Correlation of the rumen fluid microbiome and the average daily gain with a dietary supplementation of *Allium mongolicum* Regel extracts in sheep¹

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ABSTRACT: Plant extracts can affect the rumen microbiome and ADG in ruminants, and studies of the association between the rumen microbiome and ADG provide information applicable to improving ruminant growth performance. The objectives were to investigate the effects of *Allium mongolicum* Regel extracts on the rumen microbiome and ADG and their association in sheep. Forty healthy, male, small-tailed Han sheep (6 mo, 34 ± 3.5 kg body weight) were randomly assigned to 1 of the following 4 dietary treatments: basal diet as control group (CK, $n = 10$), basal diet supplemented with $3.4 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder extract as PAM group (PAM, $n = 10$), basal diet supplemented with $10 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder as AM group (AM, $n = 10$), and basal diet supplemented with $10 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder extract residue as RAM group (RAM, $n = 10$). The ADG for individual sheep was calculated using the sum of the ADGs observed during the experimental period divided by the number of days in the experimental period. At the end of the experiment, sheep were randomly selected from each treatment for slaughter

($n = 6$), and the rumen fluids were collected and stored immediately at -80°C . Illumina HiSeq was subsequently used to investigate the changes in the rumen microbiome profile, and the associations with ADG were analyzed by Spearman correlation coefficient analysis. The results demonstrated that, compared with that in CK group, the ADG in AM and RAM significantly increased ($P = 0.0171$). The abundances of Tenericutes and Mollicutes ($[\rho] = 0.5021$, $P = 0.0124$) were positively correlated with ADG. Within Mollicutes, the abundances of Anaeroplasmatales ($[\rho] = 0.5458$, $P = 0.0058$) and Anaeroplasmataceae ($[\rho] = 0.5458$, $P = 0.0058$) were positively correlated with ADG. The main negatively correlated bacteria were Saccharibacteria ($[\rho] = -0.4762$, $P = 0.0187$) and Betaproteobacteria ($[\rho] = -0.5669$, $P = 0.039$). Although Anaeroplasmatales and Anaeroplasmataceae were positively correlated with ADG, Saccharibacteria and Betaproteobacteria were negatively correlated with ADG. In conclusion, supplementation with *A. mongolicum* Regel powder and extracts will influence the rumen microbiome and increase the ADG.

Key words: *Allium mongolicum* Regel, average daily gain, extracts, sheep, rumen microbiome

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INTRODUCTION

Plant extracts are classified based on their botanical origin and composition. In terms of botanical origin, they can be classified as leaves, roots, herbs, grains, fruits, and agricultural products. In terms of composition, they can be classified as crude extracts, essential oils, and isolated compounds (Valenzuela-Grijalva et al., 2017). The use of plant extracts as dietary supplements for humans may be dangerous due to the possible side effects of such extracts (Pittler et al., 2005); however, as either crude extracts or isolated compounds, plant extracts with appetite-suppressing properties have exhibited effectiveness for body weight control (Astell et al., 2013). Extracts derived from blueberry–blackberry beverages (Johnson et al., 2016) can lead to decreased body weight gain (BWG) in mice. For ruminants, plants or plant extracts are used as feed supplements. Natural plants and their extracts affect growth performance. Most studies in cattle have evaluated the effects of the addition of essential oils during the growth and finishing phases, but the ADG was observed to be minimally modified (Vakili et al., 2013), and some types of essential oils decreased the ADG in sheep (Macías-Cruz et al., 2014). Feeding with grapefruit peel extracts (Pérez-Fonseca et al., 2016) and *Salix babylonica* extracts (Salem et al., 2014) was shown to promote the sheep's growth.

Natural plants and their extracts also influence the gastrointestinal and rumen microbiome. *Cistanches herba* water extracts (Li et al., 2017) have been investigated for their effects on the human intestinal microbiome. Grape seed extract was shown to alter the intestinal gut microbiome of mice (Griffin et al., 2017), and tea polysaccharide treatment increased the phylogenetic diversity of a high fat, diet-induced, mouse microbiome (Chen et al., 2018). In Holstein cows, ginkgo fruit and its extract were shown to increase propionate production via microbial selection (Oh et al., 2017), and plant flavonoid supplementation be effective at reducing the incidence of rumen acidosis by regulating the lactate production and consumption by bacteria in heifers (Balcells et al., 2012). Rosemary leaves and their extracts were shown to modulate the rumen microbiome and function, which are involved in protein and fiber digestion in sheep (Cobellis et al., 2016).

