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Effects of *Allium mongolicum* regel essential oil supplementation on growth performance, nutrient digestibility, rumen fermentation, and bacterial communities in sheep

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The objectives of this research were to investigate the effects of *Allium mongolicum* Regel essential oil on growth performance, nutrient digestibility, rumen fermentation, and bacterial communities in sheep. Twenty sheep were randomly divided into two dietary groups with 10 replicates each: (1) a basal diet without AMO as the control group ($n = 10$) and (2) a basal diet supplemented with 40 mg/kg AMO as the AMO group ($n = 10$). The average daily gain (ADG) was increased ($P < 0.05$), and the feed conversion ratio (FCR) was reduced ($P < 0.05$) in the AMO group compared with the control. The ruminal acetate, propionate, total volatile fatty acids (TVFA), and microbial protein (MCP) were higher ($P < 0.05$) in the AMO group than in the control. Moreover, ruminal pH and ammonia nitrogen ($\text{NH}_3\text{-N}$) were lower ($P < 0.05$) in the AMO group than in the control. The relative abundances of the phylum levels of *Firmicutes*, *Actinobacteriota*, and *Verrucomicrobiota* were higher ($P < 0.05$) in the AMO group than in the control, and the relative abundances of *Bacteroidetes* and *Spirochaetota* were lower ($P < 0.05$) in the AMO group than in the control. The relative abundance of *Prevotella* and *Prevotellaceae_UCG-003* at the genus level was increased ($P < 0.05$) in the AMO group compared with the control; however, the relative abundance of *Succiniclasicum*, *Norank_f__F082*, *Christensenellaceae_R-7_group*, and *Norank_f__Muribaculaceae* was decreased ($P < 0.05$) in the AMO group compared with the control. The activities of cellulase, α -amylase, and proteinase were higher ($P < 0.05$) in the AMO group than in the control. The apparent digestibility of dry matter (DM) and crude protein (CP) was increased

($P < 0.05$) in the AMO group compared with the control. In conclusion, AMO supplementation has the potential to improve growth performance. Moreover, supplementation with AMO improved nutrient digestibility, rumen fermentation, and bacterial communities in the rumen of sheep.

KEYWORDS

allium mongolicum regel, essential oil, growth performance, rumen fermentation, bacterial communities

Introduction

Ionophores (monensin) have been extensively used in ruminant production to reduce energy and protein losses in the rumen (1). The appearance of residues in products and the spread of antibiotic-resistant microbes have led to restrictions in the European Union and a ban in China on their use (2, 3). Consequently, the feed industry has investigated safe and effective alternatives that can functionally replace antibiotics, such as plant extracts (phytobiotics) and essential oils (EOs), which provide similar results as antibiotics in terms of feed efficiency and rumen fermentation (4).

EOs are natural bioactive compounds extracted from plants *via* distillation methods, or they can be obtained by chemical extraction (1). EOs as alternatives to antibiotics have the potential to be used in livestock feed. EOs were found to improve nutrient digestibility and increase growth performance in animals (5, 6). Laura et al. noted that a blend of EO dietary supplementation decreased the concentration of acetate and ammonia nitrogen ($\text{NH}_3\text{-N}$) in the rumen of finishing beef cattle (7). Moreover, oregano EO was shown to increase the relative abundance of *Prevotella* and *Dialister* in ruminal fluids *in vitro* (8). This capacity has become a promising research field for investigating the potential of EOs to alter rumen fermentation, resulting in improved feed efficiency in ruminants, but studies have mostly been conducted *in vitro* (9). In small ruminants, only a few *in vivo* studies have been performed to appraise the effects of EOs on animal growth performance, nutrient digestibility, rumen fermentation, and bacterial communities.

