2.1. Test Materials

Sodium diacetate and *Aspergillus oryzae* were purchased from Dahan Enzyme Biotechnology Co., Ltd., and the *Aspergillus oryzae* had a live bacteria count of 12 billion/gram.

2.2. Test Time and Location

The experiment was conducted from July 1 to August 14, 2023, at the camel breeding base of Keping County Livestock Technology Development Co., Ltd., Aksu City, Xinjiang Uygur Autonomous Region.

2.3. Experimental Animals and Experimental Design

Thirty lactating camels of similar parity and lactation stage were randomly divided into three groups: a control group (TMR), a sodium diacetate group (SDA), and an omethoate enzyme group (AO). The TMR group was fed a total mixed ration (TMR), while the SDA group received a TMR diet supplemented with 150 g/d of sodium diacetate, and the AO group received a TMR diet supplemented with 40 g/d of omethoate enzyme. Each group consisted of 10 lactating camels. The experiment lasted 45 days, including a 15-day pre-feeding period and a 30-day trial period.

2.4. Basal Diet and Nutritional Requirements

Since there are no feeding standards for lactating camels, based on the characteristics of Bactrian camels to tolerate roughage, and with reference to the NRC (2007) standard for dairy cows, a diet for lactating camels was formulated. The roughage consisted of hydroponic wheat seedlings, wheat straw, and alfalfa, which were crushed to 2-3 cm using a TMR machine and then mixed with concentrate to form the TMR diet. The composition and nutritional levels of the diet are shown in Table 1. The dry matter (DM) of the feed was determined according to GB/T 6435-2014 Determination of Moisture in Feed; crude protein (CP) was determined according to GB/T 6432 Determination of Crude Protein in Feed - Kjeldahl Method; crude fat (EE) was determined according to GB/T 6433 Determination of Crude Fat in Feed; crude ash (Ash) was determined according to GB/T 6438 Determination of Crude Ash in Feed; calcium (Ca) was determined according to GB/T 13885 Determination of Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium and Zinc Content in Feed - Atomic Absorption Spectrometry; phosphorus (P) was determined according to GB/T 6437 Determination of Total Phosphorus in Feed - Spectrophotometry; neutral detergent fiber (NDF) was determined according to GB/T 20806 Determination of Neutral Detergent Fiber (NDF) in Feed; and acid detergent fiber (ADF) was determined according to NY/T The determination was performed according to the “1459 Determination Method of Acidic Detergent Fiber in Feed”. The SOD addition amount is 1000 mg/kg according to EU standards [12] and the AO addition amount is 40 g/day based on previous research progress [13–17].

Table 1. Feed formula and nutritional composition.

Item	TMR	SDA	AO
Corn	10.98	10.98	10.98
Bran	6.34	6.34	6.34
Soybean meal	2.44	2.44	2.44
Cottonseed meal	2.68	2.68	2.68
Camel premix ¹	1.22	1.22	1.22
Salt	0.73	0.73	0.73
Wheat straw	34.9	34.9	34.9
Alfalfa	23.26	23.26	23.26
Hydroponic fodder	17.45	17.45	17.45
total	100	100	100
Nutritional Information			
Energy (MJ/kg)	14.71	14.71	14.71
Crude protein%	12.63	12.63	12.63
Crude fat%	3.25	3.25	3.25
Neutral detergent fiber	45.12	45.12	45.12
Acid detergent fiber	30.02	30.02	30.02
Calcium%	1.07	1.07	1.07
Phosphorus%	0.57	0.57	0.57
Aspergillus oryzae (g/d)	0		40
Sodium diacetate (mg/kg)		1000	

Note: 1 Premix: Each kg of premix contains vitamin A 36000 IU, vitamin D3 50000 IU, vitamin E 1600 IU, copper 300 mg, iron 900 mg, zinc 1800 mg, manganese 750 mg, selenium 12 mg, iodine 18 mg, cobalt 7.5 mg, calcium 8%, phosphorus 2.5%.

2.5. Feeding and Management

The experimental camels were kept in the same enclosures, and the temperature and humidity of the enclosures were recorded daily (Supplementary Table S1). They were allowed free access to food and water in their groups and were fed twice a day at 09:00 and 19:30, with 15% of the feed remaining each day.

2.6. Sample Collection and Methods

2.6.1. Lactation performance

The milk yield of the experimental camels was measured at 0d, 10d, 20d and 30d during the trial period. On the 30th day of the trial period, 50ml of milk sample was collected (n=10). Milk protein, milk fat, lactose, non-fat, freezing point, ash content, conductivity and temperature were detected using a milk composition analyzer.

2.6.2. Detection of 16S and ITS in Fecal Microbiota

After the experiment, three lactating camels were randomly selected from each group to collect fecal samples over three days (n=3). The samples were thoroughly mixed, stored at -80 degrees Celsius, and sent to Suzhou Nomi Metabolic Biotechnology Co., Ltd. for bacterial 16S and fungal ITS microbial flora analysis. DNA was purified from rumen fluid using the cetyltrimethylammonium bromide (CTAB) method and then amplified by PCR using specific primers as templates [18]. After quantification of the PCR products using quantitative real-time PCR (Qubit 3.0), they were sequenced on the Illumina PE300 platform of Suzhou Nomi Metabolic Biotechnology Co., Ltd. The raw data from 16S rRNA gene sequencing were processed using QIIME2 software for quality control to remove low-quality sequences and potential contaminants. The SILVA database was selected for sequence alignment. Usearch software was used to cluster [19] Reads at a similarity level of 97.0% to obtain OUT. The Alpha diversity index of the samples was evaluated using QIIME2 2020.6 software [20]. Beta diversity analysis was performed using QIIME software to compare the similarity of different samples in terms of species diversity, and PCA [21] analysis plots were generated using R language tools. Using PICRUST2, a phylogenetic tree was constructed by aligning the characteristic sequence (16S rRNA) with the reference sequence of the Integrated Microbial Genomes (IMG) database. The “nearest species” of the characteristic sequence were found, and the gene information of unknown species was predicted based on the gene types and abundance information of known species. Thus, the pathway information of the entire community was predicted by combining the KEGG pathway information of genes [22]. No LEfse differentially expressed microorganisms were detected in this experiment. The fungal flora was determined using the ITS method, and the analytical method was the same as that for bacteria.

2.6.3. Serum Biochemical Indicators and Detection Methods

After the initial testing period, fasting blood was collected from the anterior vena cava using a disposable vacuum blood collection device. After collection, the blood was centrifuged at 3500 rpm, and the serum was transferred to a 1.5 ml EP tube using a 1 ml pipette and stored in a refrigerator (-20 °C). The levels of SOD (superoxide dismutase), MDA (malondialdehyde), GSH-PX (glutathione peroxidase), CAT (catalase), and T-AOC (total antioxidant capacity) in serum were measured.