In humans and mice, some studies have examined the associations between the gut microbiome and BWG (Turnbaugh et al., 2009; Ridaura et al., 2013). Ruminant performance parameters, such

as growth, feed efficiency, carcass weight, and intramuscular fat, are closely associated with the rumen microbiome. In addition, next-generation sequencing techniques have uncovered novel features of the rumen microbiome, and integration of these results with ruminant performance parameters has led to meaningful advances in research (Morgavi et al., 2013). However, association-based studies have focused mainly on feed efficiencies for ruminants (Perea et al., 2017), and some studies have investigated the association between the cattle rumen microbiome and ADG as well as ADFI (Myer et al., 2017; Paz et al., 2018). Few studies have focused on the association between the sheep rumen microbiome and ADG.

Plants of the *Allium* genus are widely cultivated and used worldwide, particularly garlic (*Allium sativum*), onion (*Allium cepa*), shallot (*Allium ascalonicum*), leek (*Allium ampeloprasum*), and chive (*Allium schoenoprasum*; Zeng et al., 2017). *Allium mongolicum* Regel is a typical *Allium* plant that is native to and cultivated in grasslands in northern China. A previous study reported that different amounts of flavonoids extracted from *A. mongolicum* Regel may increase the ADG of sheep (Mu et al., 2017). We hypothesized that these *A. mongolicum* Regel extracts could affect the rumen microbiome and ADG. The objectives of this study were to evaluate the effects of *A. mongolicum* Regel extracts on ADG and the rumen microbiome. In addition, the association between the rumen microbiome and ADG was also investigated.

MATERIALS AND METHODS

This study was carried out in accordance with the recommendations of the Instructive Notions with Respect to Caring for Experimental Animals, Ministry of Technology of China. The protocol was approved by the Ethical Committee of the College of Animal Science of Inner Mongolia Agricultural University.

Extraction Process of A. mongolicum Regel Powder Extracts

Allium mongolicum Regel powder was purchased from Alashan Haohai Biotechnology Co., Ltd. (Alashan League, Inner Mongolia, China). The powder was mixed with distilled water at a ratio of 1:20 and oscillated by water bath shake at 80 °C for 8 h. To obtain the *A. mongolicum* Regel powder extract, the supernatant was collected and concentrated using a rotary evaporator (IKA Laboratory

Technology, Germany) and lyophilized in a freeze dryer (Millrock Technology, New York, NY), then pulverized. The residue was dried in 65 °C and pulverized to be used as *A. mongolicum* Regel powder extract residue.

Animals, Diets, Experimental Design and Procedure

Forty healthy, male, small-tailed Han sheep (6 mo, 34 ± 3.5 kg body weight) were randomly assigned to 1 of the following 4 dietary treatments: basal diet as control group (**CK**, $n = 10$), basal diet supplemented with $3.4 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder extract as PAM group (**PAM**, $n = 10$), basal diet supplemented with $10 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder as AM group (**AM**, $n = 10$), and basal diet supplemented with $10 \text{ g} \cdot \text{sheep}^{-1} \cdot \text{d}^{-1}$ *A. mongolicum* Regel powder extract residue as RAM group (**RAM**, $n = 10$). Fifty-gram concentrate of basal diet was mixed with 3.4 g of *A. mongolicum* Regel powder extract, 10 g of *A. mongolicum* Regel powder, or 10 g *A. mongolicum* Regel powder extract residue, respectively, and divided the each mixture into 2 equal amounts then they were provided for individual sheep twice daily to make sure the supplements were completely consumed by each individual sheep. The experiment was conducted at Fuchuan Feed Science and Technology Co., Ltd., in Bayannaoer city, Inner Mongolia, China. The experiment lasted for 75 d, including a 15-d preliminary feeding period for adaptation and a 60-d experimental feeding period.