The *Allium* genus consists of many species, including shallot (*Allium ascalonicum*), leek (*Allium ampeloprasum*), garlic (*Allium sativum*), onion (*Allium cepa*), and chive (*Allium schoenoprasum*) (10). *Allium mongolicum* Regel (AM) is an *Allium* plant that grows widely in northern China (11). AM is a precious source of forage as it offers extensive nutrients and the contents of protein, ether extract (EE), flavonoids, minerals, amino acids, essential trace elements, and other components (12). A recent study noted that the use of AM water extract could increase the average daily gain (ADG) and decrease the feed conversion ratio (FCR) in sheep (13). In addition, Zhao

et al. found that AM ethanol extract at doses of 2.8 g with diets lowered ruminal $\text{NH}_3\text{-N}$ values after feeding in sheep (14). AM could increase the dry matter digestibility (DMD) and acid detergent fiber digestibility (ADFD) of Simmental calves (15). According to our previous literature, the use of polysaccharides from AM could decrease the abundances of *f-Prevotellaceae* and *g-Prevotellaceae_UCG-001* in the Bacteroidetes of sheep (16).

To date, no study has assessed the possible impact of *Allium mongolicum* Regel essential oil (AMO) on growth performance, nutrient digestibility, rumen fermentation, and bacterial communities in sheep. The purpose of this study, therefore, was to fill the gap in knowledge in this field and explore the potential of AMO as an antibiotic substitute for sheep.

Materials and methods

Ethics approval and consent to participate

All the animal procedures were carried out according to the protocols approved by the College of Animal Science, Inner Mongolia Agricultural University, China. We confirm that the authors complied with the Animal Research Reporting of *in vivo* Experiments (ARRIVE) guidelines.

Extraction process of AMO

Dried powder derived from AM leaves was purchased from Hao Hai Biological Co., Ltd. (Alashan League, Inner Mongolia, China). AMO was processed from the AM by hydrodistillation (1 kg in 5 L of distilled water) using a Clevenger-type apparatus for 3 h in line with the procedure noted in the European Pharmacopeia (17). The oil acquired above the aqueous distillate was dried with anhydrous sodium sulfate (1 mg/2 mL). The AMO was kept in a stainless sealed container at 4°C until use. AMO was analyzed for its chemical compounds using a GC/MS apparatus at the Atomic and Molecular Physics Unit. The major components of AMO

are anethole (37.63%), aromatic hydrocarbon (30.52%), and gingerol (12.60%).

Animals, diets, and experimental design

Twenty healthy male Dorper sheep with a mean BW (\pm SD) of 34.5 kg (\pm 2.5 kg) and an average age of 6 months were used. The sheep were randomly divided into two dietary groups with 10 replicates each: (1) a basal diet without AMO as the control group ($n = 10$) and (2) a basal diet supplemented with 40 mg/kg AMO as the AMO group ($n = 10$). The doses of the AMO were determined based on rumen fermentation using *in vitro* 24-h batch incubation (18). The basal diet was formulated to meet the standard nutrient requirements of sheep according to NRC recommendations (19). The dietary ingredients and nutrient levels of the diet are presented in Table 1. The AMO was completely mixed with the concentrate portion of the TMR before being mixed with the forage component. The diets were provided *ad libitum* twice per day at 07:00 and 18:00. Fresh water was available for the sheep during the whole feeding trial. The experiment lasted 75 days and comprised 15 days for the adaptation period and 60 days for the data and sample collection period.

Data and sample collection

During the trial, sheep were measured for health status, and their body weight was measured at 15-day intervals. Feed was suspended before initial and final weighing. The ADG and dry matter intake (DMI) of sheep were recorded, and the FCR was computed. FCR was determined as average daily DMI/ADG.

The rumen fluid was sampled at 2 h after the morning feeding *via* a stomach tube linked to an Erlenmeyer flask and a vacuum pump on the 60th day of the experimental period. The first 100 mL rumen fluid collected was discarded to prevent saliva contamination, and 100 mL rumen fluid was acquired. pH was immediately determined using an electric handheld pH meter (PHS-3C, Beijing Huoze Experimental Instrument Co., Ltd., Beijing, China). After that, the rumen fluid was then strained immediately through four layers of cheesecloth. One tube of the filtrate with 250 g/L (w/v) metaphosphoric acid (8: 2, v/v) was stored (-20°C) to detect the volatile fatty acids (VFA), and another tube of 10 mL filtrate was preserved by adding 2 mL of 20 g/L (w/v) H_2SO_4 for $\text{NH}_3\text{-N}$ determination in another tube. The third tube of the filtrate was kept at -20°C to determine the microbial protein (MCP), cellulase, β -glucanase, α -amylase, lipase, and proteinase contents. The fourth tube of the filtrate was stored at -80°C for analysis of the bacterial composition.