2.6.4. Dairy Quality Testing

On day 30, the sample was sent to Novogene Biotechnology Co., Ltd. to determine the content of camel milk fatty acids, minerals (Na, Mg, K, Ca, Fe), vitamin E, insulin and vitamin C.

2.6.5. Fatty Acid Detection

Total fatty acids (FAs) were extracted from frozen meat samples following the procedure of Liang et al. FA separation was performed using gas chromatography (GC-450; Varian Co., Walnut Creek, CA, USA), with peaks identified based on retention time. Individual FA concentrations were quantified against standard curves prepared from a known methyl ester mixture (C4–C24; Sigma-Aldrich, St. Louis, MO, USA)

2.7. Data Analysis

The experimental data were initially processed using Excel 2016, and then a two-way ANOVA was performed using SPSS 26.0 statistical analysis software. Multiple comparisons were then performed using Duncan’s method. $P < 0.01$ indicated extremely significant differences, $P < 0.05$ indicated significant differences, and $P > 0.05$ indicated no significant differences.

3. Results and Analysis

3.1. Effects of Different Feed Additives on Milk Production Performance In Lactating Camels

As shown in Table 3, the addition of sodium diacetate and kojiase to the camel diet had no significant effect on the milk production of lactating camels ($P>0.05$).

Table 3. Differences in milk production performance of lactating camels with different additive groups(n=10).

Time	Group			SEM	p-Value
	TMR	AO	SDA		
0d	2.71	2.74	2.66	0.124	0.99
10d	2.78	2.47	2.67	0.129	0.28
20d	2.43	2.57	2.73	0.124	0.48
30d	2.46	2.67	2.56	0.128	0.29

a, b, c Different superscripts indicate significant differences within a row ($P < 0.05$).

3.2. Effects of Different Feed Additives on the Quality of Milk from Lactating Camels

As shown in Table 4, the addition of sodium diacetate and kojimaase to the diet had no significant effect on any of the indicators in the morning and evening milk of lactating camels after 30 days ($p>0.05$).

Table 4. Effects of different feed additives on milk quality.

Item (%)	Time	Group			SEM	p-Value
		TMR	AO	SDA		
Fat	Morning	4.85	5.09	5.31	0.10	0.39
	Night	5.37	5.3	5.54	0.14	0.93
Non-fat	Morning	9.52	9.67	9.57	0.10	0.16
	Night	10.14	9.64	9.84	0.11	0.41
Lactose	Morning	5.2	5.29	5.23	0.05	0.15
	Night	5.53	5.27	5.37	0.06	0.41
Conductivity	Morning	2.6	2.5	2.43	0.51	0.48
	Night	2.58	2.52	2.42	0.04	0.49
Milk Protein	Morning	3.6	3.66	3.62	0.04	0.19
	Night	3.83	3.64	3.72	0.04	0.43
pH	Morning	4.04	4.05	4.04	0.00	0.98
	Night	4.04	4.03	4.04	0.00	0.57
Freezing Point	Morning	61.27	62.01	61.38	0.54	0.12
	Night	64.17	59.47	62.39	0.85	0.28
Ash	Morning	0.72	0.73	0.73	0.01	0.14
	Night	0.77	0.73	0.75	0.01	0.44

a, b, c Different superscripts indicate significant differences within a row ($P < 0.05$).

3.3. Effects of Different Feed Additives on the Composition of Milk from Lactating Camels

As shown in Table 5, the vitamin E content in the milk of the sodium diacetate group was significantly higher than that of the basal diet TMR group and the kojimab group ($P < 0.01$), and the

vitamin E content in the milk of the kojimab group was significantly higher than that of the basal TMR group ($P < 0.01$); the vitamin C content in the milk of the sodium diacetate group was significantly higher than that of the basal TMR group ($P < 0.01$); there was no significant difference in insulin content among the three groups ($P > 0.05$).

Table 5. Effects of different feed additives on bioactive substances in lactating camel milk.

Item	Group			SEM	p-Value
	TMR	AO	SDA		
VE	5.28 ^{Cc}	6.69 ^{Bb}	7.61 ^{Aa}	0.22	0.001
Insulin	2.23	3.49	3.10	0.29	0.494
VC	90.89 ^{Bb}	101.82 ^{AaBb}	106.32 ^{Aa}	3.17	0.001

A, B, C Different superscripts indicate significant differences within a row ($p < 0.01$);^{a, b, c} Different superscripts indicate significant differences within a row ($P < 0.05$).

As shown in Table 6, there were no significant differences in the content of sodium, magnesium, potassium, calcium and iron in camel milk among the three groups ($P > 0.05$).

Table 6. Effects of different feed additives on trace elements in lactating camel milk.

Item	Group			SEM	p-Value
	TMR	AO	SDA		
Na	775.02	703.78	721.31	17.7	0.55
Mg	137.10	140.66	140.23	3.51	0.38
K	2120.06	1987.92	1850.58	53.42	0.32
Ca	1122.70	1213.14	1205.38	43.73	0.89
Fe	1.62	1.20	1.69	0.11	0.19

As shown in Table 7, the C21:0 content in the milk of the SDA group was significantly higher than that of the basal diet group and the kojimab group ($P < 0.01$), and the C22:6N3 content was significantly higher than that of the basal diet group and the kojimab group ($P < 0.05$). The C20:3N6 content in the milk of the sodium diacetate group and the basal TMR group was significantly higher than that of the kojimab group ($P < 0.01$), and the C20:5N3 content was significantly higher than that of the kojimab group ($P < 0.05$). The C24:1N9 content in the milk of the basal TMR group and the kojimab group was significantly higher than that of the sodium diacetate group ($P < 0.01$).

Table 7. Effects of different feed additives on fatty acid content in lactating camel milk.