The sheep were fed a finishing diet comprised 35% whole plant corn silage, 25% alfalfa hay, corn 19%, wheat bran 2.2%, sunflower meal 10%, soybean meal 4%, cottonseed meal 3%, 0.8% salt, and 1% vitamin and mineral supplement (DM basis). *Allium mongolicum* Regel extracts were provided by the Animal Nutrition and Immunology Laboratory of Inner Mongolia Agricultural University. Respective treatment's diets were offered to the animals twice daily at 0700 and 1800 h, and the body weights were measured on the first and last days of the experimental feeding period before the morning meal. The ADG for individual sheep was calculated using the sum of the ADGs determined during the experimental period divided by the number of days during the experimental period. After the experiment, on the day of slaughtering, sheep without a morning diet were randomly selected from each treatment ($n = 6$). The rumen fluid of each sheep was collected in a 5-mL cryopreservation tube and stored immediately at -80 °C until DNA extraction.

Microbial DNA Extraction, PCR Amplification, and Illumina HiSeq Sequencing

Total genomic DNA was extracted from the samples using the hexadecyltrimethyl ammonium bromide method. The DNA concentration and purity were monitored on 1% agarose gels. On the basis of its concentration, the DNA was diluted to $1 \text{ ng}/\mu\text{L}$ using sterile water. The V3–V4 regions of the 16S rRNA genes were amplified using the specific primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT). All PCRs were carried out with Phusion high-fidelity PCR master mix (New England Biolabs). An equal volume of $1\times$ loading buffer was mixed with the PCR products, and electrophoresis was performed on a 2% agarose gel for detection. Samples with bright main bands between 400 and 450 bp were selected for additional experiments. The PCR products were mixed together, after which the mixture was purified with the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries was generated using a TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA) following the manufacturer's instructions, and index codes were added. Library quality was assessed on a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Madison, WI). The library was ultimately sequenced on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA), and 250-bp paired-end reads were generated.

Sequencing Data Processing and Bioinformatics

Paired-end reads were assigned to samples based on their unique barcodes and truncated by removal of the barcode and primer sequences. The paired-end reads were merged using FLASH (v 1.2.7; Magoč and Salzberg, 2011), which is a rapid and accurate analytical tool designed to merge paired-end reads when at least some of the reads overlap with the reads generated from the opposite end of the same DNA fragment; the spliced sequences are referred to as raw tags. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags (Bokulich et al., 2013) according to the Quantitative Insights into Microbial Ecology (QIIME; v 1.7.0; Carmody et al., 2015) quality control process. The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using the UCHIME algorithm (Edgar et al., 2011) to detect chimeric sequences, which were then removed (Haas et al., 2011). Effective tags were

ultimately obtained. Sequencing analysis was performed using UPARSE software (UPARSE v 7.0.1001; Edgar, 2013), and sequences with $\geq 97\%$ similarity were assigned to the same operational taxonomic unit (OTU). A representative sequence for each OTU was screened for further annotation. For each representative sequence, the Greengenes database (<http://greengenes.lbl.gov/Download/>; DeSantis et al., 2006) was used based on the QIIME's Ribosomal Database Project classifier (v 2.2; Wang et al., 2007) algorithms for annotation of taxonomic information. To study the phylogenetic relationships of different OTUs, multiple sequence alignment was conducted using MUSCLE software (v 3.8.31; Edgar, 2004), and OTU abundance was normalized using a standard sequence number corresponding to the sample with the lowest number of sequences.

Statistical Analysis

Principal coordinate analysis (PCoA) of the rumen microbial communities was performed on the basis of weighted UniFrac distance metrics by using the FactoMineR package and visualized by using the ggplot2 package in R software (v 2.15.3). On the basis of all the rumen fluid samples, the relative abundances of the rumen microbiome were ranked by QIIME (v 1.7.0) at each taxonomic level.

One-way ANOVA of the ADGs of the slaughtered sheep in each treatment ($n = 6$) was performed by SAS (v 9.2) for the 4 treatments. On the basis of all the rumen fluid samples, at each taxonomic level, the rumen microbes with the top 35 relative abundances at each taxonomic level were selected, and ANOVA of the top 35 microbes at each level was performed by SAS (v 9.2). Spearman's rank correlation coefficients analysis between all the detected phyla, the top 35 rumen microbes at each level, and ADG were analyzed via the corr. test of the psych package in R software (v 2.15.3) and visualized using the pheatmap package ($n = 24$).