Approximately 100 g of fecal samples was collected from the rectum at 08:00, 11:00, 14:00, and 17:00 on the

TABLE 1 Composition and nutrient levels of basal diets of the sheep (DM basis).

Ingredients	Content%
Alfalfa	27.78
Corn	19.25
Whole corn silage	15.52
Gourd seed skin	12.15
DDGS ¹	4.33
Flax seed meal	4.62
Sunflower seed meal	6.09
Wheat bran	4.29
Red dates	1.68
Limestone	1.48
CaHPO ₄	0.69
NaCl	0.72
Premix ²	1.40
Total	100.00
Nutrient levels ³	
DE ⁴ (MJ/kg)	16.83
DM%	91.20
CP%	15.32
ADF%	29.69
NDF%	50.98
EE%	3.09
Ca%	1.02
P%	0.55

¹Distillers Dried Grains with Soluble.

²The premix provided the following per kilogram of diets: 25 mg iron (as FeSO_4), 29 mg zinc (as ZnSO_4), 8 mg copper (as CuSO_4), 30 mg manganese (as MnSO_4), 0.04 mg iodine (as KI), 0.1 mg cobalt (as CoSO_4), 3200 IU vitamin A, 1200 IU vitamin D, and 20 IU vitamin E.

³Digestible energy was a calculated value, whereas the others were measured values.

⁴DE, digestible energy; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; EE, ether extract; Ca, calcium; P, phosphorus.

58th, 59th, and 60th days of the experimental period to estimate the DMD, organic matter digestibility (OMD), crude protein digestibility (CPD), EE digestibility (EED), neutral detergent fiber digestibility (NDFD), and ADFD. Fecal yield was determined using acid-insoluble ash as an internal indicator (20).

Sample analysis

Ruminal $\text{NH}_3\text{-N}$, MCP, and VFA

The VFA contents were measured using gas chromatography (Trace 1100; Thermo Fisher Scientific Co., Ltd., Beijing, China) as described by Zhou et al. (21). The $\text{NH}_3\text{-N}$ contents were determined using the method described by the Kjeldahl assay (22). The concentration of MCP yield was determined by urinary purine derivative excretion according to the method described

by Hu et al. (23). The cellulase, β -glucanase, α -amylase, lipase, and proteinase activities were analyzed using kits (Nanjing Jiancheng Biological Technology Co. Ltd., Nanjing, China) according to the method of Laura et al. (7).

DNA extraction and PCR amplification

Microbial community genomic DNA was extracted from ruminal samples using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The DNA was extracted according to Jiang (24). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with the primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp[®] 9700 PCR thermocycler (ABI, CA, USA) (8). The PCR amplification of the 16S rRNA gene was performed according to Liu (25). The PCR mixtures refer to Wang (26). The PCR product was extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using a Quantus[™] Fluorometer (Promega, USA) (26).

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0 (27), and merged by FLASH version 1.2.7 (28).

Feed and fecal chemical analyses

The fecal samples and diets were dried in a forced-air oven at 55°C for 72 h and ground using a 1 mm screen. All feeds were analyzed in duplicate for DM, calcium (Ca), phosphorus (P), EE, CP, and ADF (29). The NDF content was analyzed with sodium sulfite and α -amylase (30). The total fecal output for each sheep was measured based on the content of indigestible neutral detergent fiber (iNDF) and used as an endogenous indicator. The iNDF content was calculated to determine *in*

TABLE 2 Effects of AMO supplementation in sheep diet on growth performance.

Items ²	Treatments ¹		SEM ³	P-value
	CON	AMO		
Initial body weight	34.23	35.19	0.734	0.376
Final body weight	46.55	48.62	3.103	0.535
ADG / (kg/d)	0.167	0.178	0.004	0.043
DMI / (g/d)	1380.66	1406.67	25.353	0.363
FCR	8.27	7.89	0.077	0.005

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio.