Item	Group			SEM	p-Value
	TMR	AO	SDA		
C18:0	99	98.81	113.07	5.54	0.79
C18:1TN9	426.95	424.82	486.17	23.78	0.80
C18:1N9	187.99	168.05	212.26	9.49	0.39
C18:2TTN6	2.03	1.85	2.01	0.15	0.78
C18:2N6	47.6	41.96	50.65	2.41	0.37
C18:3N6	1.73	1.86	1.83	0.03	0.40
C18:3N3	15.24	12.31	15.93	1.15	0.14
C20:0	3.24	3.01	3.65	0.23	0.81
C20:1N9	2.48	2.04	2.38	0.25	0.62
C20:2N6	5.43	5.07	5.97	0.34	0.46
C21:0	0.84 ^{Bb}	0.75 ^{Bb}	1.06 ^{Aa}	0.07	0.006
C20:3N6	5.21 ^{Aa}	4.37 ^{Bb}	5.09 ^{Aa}	5.74	0.007
C20:4N6	7.61	6.49	7.37	0.46	0.33
C20:5N3	0.69 ^a	0.55 ^b	0.68 ^a	0.04	0.05

C22:0	3.37	3.24	4.00	0.2	0.46
C23:0	8.98	9.25	9.8	0.66	0.13
C22:4N6	1.82	1.65	1.62	0.17	0.17
C22:5N6	0.24	0.23	0.31	0.02	0.70
C24:0	13.92	13.62	14.85	1.00	0.45
C22:5N3	1.8	1.62	1.95	0.15	0.19
C22:6N3	0.22 ^b	0.25 ^b	0.39 ^a	0.03	0.07
C24:1N9	24.97 ^{Aa}	21.53 ^{Aa}	15.88 ^{Bb}	1.97	0.007

3.4. Effects of Different Feed Additives on Antioxidant Indices in Lactating Camels

As shown in Table 8, the addition of different types of additives to the feed had no significant effect on the antioxidant indicators in the serum of lactating camels ($p>0.05$).

Table 8. Effects of different feed additives on serum antioxidant levels in lactating camels.

Item	Group			SEM	P-Value
	TMR	SDA	AO		
SOD	196.58	187.17	91.97	3.96	0.65
MDA	1.76	1.76	1.48	0.10	0.46
GSH-PX	127.57	118.83	124.27	7.19	0.89
CAT	0.97	1.07	0.82	0.07	0.12
T-AOC	1.47	1.47	1.50	0.02	0.86

a, b, c Different superscripts indicate significant differences within a row ($P < 0.05$).

3.5. Effects of Different Feed Additives on the Fecal Microbiota of Lactating Camels

As shown in Figure 1A, there are 1514 unique bacterial microorganisms in the TMR group, 2122 in the SDA group, and 1992 in the AO group, for a total of 659 microorganisms across the three groups. As shown in Figure 1B, there are 90 unique fungal microorganisms in the TMR group, 67 in the SDA group, and 84 in the AO group, for a total of 11 fungal microorganisms across the three groups.

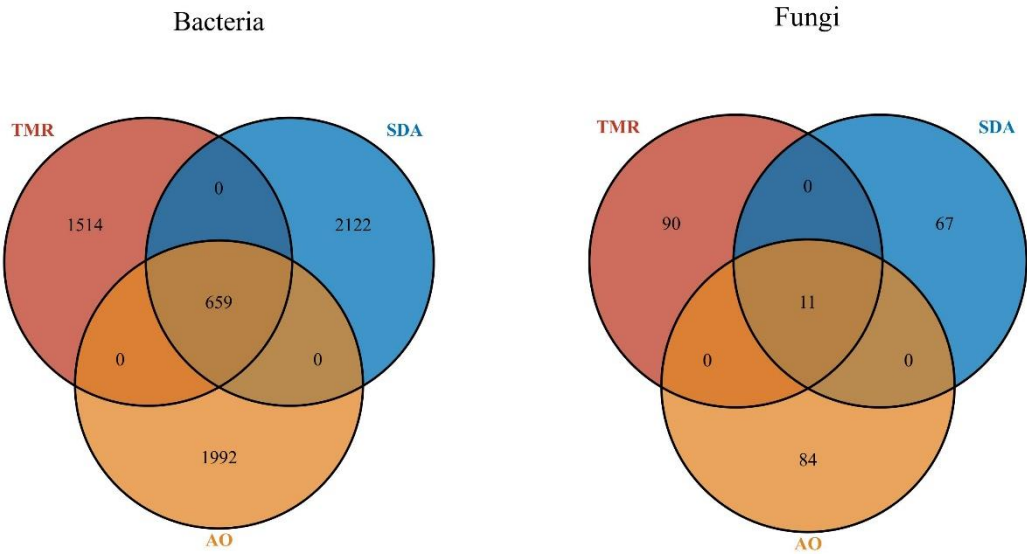


Figure 1. Venn diagram of the microbial flora of lactating camels with different feed additives. (a) Bacteria, (b) Fungi.

As shown in Figure 2, there were no significant differences in Alpha diversity among the three groups of bacteria and fungi ($P>0.05$).

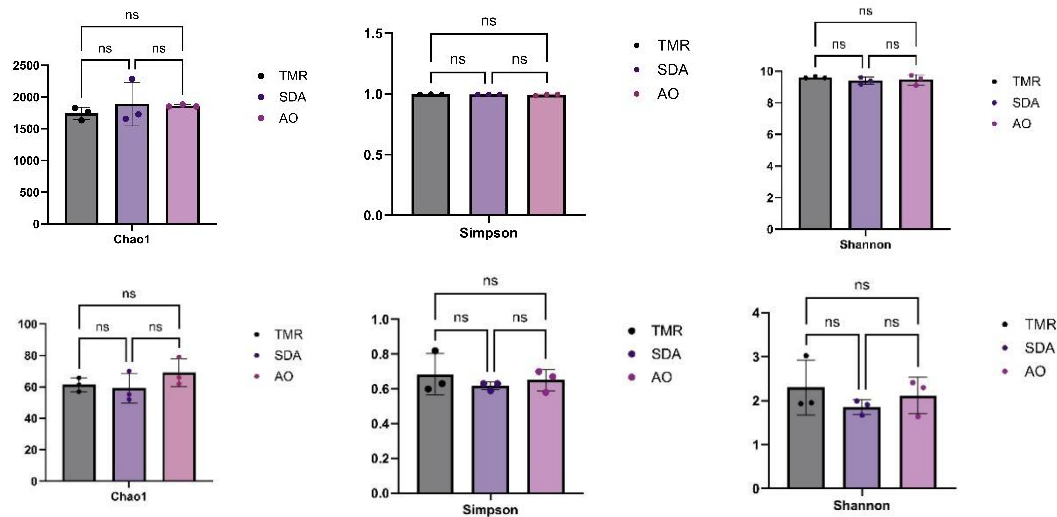


Figure 2. Alpha diversity analysis of microorganisms in feces of lactating camels with different feed additives. (a) Bacterial Chao1 index, (b) Bacterial Simpson index, (c) Bacterial Shannon index, (d) Fungal Chao1 index, (e) Fungal Simpson index, (f) Fungal Shannon index.