RESULTS

Effects of Supplementation with *A. mongolicum* Regel Extracts on ADG in Sheep

The ADG was lower for CK than for the other groups ($P = 0.0171$; Table 1). The ADG was 139.0 g for CK group and increased to 158.5 g for PAM. The value significantly increased to 176.8 g for AM and 181.6 g for RAM.

Rumen Microbiome Sequences and Taxonomy

In total, 1,414,402 clean sequences were obtained for bacterial and archaeal 16S rRNA genes by sequencing. The average sequence length was 416.38 bases per read, and the average coverage was 58,933 sequences per sample. In total, 2,566 OTUs were clustered based on 97% identity. Via taxonomic analysis, 21 taxa were identified at the phylum level, 40 at the class level, 61 at the order level, 91 at the family level, and 102 at the genus level.

Weighted PCoA of the Rumen Microbiome

The PCoA with the weighted UniFrac distance metrics showed that the AM and RAM groups were clustered together and that the CK and PAM groups were clustered together. The PCoA axis 1 accounted for 42.84% of the variation, and the PCoA axis 2 accounted for 19.11% of the variation (Fig. 1).

Changes in Rumen Microbial Ecology

In the present study, on the basis of all the samples, the top 35 relative abundances were selected at each taxonomic level, and this study was based on these top 35 relatively abundant taxa, except at the phylum level as only 21 phyla were identified. For each treatment, the taxa that exhibited relative

Table 1. Effects of supplementation with *A. mongolicum* Regel extracts on average daily gain (ADG) in sheep

Item	Treatment ^{1,2}				SEM	P-value
	CK	PAM	AM	RAM		
ADG ³ , g/d	139.0 ^b	158.5 ^{ab}	176.8 ^a	181.6 ^a	5.60	0.0171

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹Treatments: CK = basal diet; PAM = basal diet supplemented with 3.4 g·sheep⁻¹·d⁻¹ of *A. mongolicum* Regel powder extract; AM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder; RAM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel extract residue.

²*A. mongolicum* Regel extracts = *A. mongolicum* Regel extract and residue.

³ADG = average daily gain.

Figure 1. Principal coordinate analysis (PCoA) of the rumen microbial communities on the basis of weighted UniFrac distance metrics. PC1 represents PCoA axis 1, and PC2 represents PCoA axis 2 (the percentages show the contributions of the principal components to the differences among samples). CK (red squares represent the samples in the CK group, $n = 6$), basal diet; PAM (the purple circles represent the samples in the PAM, $n = 6$), basal diet supplemented with 3.4 g-sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder extract; AM (the green triangles represent the samples in the AM group, $n = 6$), basal diet supplemented with 10 g-sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder; RAM (the pink squares represent the samples in the RAM group, $n = 6$), basal diet supplemented with 10 g-sheep⁻¹·d⁻¹ *A. mongolicum* Regel extract residue.

abundances greater than 0.5% and were within the top 5 relatively abundant taxa were defined as the predominant taxa at each taxonomic level. At the phylum, class, and order levels, taxa whose total relative abundances were greater than 0.1% were defined as dominant taxa, whereas those whose values were less than 0.1% were defined as taxa with low relative abundances. At the family and genus levels, taxa whose total relative abundances were greater than 0.5% were defined as dominant taxa, whereas those whose values were less than 0.5% were defined as taxa with low relative abundances. Phylum, class, order, family, and genus are abbreviated to p-, c-, o-, f-, and g-, respectively.

The total Bacteria, total Archaea, and predominant taxa at the phylum, class, and order levels are presented in Table 2. The relative abundance of the total Bacteria was not significantly different among the treatments: 99.82% for CK, 99.72% for PAM, 99.79% for AM, and 99.93% for

RAM. The same trend was observed for the total Archaea. The predominant shared phyla were Bacteroidetes, Firmicutes, and Proteobacteria. Tenericutes was dominant in all groups except CK, and Candidate_division_SR1 was dominant only in AM. At the class level, the predominant shared taxa were Bacteroidia, Clostridia, Erysipelotrichia, and Deltaproteobacteria. Negativicutes was predominant in CK and PAM, whereas Mollicutes was predominant in AM and RAM. At the order level, the predominant shared taxa were Bacteroidales, Clostridiales, and Erysipelotrichales. Selenomonadales was predominant in all groups except AM, whereas Desulfovibrionales was predominant in all groups except CK and AM. Mycoplasmatales and Mollicutes_RF9 were predominant in only AM.