³Standard error of mean.

in vivo digestibility using the following equation from Menezes et al. (31).

$$\text{iNDF (\% DM)} = \text{iNDF (g)/DM (\%)} \times 100$$

Statistical analysis

The data were analyzed using SAS software (version 9.0, SAS Inst. Inc., Cary, NC). Before analysis, all data were examined for normality using the UNIVARIATE procedure. Data on growth performance, rumen fermentation parameters, rumen enzyme activities, bacterial relative abundance, and nutrient digestibility were analyzed separately using a one-way analysis of variance (ANOVA) with dietary treatments used as the main factor.

Statistically significant differences among treatments were evaluated using Tukey's multiple range test. Probability values of $P \leq 0.05$ were declared significant, and probability values were declared statistical trends when $0.05 < P \leq 0.10$.

Results

Effects of AMO on growth performance and feed efficiency

The effects of AMO on the growth performance and feed efficiency of sheep are shown in Table 2. Dietary supplementation with AMO increased ($P < 0.05$) ADG compared with the control. Dietary supplementation with AMO decreased ($P < 0.05$) FCR compared with the control. There was no effect of AMO inclusion in the diet on DMI ($P > 0.05$).

Effects of AMO on rumen fermentation parameters

The effects of AMO on the rumen fermentation parameters of sheep are shown in Table 3. Dietary supplementation with

TABLE 3 Effect of AMO on ruminal fermentation in sheep.

Items ²	Treatments ¹		SEM ³	P-value
	CON	AMO		
pH	6.61	6.49	0.026	0.010
NH ₃ -N mg/dL	18.83	16.80	0.272	0.005
TVFA mmol/L	97.13	100.76	1.054	0.034
MCP mg/dL	163.66	175.65	3.986	0.040
Acetate mmol/L	59.37	63.66	0.909	0.016
Propionate mmol/L	23.07	25.33	0.625	0.037
Butyrate mmol/L	10.94	9.10	2.285	0.387
Isobutyrate mmol/L	1.37	1.46	0.082	0.109
Valerate mmol/L	1.27	1.22	2.049	0.570
Isovalerate mmol/L	1.13	1.20	3.029	0.061
A/P	2.57	2.51	0.070	0.459

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²NH₃-N, ammonia nitrogen; TVFA, total VFA; MCP, microbial protein; A/P, Acetate/Propionate.

³Standard error of mean.

TABLE 4 Effect of AMO on rumen bacterial diversity.

Items	Treatments ¹		SEM ²	P-value
	CON	AMO		
OTU ³	1417	1452	18.70	0.179
Shannon	4.88	5.14	0.169	0.268
Simpson	0.03	0.02	0.01	0.770
Ace	1057.66	1155.32	38.32	0.060
Chao1	1079.29	1169.51	60.31	0.273
Coverage	0.95	0.96	0.01	0.770

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²Standard error of mean.

³OTU, Operational Taxonomic Unit.

AMO increased ($P < 0.05$) the concentrations of MCP, TVFA, acetate, and propionate compared with the control. Moreover, a lower rumen pH ($P < 0.05$) and a lower NH₃-N ($P < 0.05$) concentration were noted in the AMO group. There were no effects of AMO inclusion in the diet on the acetate-to-propionate ratio or the concentrations of butyrate, isobutyrate, valerate, and isovalerate ($P > 0.05$).

Effects of AMO on the rumen bacterial community

Table 4 shows that the OTU (Operational Taxonomic Unit), Shannon, Simpson, Ace, Chao1, and coverage indices were not affected ($P > 0.05$) by AMO. Table 5 shows that the relative abundance of the phylum level *Bacteroidetes* and *Spirochaetota* in the AMO group was lower than that in the

TABLE 5 Effect of AMO on rumen bacterial composition at phylum level (%).

Items	Treatments ¹		SEM ²	P-value
	CON	AMO		
<i>Bacteroidetes</i>	50.50	58.45	0.859	0.001
<i>Firmicutes</i>	45.12	37.65	0.722	0.001
<i>Actinobacteriota</i>	1.52	0.71	0.084	0.001
<i>Desulfobacterota</i>	0.65	0.66	0.056	0.827
<i>Spirochaetota</i>	0.27	0.85	0.049	0.000
<i>Verrucomicrobiota</i>	0.43	0.23	0.018	0.001
<i>Synergistota</i>	0.33	0.31	0.054	0.690
<i>Patescibacteria</i>	0.35	0.37	0.047	0.743
<i>Unclassified_k_norank_d_bacteria</i>	0.34	0.33	0.029	0.754
<i>Chloroflex</i>	0.08	0.08	0.010	0.770

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²Standard error of mean.

control. Conversely, the relative abundances of *Firmicutes*, *Actinobacteriota*, and *Verrucomicrobiota* of sheep fed the AMO diet were higher ($P < 0.05$) than those fed the control diet. Furthermore, no differences were noted for the relative abundances of other rumen bacterial phyla between treatments.