As shown in Figure 3a, there is a clear separation between the bacterial microbial communities in the AO group and the TMR group; as shown in Figure 3), there is a clear separation between the fungal microbial communities in the AO group and the SDA group.

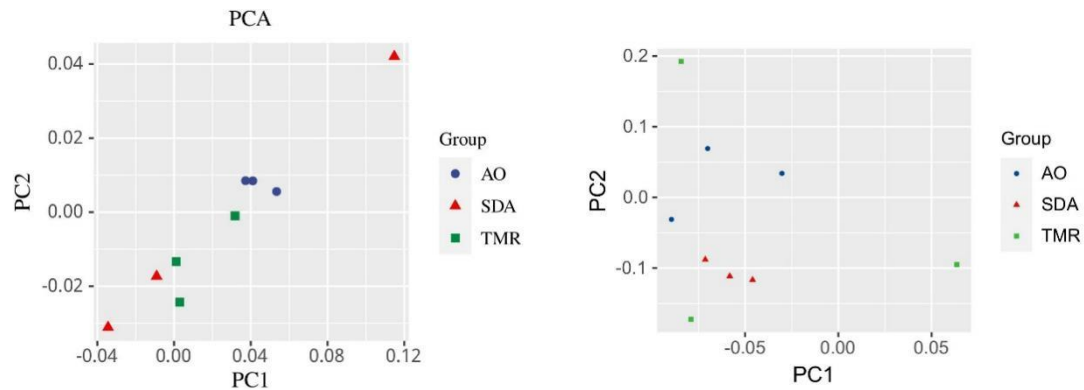


Figure 3. PCA diagram of fecal microorganisms in lactating camels with different feed additives. (a) Bacteria, (b) Fungi.

As shown in Table 9 and Figure 4a, the Actinobacteria phylum in the TMR group was significantly higher than that in the other two groups ($P < 0.01$), while there were no significant differences among the groups in other bacterial phyla ($P > 0.05$). As shown in Table 10 and Figure 4b, there were no significant differences among the groups in any bacterial genus ($P > 0.05$). As shown in Table 11 and Figure 4c, there were no significant differences among the three groups in any fungal phylum ($P > 0.05$). As shown in Table 12 and Figure 4d, the Melanocarpus genus in the SDA group was significantly higher than that in the other two groups ($P < 0.01$), while there were no significant differences among the other fungal genera.

Table 9. Differences in the level of bacterial phylum in feces of lactating camels with different feed additives.

Item	Group			SEM	p-Value
	TMR	SDA	AO		
Firmicutes	0.63	0.61	0.59	0.02	0.70
Bacteroidetes	0.23	0.29	0.08	0.05	0.39

Verrucomicrobia	0.06	0.05	0.05	0.01	0.43
Spirochaetes	0.03	0.02	0.02	0.003	0.84
Proteobacteria	0.01	0.01	0.01	0.003	0.80
Tenericutes	0.01	0.01	0.01	0.001	0.57
Actinobacteria	0.02Aa	0.005Bb	0.005Bb	0.002	0.01
Fibrobacteres	0.001	0.003	0.002	0.001	0.76
TM7	0.002	0.002	0.001	0.0002	0.09
other	0.004	0.004	0.006	0.001	0.35

A, B, C Different superscripts indicate significant differences within a row ($p < 0.01$);^{a, b, c} Different superscripts indicate significant differences within a row ($P < 0.05$).

Table 10. Differences in bacterial genera and microorganism levels in feces of lactating camels with different feed additives.

Item	Group			SEM	p-Value
	TMR	SDA	AO		
<i>Ruminococcaceae</i>	0.22	0.20	0.20	0.010	0.43
<i>Bacteroidales</i>	0.10	0.13	0.17	0.180	0.31
<i>unidentified_Clostridiales</i>	0.10	0.11	0.10	0.002	0.86
<i>unclassified_Clostridiales</i>	0.06	0.06	0.06	0.002	0.90
<i>Akkermansia</i>	0.06	0.04	0.04	0.010	0.40
<i>Lachnospiraceae</i>	0.04	0.06	0.03	0.010	0.63
<i>Christensenellaceae</i>	0.03	0.02	0.02	0.002	0.99
<i>Ruminococcus</i>	0.03	0.02	0.02	0.003	0.93
<i>Treponema</i>	0.03	0.02	0.02	0.003	0.84
<i>other</i>	0.33	0.32	0.32	0.010	0.91

Table 11. Differences in the level of fungi in feces from lactating camels with different feed additives.

Item	Group			SEM	p-Value
	TMR	SDA	AO		
Ascomycota	0.99	0.99	0.99	0.003	0.64
Fungi_phy_Incertae_sedis	0.003	0.001	0.004	0.001	0.62
Mucoromycota	0.004	0.005	0.006	0.001	0.91
Basidiomycota	0.002	0.001	0.002	0.001	0.73
Neocallimastigomycota	0.003	0.001	0.001	0.001	0.36
unclassified_Fungi	0.0003	0.00	0.00004	0.0001	0.39
Mortierellomycota	0.00	0.00	0.00001	0.000003	0.42
Chytridiomycota	0.000006	0.00	0.00	0.000002	0.42
Rozellomycota	0.00	0.000006	0.00	0.000002	0.42
Ascomycota	0.99	0.99	0.99	0.003	0.64

Table 12. Differences in the level of fungal genera in feces of lactating camels with different feed additives.

Item	Group			SEM	p-Value
	TMR	SDA	AO		
<i>Aspergillus</i>	0.49	0.46	0.56	0.03	0.57
<i>Thermomyces</i>	0.37	0.46	0.37	0.03	0.44
<i>Acaulium</i>	0.00	0.00	0.0009	0.0003	0.42
<i>Pichia</i>	0.09	0.0007	0.008	0.03	0.39
<i>Melanocarpus</i>	0.0008Bb	0.05Aa	0Bb	0.009	0.002
<i>Mycothermus</i>	0.00	0.01	0.02	0.006	0.59
<i>Fungi_gen_Incertae_sedis</i>	0.003	0.001	0.004	0.001	0.62
<i>Diutina</i>	0.03	0.00	0.00	0.009	0.42

other	0.02	0.02	0.04	0.008	0.48
Aspergillus	0.49	0.46	0.56	0.03	0.57

A, B, C Different superscripts indicate significant differences within a row ($p < 0.01$),^{a, b, c} Different superscripts indicate significant differences within a row ($P < 0.05$).

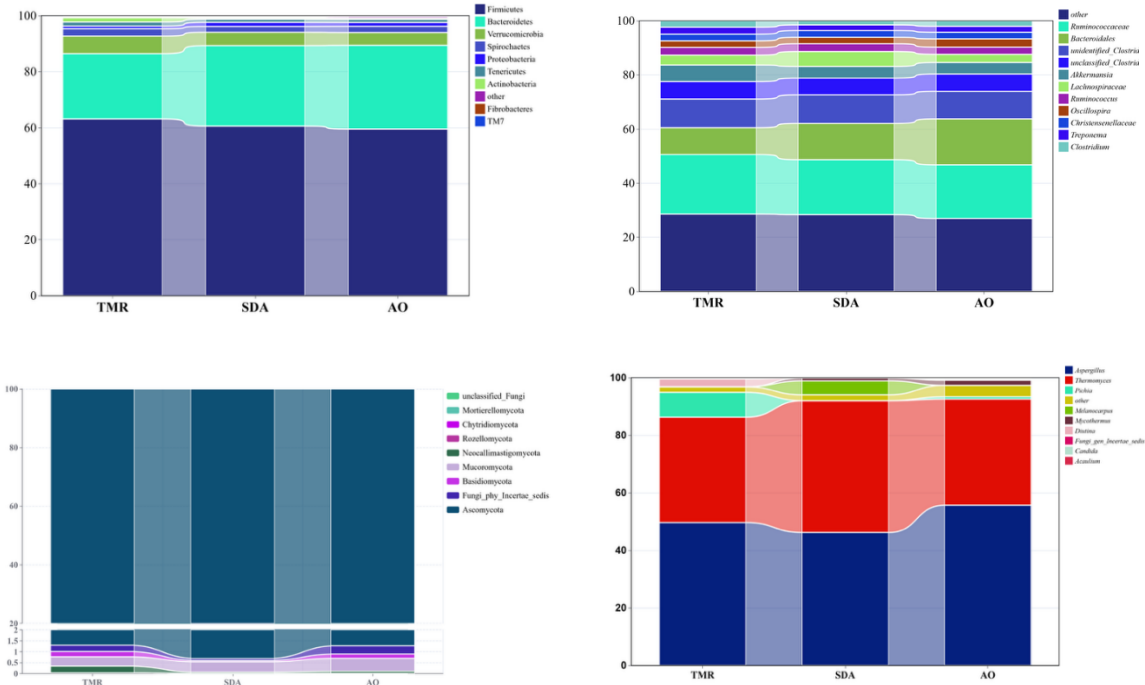


Figure 4. Microbial abundance in feces of lactating camels with different feed additives. (a) Phylum level, (b) Genus's level, (c) Phylum level, (d) Genus's level.

As shown in Figure 5a, compared with the SDA group, the Zeatin_biosynthesis pathway and Systemic_lupus_erythematosus pathway were significantly upregulated in the TMR group ($P < 0.01$); as shown in Figure 5b, compared with the AO group, the Photosynthesis_antenna-proteins pathway was significantly upregulated in the TMR group ($P < 0.05$), and the Polycyclic_aromatic_hydrocarbon_degradation pathway was significantly downregulated ($P < 0.001$).

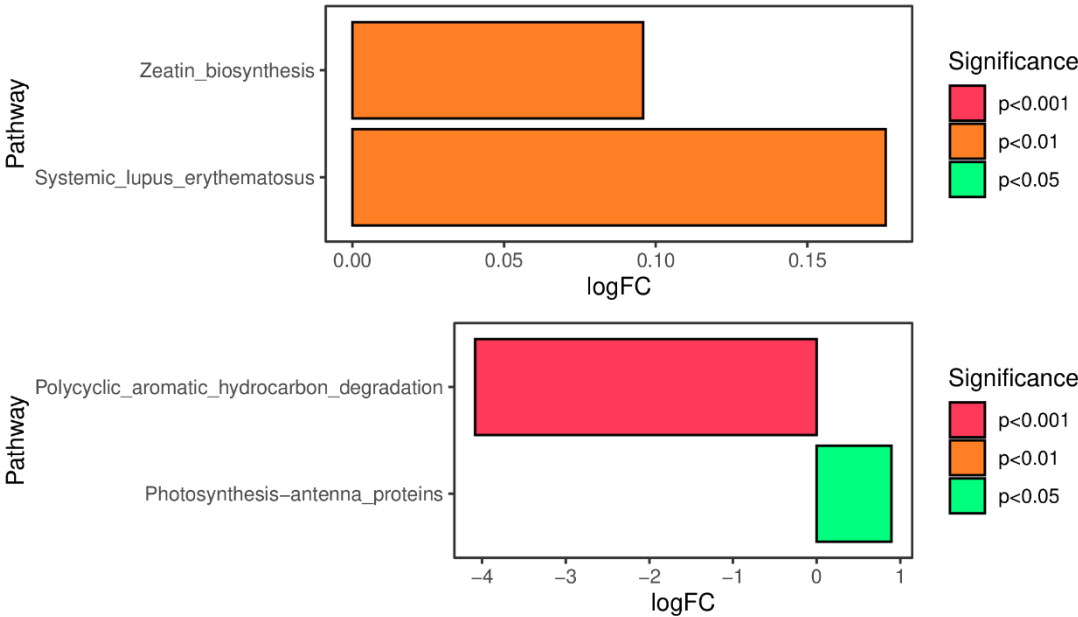


Figure 5. Differential metabolic pathways of microorganisms in feces of lactating camels with different feed additives. (a) Bacterial TMR vs SDA, (b) Bacterial TMR vs AO.

As shown in Figure 6a, at the bacterial level, there are significant positive correlations among the Firmicutes, Bacteroidetes, Verrucomicrobia, and Proteobacteria phyla, indicating a strong synergistic effect in the rumen of lactating camels. Figure 6b shows that at the fungal level, there are high levels of synergistic and antagonistic effects among the Ascomycota phyla. Ascomycota is significantly positively correlated with Neocallimastigomycota and Mucoromycota phyla, and significantly negatively correlated with Basidiomycota phylum.