Table 6 shows that the relative abundances of the genera *Prevotella* ($P < 0.05$) and *Prevotellaceae_UCG-003* ($P < 0.05$) were increased in the AMO group compared with those in the control. In contrast, sheep fed the AMO diet decreased the relative abundance of *Succiniclaticum*, *norank_f_F082*, *Christensenellaceae_R-7_group*, and *norank_f_Muribaculaceae* ($P < 0.05$). Moreover, no differences were noted for the relative abundances of other rumen bacterial genera between treatments.

Effects of AMO on rumen enzyme activities

The effects of AMO on the rumen enzyme activities of sheep are shown in Table 7. Dietary supplementation with AMO increased ($P < 0.05$) the activities of cellulase, α -amylase, and proteinase. In addition, no difference was found for the activities of rumen lipase and β -glucanase ($P > 0.05$).

Effects of AMO on nutrient digestibility

The effects of AMO on the apparent digestibility of sheep are shown in Table 8. Dietary supplementation with AMO increased ($P < 0.05$) the apparent digestibility of DM and CP. No difference was found for the apparent digestibility of OM, EE, ADF, and NDF ($P > 0.05$).

Discussion

Effects of AMO on growth performance and feed efficiency

As one of the main components of AM, AM flavonoids have been used as feed additives to increase the growth hormone (GH) level in the serum and ADG of sheep (32). Du et al. reported an improvement in the growth performance of sheep with dietary inclusion of AM extract (3.4 g/sheep/day) (16). However, scarce literature is available with regard to the effects of dietary supplementation with AMO on the DMI and ADG of sheep. The present research investigated whether sheep-fed AMO had a change in DMI compared with the control group. However, in contrast to this finding, an enhancement in ADG was found for sheep fed the diet supplemented with AMO. In the current study, the addition of AMO to the diet of sheep clearly decreased FCR, resulting from the increased ADG and similar DMI. These results are consistent with Zhao et al., who stated that diets supplemented with AM water extract in sheep resulted in changes in ADG and FCR (14). Such favorable effects on ADG and FCR were likely due to an increase in the relative abundance of bacteria involved in carbohydrate metabolism in the rumen, thereby enhancing the digestibility of DM and CP. Furthermore, the increases in growth performance may be partly related to the immunoenhancement and antioxidative effects of AMO (33).

Effects of AMO on rumen fermentation parameters

The decrease in ruminal Ph following AMO supplementation was in accordance with the increase in TVFA content (34). A lower ruminal pH was reported in sheep receiving AMO addition and was 6.49, which was suitable for rumen microbial growth and nutrient degradation. The major products of carbohydrate fermentation in the rumen are VFAs, which are the primary energy sources of ruminants (24). The total and molar proportions of VFA generated in the rumen depend on the substrate composition, nutrient availability, ruminal microbial community, and rumen pH reached (35). These alterations in TVFA proportions could be due to the stimulating action of AMO on propionate- and acetate-producing bacteria, indicating that AMO increased the fermentation of carbohydrates. It was also noted that AM water extract and AM ethanol extract could increase the quantity of amylolytic bacteria and cellulolytic bacteria in the rumen (14). This could be used for carbohydrate fermentation, generating high VFA. These current results differ in part from those of Ding et al., who did not observe a difference in the ruminal concentrations of TVFA or acetic and propionic acids in the rumen fluid of sheep-fed AM diets (36). Zhao et al. also found that feeding sheep with AM water extract increased the content

TABLE 6 Effect of AMO on rumen bacterial composition (%) at genus level.