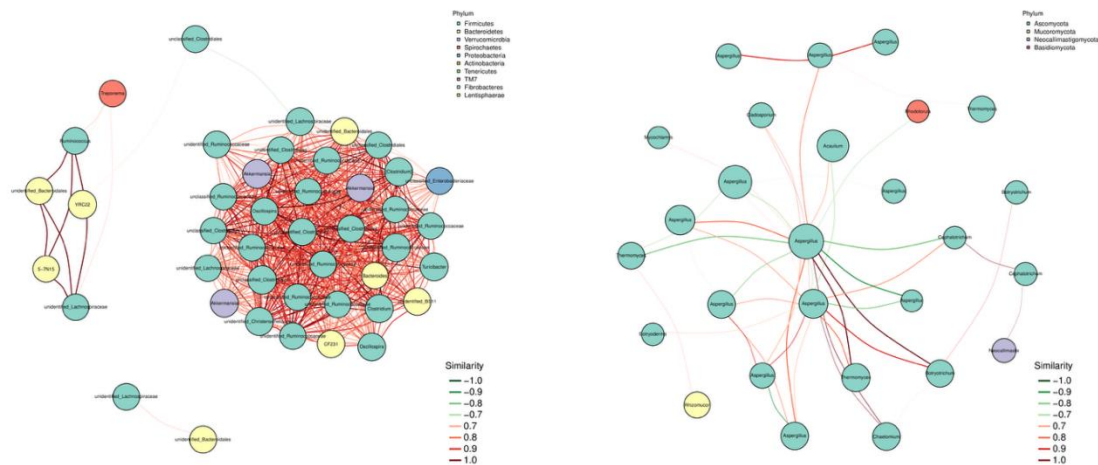


Figure 6. Co-expression network of microorganisms in feces of lactating camels with different feed additives. (a) Bacteria, (b).

As shown in Figure 7, the C24:1N9 content in camel milk was significantly positively correlated with the abundance of Tenericutes bacteria ($P<0.05$).

4. Discussion

Camels, due to their unique physiological structure, can survive for a long time in arid conditions, and their milk provides a good source of nutrition for local herders. Currently, common camel diet additives mainly include enzyme preparations and probiotic preparations. Adding feed additives to the diet can significantly improve the camel’s productivity, improve the rumen environment, and reduce acidosis [5]. Sodium diacetate, as a common feed additive and preservative, is widely used in the production and preservation of silage for ruminants and has the effect of improving the nutritional value and aerobic stability of silage [23]. The addition of sodium diacetate in this experiment had no significant effect on milk yield or routine milk quality in lactating camels, which is consistent with the results of Shockey’s study in dairy cows [24]. *Aspergillus oryzae*, a fungal enzyme, has been widely used in dairy production. Adding *aspergillus oryzae* to the diet can significantly increase cellulose-decomposing bacteria in the rumen and improve the apparent digestibility of dry matter [15]. Zhang [25] research shows that adding kojimab to dairy cow diets can significantly increase milk yield, milk protein and lactose content, and alter the composition of the hindgut microbiota. This differs from some of the results of this experiment. In this experiment, the addition of kojimab did not significantly affect the milk yield and routine milk production of lactating camels, but the microbiological results were more consistent. There was a clear separation of the fungal flora among the different groups in the feces. This may be related to the fungal properties of kojimab. Camels themselves have a strong ability to digest fiber and abundant fiber-digesting bacteria. The addition of kojimab has little effect on the fiber-digesting ability of bacteria [26]. In this

experiment, although the addition of sodium diacetate and kojimab had no significant effect on the milk yield and conventional milk quality of lactating camels, the addition of kojimab and sodium diacetate significantly increased the vitamin E and vitamin C content in camel milk, and there were significant differences in the content of polyunsaturated fatty acids (Table 7), with the addition of sodium diacetate showing the best effect. However, there is currently limited research on the deposition mechanisms of vitamin E, vitamin C, and fatty acids in camel milk, which could be the focus of future research. The correlation analysis showed a high positive correlation between *Tenericutes* phylum and C24:1N9. While the exact nature of *Tenericutes* phylum is still unclear, it is believed to be a potential mutualistic symbiotic organism in the host gut, increasing lignin digestibility[27]. However, there was no significant difference in *Tenericutes* phylum among the three groups, suggesting that the mechanism of action of adding sodium diacetate and kojimab is not to directly affect microbial abundance, but rather to further increase C24:1n9 content through synergistic effects between microorganisms. In the fatty acid results (Table 7), the C24:1n9 content in camel milk was significantly higher in the TMR and AO groups than in the SOD group. Therefore, we can speculate that *Tenericutes* phylum may have an antagonistic effect with sodium diacetate but a synergistic effect with kojimab.

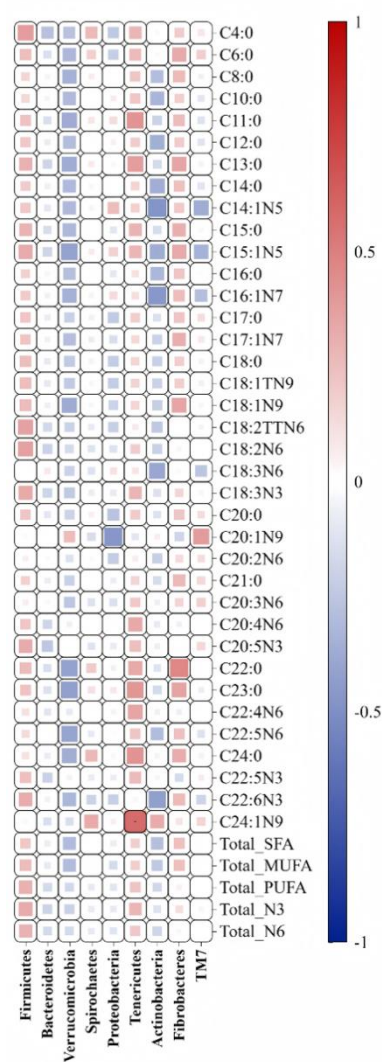


Figure 7. Association analysis of fatty acids and bacterial microorganisms in lactating camels with different feed additives.

Analysis of the fecal microbiota in this experiment showed that the abundance of *Melanocarpus* in the fecal fungi of the SOD group was significantly higher than that of the TMR and AO groups. Current research on *Melanocarpus* shows that as a xylanase-producing microorganism, it can effectively decompose arabinoxylan in the diet, reduce feed viscosity, and improve the digestibility of ruminant feed [28]. This indicates that adding SOD can improve the ability of bacteria in the camel's gastrointestinal tract to digest fiber, thereby promoting feed digestion and metabolism. KEGG analysis of the gut microbiota revealed that the expression of the zeatin biosynthesis pathway and Systemic_lupus_erythematosus pathway was significantly lower in the SOD group compared to the TMR group. The zeatin biosynthesis pathway can regulate the utilization of carbohydrates by the microbial community [29]. The Systemic_lupus_erythematosus pathway is closely related to the gastrointestinal microbiota [30]. Differences in the gastrointestinal flora led to metabolic dysfunction, affecting local gastrointestinal function, which in turn affects the expression of the Systemic_lupus_erythematosus pathway [31]. This reduces the animal's immune system capacity. Therefore, we can infer that adding sodium diacetate to the diet can effectively improve the stability of the gastrointestinal flora in lactating camels and improve the utilization efficiency of carbohydrates. The comparison of KEGG differential pathways between the AO group and the TMR group shows that the Photosynthesis_antenna-proteins pathway was significantly upregulated in the TMR group and significantly downregulated in the Photosynthesis_antenna-proteins pathway. Polycyclic aromatic hydrocarbons (PAHs) are dangerous environmental pollutants that have been classified as carcinogens and affect several diseases in humans [32]. Current research shows that when dairy cows are exposed to PAH contamination, PAH residues remain in their milk, posing a significant risk of transfer from dairy products to humans [33]. However, in this study, the PAH degradation pathway was significantly enhanced in the AO group, indicating that adding kojimab to the diet can effectively improve the degradation rate of PAH in camels, thereby improving the safety of camel milk.

The fecal microbial network analysis results showed that Firmicutes were the central phylum in the bacterial network, which may mean that Firmicutes plays a major core role in the three groups of lactating camel fecal bacteria. There was also a significant positive correlation between Firmicutes and Bacteroidetes. Currently, Firmicutes and Bacteroidetes are the main phyla in ruminant microbiology research in lignin decomposition and carbohydrate binding [34]. This is consistent with the results of this experiment, indicating that there is a high degree of synergy among lactating camel microbes and that they may produce the same metabolites. Fungal results showed that Ascomycota plays a major core role in fungal communities and is crucial in cellulose degradation [35]. Ascomycota, along with Basidiomycota, is typically a major fungal community in the gastrointestinal tract of ruminants. However, in the expression network of this study, Ascomycota and Basidiomycota exhibited some antagonistic activity. Both Ascomycota and Basidiomycota are non-anaerobic fungi and were previously considered dominant fungi in the gastrointestinal tract of ruminants [36]. The antagonistic activity between Ascomycota and Basidiomycota in this study may be related not only to competition for rumen oxygen, but also to competition between the addition of sodium diacetate and fungal kojimaase to the diet, leading to niche competition between the two fungal communities.

Therefore, this experiment used sodium diacetate and kojimab as feed additives to study their effects on milk yield, milk quality, and fecal microbiota in lactating camels. However, the experiment still has limitations. The optimal ratio of the two additives and the main metabolic differences between the additives are not yet clear. But the main purpose is to provide ideas for future research to find the metabolic mechanisms of sodium diacetate and kojimab, as well as their optimal feeding amounts, and thus improve the quality of camel milk from lactating camels.

5. Conclusions

The results of this study show that, under the same feed conditions, adding sodium diacetate or kojimab to the diet can improve the quality of camel milk to a certain extent without affecting milk yield, and adding 1000 mg of sodium diacetate has the best effect.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, Zhijun Zhang, Wenxin Zheng; methodology, Ziting Wang; software, Ziting Wang; validation, Qiyuan Deng, and Jingjing Wu; formal analysis, Ziting Wang; investigation, Ziting Wang; resources, Ziting Wang; data curation, Weiting Xing, Yong Tuo; writing—original draft preparation, Ziting Wang; writing—review and editing, Dehang Song; visualization, Har Ali; supervision, Zhijun Zhang; project administration, Zhijun Zhang; funding acquisition, Wenxin Zheng. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Technology Integration and Demonstration for Efficient Conversion and Utilization of Cotton Straw and Coordinated Development of Grass-Livestock Industry (AKS-2025-AGTI-F09); Sub-task of the 6th major special project of the autonomous region: “Investigation and basic parameter collection of rapid detection instruments for pesticide and veterinary drug residues” (2022A02006-6-2).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Institute of Feed Research, Xinjiang Academy of Animal Science (protocol code NO. 11 20231228 and 11 November 2023 date of approval).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Muthukumaran MS, Mudgil P, Baba WN, Ayoub MA, Maqsood S. A comprehensive review on health benefits, nutritional composition and processed products of camel milk. *Food Rev Int.* 2023;39(6):3080-3116. doi:10.1080/87559129.2021.2008953
2. Vincenzetti S, Cammertoni N, Rapaccetti R, et al. Nutraceutical and Functional Properties of Camelids' Milk. *Beverages.* 2022;8(1):12. doi:10.3390/beverages8010012
3. Ait El Alia O, Zine-Eddine Y, Chaji S, Boukrouh S, Boutoia K, Faye B. Global camel milk industry: A comprehensive overview of production, consumption trends, market evolution, and value chain efficiency. *Small Rumin Res.* 2025;243:107441. doi:10.1016/j.smallrumres.2025.107441
4. Nastoh NA, Waqas M, Çınar AA, Salman M. The impact of phytogenic feed additives on ruminant production: A review. doi:10.22358/jafs/191479/2024
5. Sallam SMA, Abo-Baker FA, Attia MFA, et al. Effect of microbial feed additives on performance of Maghrebian she-camels under Egyptian conditions. *Small Rumin Res.* 2025;252:107574. doi:10.1016/j.smallrumres.2025.107574
6. Ghazzawy HS, Alqahtani NK, Sheikh A, et al. Phoenix dactylifera seed-derived biochar as a sustainable and environmentally feed supplement in camel: impacts gas production, methane emissions, nutrient degradability and fermentation parameters, performance predictions. *Front Vet Sci.* 2025;12. doi:10.3389/fvets.2025.1632447
7. Wang S, Liu H, Zhao J, Dong Z, Li J, Shao T. Influences of Organic Acid Salts and Bacterial Additives on Fermentation Profile, Aerobic Stability, and In Vitro Digestibility of Total Mixed Ration Silage Prepared with Wet Hulless Barley Distillers' Grains. *Agronomy.* 2023;13(3):672. doi:10.3390/agronomy13030672
8. 添加双乙酸钠对裹包 TMR 贮存效果及奶牛生产性能的影响_国卫杰.
9. Uwineza C, Parchami M, Bouzarjomehr M, Taherzadeh MJ, Mahboubi A. Recent Developments in the Application of Filamentous Fungus *Aspergillus oryzae* in Ruminant Feed. *Animals.* 2024;14(16):2427. doi:10.3390/ani14162427
10. Effects of feeding *Aspergillus oryzae* fermentation product to transition Holstein cows on performance and health. Accessed December 3, 2025. <https://cdnsiencepub.com/doi/10.1139/cjas-2018-0037>