Items	Treatments ¹		SEM ²	P-value
	CON	AMO		
<i>Prevotella</i>	15.82	26.20	1.24	0.004
<i>Rikenellaceae_RC9_gut_group</i>	17.67	16.59	0.736	0.218
<i>Succiniclacticum</i>	7.51	3.85	0.40	0.007
<i>Norank_f_F082</i>	6.68	4.70	0.172	0.006
<i>Christensenellaceae_R-7_group</i>	4.71	4.42	0.049	0.015
<i>Norank_f_Muribaculaceae</i>	3.63	2.40	0.317	0.033
<i>NK4A214_group</i>	2.74	2.62	0.144	0.432
<i>Norank_f_UCG-011</i>	2.85	2.75	0.098	0.355
<i>Prevotellaceae_UCG-003</i>	1.99	2.66	0.074	0.004
<i>Quinella</i>	2.79	2.72	0.355	0.846
<i>Norank_f_Bacteroidales_UCG-001</i>	2.02	2.10	0.104	0.519

¹Treatments: CON = basal diet; AMO = *Allium mongolicum* Regel essential oil.

²Standard error of mean.

TABLE 7 Effect of AMO on enzyme activities in sheep.

Items	Treatments ¹		SEM ²	P-value
	CON	AMO		
Cellulase (U/L)	419.82	477.68	6.140	0.000
β -glucanase (U/mL)	6.30	6.36	0.217	0.484
α -amylase (U/dL)	47.92	59.83	0.897	0.000
Lipase (U/L)	87.59	91.80	2.664	0.145
Proteinase (U/dL)	80.34	83.10	0.913	0.024

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²Standard error of mean.

TABLE 8 Effect of AMO on nutrient digestibility in sheep (%).

Items ²	Treatments ¹		SEM ³	P-value
	CON	AMO		
DMD	66.51	69.66	0.907	0.026
OMD	67.46	69.39	0.843	0.084
CPD	61.58	63.43	0.459	0.016
EED	80.53	79.23	0.625	0.105
NDFD	45.8	45.92	0.799	0.891
ADFD	27.04	25.95	0.701	0.195

¹Treatments: CON, basal diet; AMO, *Allium mongolicum* Regel essential oil.

²DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; EED, ether extract digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility.

³Standard error of mean.

of butyric acid and isobutyric acid (14). The difference between studies could be related to the source of AMO and AM water extract, the amount consumed, and the basic diet.

Our previous research found that the use of AMO decreases the content of $\text{NH}_3\text{-N}$ in *in vitro* rumen fermentation (18). Consistent with the above study, the addition of AMO decreased the $\text{NH}_3\text{-N}$ content in the rumen of sheep. Wallace et al. affirmed that a rumen $\text{NH}_3\text{-N}$ content between 15 and 30 mg/dL was more reasonable (37). Consequently, we reported that using AMO as a dietary additive could accelerate nitrogen metabolism in the rumen within a legitimate range (16.80 mg/dL) in the present study. MCP is synthesized by rumen microorganisms using $\text{NH}_3\text{-N}$, peptide, and amino acids in the rumen, and energy is necessary during the whole process of MCP synthesis (24). The contents of MCP and $\text{NH}_3\text{-N}$ maintain a balance in the rumen, and excessive $\text{NH}_3\text{-N}$ will increase the loss of nitrogen in the nitrogen cycle, while less $\text{NH}_3\text{-N}$ will influence the production of MCP (24). The present research found that the output of MCP in the rumen was increased by feeding a diet with AMO to sheep, which indicates that AMO addition supplies an adequate nitrogen source, carbon skeleton, and energy for MCP synthesis. A similar consequence was acquired with dietary supplementation with different levels of AMO in the diet, which enhanced the rumen MCP yield *in vitro* (18).

Effects of AMO on the rumen bacterial community

The rumen is colonized by some microorganism communities (38), which play significant roles in fermenting and digesting nutrients to supply protein and energy resources (39), improving resistance to external stress, and enhancing growth and performance (40). The most significant microorganisms in the rumen were bacteria, as were also mentioned in previous literature (41). These bacterial communities live in dynamic conditions that could be influenced by several factors, such as diet, animal age, and feed additives (42). In the current study, there was no significant difference in OTU, Ace, Chao1, Simpson, Shannon, and coverage, which suggested that the addition of AMO had no negative effect on the alpha diversity of rumen microbes.