11. Sallam SMA, Abdelmalek MLR, Kholif AE, et al. The effect of *Saccharomyces cerevisiae* live cells and *Aspergillus oryzae* fermentation extract on the lactational performance of dairy cows. *Anim Biotechnol.* 2020;31(6):491-497. doi:10.1080/10495398.2019.1625783
12. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Scientific Opinion on the safety and efficacy of acetic acid, sodium diacetate and calcium acetate as preservatives for feed for all animal species. *EFSA J.* 2012;10(2):2571. doi:10.2903/j.efsa.2012.2571
13. Tricarico JM, Johnston JD, Dawson KA. Dietary supplementation of ruminant diets with an *Aspergillus oryzae* α -amylase. *Anim Feed Sci Technol.* 2008;145(1):136-150. doi:10.1016/j.anifeedsci.2007.04.017
14. Guo L, Zhang D, Du R, Li F, Li F, Ran T. Supplementation of *Aspergillus oryzae* Culture Improved the Feed Dry Matter Digestibility and the Energy Supply of Total Volatile Fatty Acid Concentrations in the Rumen of Hu Sheep. *Front Nutr.* 2022;9. doi:10.3389/fnut.2022.847156
15. Higginbotham GE, Santos JEP, Juchem SO, DePeters EJ. Effect of feeding *Aspergillus oryzae* extract on milk production and rumen parameters. *Livest Prod Sci.* 2004;86(1):55-59. doi:10.1016/S0301-6226(03)00168-4
16. Chiquette J. *Saccharomyces cerevisiae* and *Aspergillus oryzae*, used alone or in combination, as a feed supplement for beef and dairy cattle. *Can J Anim Sci.* 1995;75(3):405-415. doi:10.4141/cjas95-060
17. Gomez-Alarcon RA, Dudas C, Huber JT. Influence of Cultures of *Aspergillus oryzae* on Rumen and Total Tract Digestibility of Dietary Components1. *J Dairy Sci.* 1990;73(3):703-710. doi:10.3168/jds.S0022-0302(90)78723-1
18. Terzi V, Infascelli F, Tudisco R, Russo G, Stanca AM, Faccioli P. Quantitative detection of *Secale cereale* by real-time PCR amplification. *LWT - Food Sci Technol.* 2004;37(2):239-246. doi:10.1016/j.lwt.2003.08.005
19. UPARSE: highly accurate OTU sequences from microbial amplicon reads | *Nature Methods*. Accessed December 3, 2025. <https://www.nature.com/articles/nmeth.2604>
20. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019;37(8):852-857.
21. Multiple common variants for celiac disease influencing immune gene expression | *Nature Genetics*. Accessed December 3, 2025. <https://www.nature.com/articles/ng.543>
22. STAMP: statistical analysis of taxonomic and functional profiles | *Bioinformatics* | Oxford Academic. Accessed December 3, 2025. <https://academic.oup.com/bioinformatics/article/30/21/3123/2422240>
23. Li YF, Wang LL, Jeong EC, Kim HJ, Ahmadi F, Kim JG. Effects of sodium diacetate or microbial inoculants on aerobic stability of wilted rye silage. *Anim Biosci.* 2022;35(12):1871-1880. doi:10.5713/ab.22.0150
24. Shockey WL, Heinrichs AJ, Weiss WP, Conrad HR. Evaluation of Sodium Diacetate-Treated Alfalfa Silage for Lactating Dairy Cows. *J Prod Agric.* 1990;3(3):381-385. doi:10.2134/jpa1990.0381
25. Response of Milk Performance, Rumen and Hindgut Microbiome to Dietary Supplementation with *Aspergillus oryzae* Fermentation Extracts in Dairy Cows | *Current Microbiology*. Accessed December 3, 2025. <https://link.springer.com/article/10.1007/s00284-022-02790-z>
26. Rabee AE, Forster R, Sabra EA. Lignocellulolytic activities and composition of bacterial community in the camel rumen. *AIMS Microbiol.* 2021;7(3):354-367. doi:10.3934/microbiol.2021022
27. Exploring age-related changes in gut bacterial community composition of yak: insights from different age groups | *BMC Microbiology*. Accessed December 3, 2025. <https://link.springer.com/article/10.1186/s12866-025-04011-6>
28. Phuyal M, Budhathoki U, Bista D, Shakya S, Shrestha R, Shrestha AK. Xylanase-Producing Microbes and Their Real-World Application. *Int J Chem Eng.* 2023;2023(1):3593035. doi:10.1155/2023/3593035
29. Zhang R, Bao X, Shi X, et al. In Vitro Evaluation of Enzymatically Transformed Alfalfa Saponins on Methane Reduction, Rumen Microbes and Metabolomics in Goats. *Animals.* 2025;15(11):1516. doi:10.3390/ani15111516
30. Wang J, Fan H, Li M, et al. Integration of Non-Coding RNA and mRNA Profiles Reveals the Mechanisms of Rumen Development Induced by Different Types of Diet in Calves. *Genes.* 2023;14(5):1093. doi:10.3390/genes14051093
31. Effect of *Zanthoxylum bungeanum* essential oil on rumen enzyme activity, microbiome, and metabolites in lambs | *PLOS One*. Accessed December 3, 2025. <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0272310>

32. Straif K, Baan R, Grosse Y, Secretan B, Ghissassi FE, Coglianò V. Carcinogenicity of polycyclic aromatic hydrocarbons. *Lancet Oncol.* 2005;6(12):931-932. doi:10.1016/S1470-2045(05)70458-7
33. Evaluation of the Risk of PAHs and Dioxins Transfer to Humans via the Dairy Ruminant | SpringerLink. Accessed December 3, 2025. https://link.springer.com/chapter/10.1007/3-540-26531-7_38
34. da Silva Pereira M, Alcantara LM, de Freitas LM, de Oliveira Ferreira AL, Leal PL. Microbial Rumen proteome analysis suggests Firmicutes and Bacteroidetes as key producers of lignocellulolytic enzymes and carbohydrate-binding modules. *Braz J Microbiol.* 2025;56(2):817-833. doi:10.1007/s42770-025-01627-8
35. Li Y, Li X, Liu Y, et al. Comparison of Bacterial and Fungal Community Structure and Potential Function Analysis of Yak Feces before and after Weaning. *BioMed Res Int.* 2022;2022(1):6297231. doi:10.1155/2022/6297231
36. Cui X, Liu Y, Wu H, et al. Dynamic changes in the yak rumen eukaryotic community and metabolome characteristics in response to feed type. *Front Vet Sci.* 2022;9. doi:10.3389/fvets.2022.1027967

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.