In the present research, *Bacteroidetes* and *Firmicutes* were the superior phyla in the ruminal microbial community, which is in accordance with previous literature in sheep (43, 44). In the current study, AMO addition in diets increased the relative abundance of the phylum *Firmicutes* and decreased the relative abundance of the phylum *Bacteroidetes*, consequently increasing the *Firmicutes* to *Bacteroidetes* (F:B) ratio, which could be beneficial for energy utilization and growth performance (45). The increased F:B ratio might be due to the acceleration of growth of *Firmicutes* by the antimicrobial function of the active ingredient in AMO.

In ruminants, *Prevotella* is the genus of the phylum *Bacteroidetes* with the highest relative abundance (26). *Prevotella*

plays an important role in secreting the enzyme xylanase, which contributes to polysaccharide degradation (46). Some species of *Prevotella* are involved in the degradation and utilization processes of fiber, hemicellulose, pectin, and protein (47). In agreement with these results, our study found that the relative abundance of *Prevotella* seemed to be the most abundant in all rumen fluid samples at the genus level. Furthermore, the current work also found that the use of AMO in the sheep diet could promote the relative abundance of *Prevotellaceae_UCG-003*. The connection between the substrate and growth of *Prevotella* is not apparent. Previous studies have confirmed that *Prevotellaceae_UCG-003* participates in glucose metabolism, generating acetic acids as the primary fermentation final products (25). This explains why acetic acid was present at a higher level in the AMO group.

Effects of AMO on enzyme activity

Meanwhile, the rumen cellulase, α -amylase, and proteinase activity apparently increased in the AMO group compared with the control. This could be sensed by a higher rumen *Prevotella* and *Prevotellaceae_UCG-003* in the AMO group, which contribute to the production of cellulase, α -amylase, and proteinase. Similar to the results observed in this study by Zhao et al., AM extracts could enhance the activities of cellulase, α -amylase, and proteinase in the rumen (14).

Effects of AMO on nutrient digestibility

Because AMO has been observed to decrease $\text{NH}_3\text{-N}$ accumulation and change microbial community structure, we supposed that AMO would enhance nutrient digestibility in sheep by improving rumen microbial synthesis efficiency. Qiao et al. suggested that dietary AMO addition promoted the apparent total tract digestibility of CP in sheep (48). In addition, Yaxing et al. (33) noted increased DM digestibility in *in vitro* batch cultures with rumen fluid of sheep supplemented with increasing levels of AMO. Data from our research found that the digestibility of DM and CP in sheep with AMO supplementation was increased, which was similar to what we hypothesized. Similarly, several studies have reported that nutrient digestibility is enhanced by cinnamon oil in chickens (49), by oregano essential oil in lambs (8), and by essential oil in beef cattle (50). There are three reasons for the results of the present study. First, the decreased ruminal $\text{NH}_3\text{-N}$ with sheep AMO inclusion suggested that the synthesis of MCP had accelerated and led to the increased CP digestibility. Second, Dowd et al. observed that *Prevotella* was very significant for the degradation and absorption of proteins as well as for the fermentation of peptides in the rumen (51). Based on the above findings, our present research detected that the relative abundance of

Prevotella was most abundant in the rumen fluid of sheep in the two treatments at the genus level. Moreover, the current research also demonstrated that supplementation with AMO in the diet could increase the relative abundance of *Prevotella* and *Prevotellaceae_UCG-003*. These results revealed that dietary addition of AMO had a favorable effect on enhancing the degradation of CP in the rumen, thereby boosting rumen fermentation. Third, we surmised that the improved digestibility of DM and CP was partially due to the elevated ruminal proteinase enzyme activity.

Conclusion

In general, supplementation with AMO in sheep diets increased growth performance by increasing ADG and decreasing FCR, promoted rumen fermentation, diversified the bacterial community composition in the rumen, enhanced the apparent digestibility of DM and CP, and improved rumen enzyme activities.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI; PRJNA833585.

Ethics statement

The animal study was reviewed and approved by College of Animal Science, Inner Mongolia Agricultural University, China. Written informed consent was obtained from the owners for the participation of their animals in this study.

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Author contributions

ZY wrote the main manuscript. KE and BZ prepared Tables 1–4, and BC prepared Tables 5–8. AC edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2022.926721/full#supplementary-material>

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