The dominant taxa that significantly changed among treatments at the phylum, class, and order levels are also presented in Table 2. Within Saccharibacteria, the abundances of p-unidentified_

Table 2. Effects of supplementation with *A. mongolicum* Regel extracts on relative abundances of total Bacteria, total Archaea, predominant taxa, and dominant taxa (expressed as percentages) at the phylum, class, and order levels of sheep rumen microbiome

Taxa	Treatment ^{1,2}				SEM	P-value
	CK	PAM	AM	RAM		
Total Bacteria	99.82	99.72	99.79	99.92	0.04	0.3216
Total Archaea	0.17	0.28	0.20	0.07	0.04	0.3349
Predominant taxa ³						
p-Bacteroidetes	53.85	47.26	49.12	55.27	1.78	0.3511
p-Firmicutes	42.91	48.39	45.91	40.63	1.68	0.4023
p-Tenericutes	0.46	0.86	1.67	1.17	0.20	0.1842
p-Proteobacteria	1.00	1.49	0.96	1.16	0.15	0.6148
p-Candidate_division_SR1	0.34	0.38	0.57	0.19	0.07	0.2705
c-Bacteroidia	53.77	47.19	49.05	55.17	1.78	0.3529
c-Clostridia	40.94	46.19	43.46	38.97	1.66	0.4691
c-Mollicutes	0.46	0.86	1.67	1.17	0.20	0.1842
c-Erysipelotrichia	1.33 ^b	1.16 ^b	1.91 ^a	1.01 ^b	0.10	0.0035
c-Negativicutes	0.62	1.01	0.51	0.59	0.09	0.2195
c-Deltaproteobacteria	0.64	0.93	0.71	0.83	0.10	0.7465
o-Bacteroidales	53.76	47.17	49.05	55.16	1.78	0.3521
o-Clostridiales	40.94	46.19	43.46	38.97	1.66	0.4691
o-Mycoplasmatales	0.02	0.17	0.93	0.49	0.19	0.3554
o-Erysipelotrichales	1.33 ^b	1.16 ^b	1.91 ^a	1.01 ^b	0.10	0.0035
o-Selenomonadales	0.62	1.01	0.51	0.59	0.09	0.2196
o-Desulfovibrionales	0.45 ^a	0.80 ^a	0.50 ^a	0.71 ^a	0.09	0.4960
o-Mollicutes_RF9	0.40	0.63	0.64	0.55	0.06	0.4475
Dominant taxa ⁴						
p-Fibrobacteres	0.04 ^b	0.04 ^b	0.06 ^b	0.38 ^a	0.05	0.0154
p-Saccharibacteria	0.30 ^a	0.38 ^a	0.24 ^{ab}	0.08 ^b	0.04	0.0309
c-Fibrobacteria	0.04 ^b	0.04 ^b	0.06 ^b	0.38 ^a	0.05	0.0154
c-unidentified_Saccharibacteria	0.30 ^a	0.38 ^a	0.25 ^{ab}	0.09 ^b	0.05	0.0302
o-Fibrobacterales	0.04 ^b	0.04 ^b	0.06 ^b	0.38 ^a	0.05	0.0154
o-unidentified_Saccharibacteria	0.30 ^a	0.38 ^a	0.25 ^{ab}	0.09 ^b	0.04	0.0302

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹Treatments: CK = basal diet; PAM = basal diet supplemented with 3.4 g·sheep⁻¹·d⁻¹ of *A. mongolicum* Regel powder extract; AM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder; RAM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel extract residue.

²*A. mongolicum* Regel extracts = *A. mongolicum* Regel extract and residue.

³For each treatment, taxa with relative abundances greater than 0.5% and within the top 5 relatively abundant taxa were defined as the predominant taxa at each taxonomic level p = phylum; c = class; o = order.

⁴Dominant taxa were defined as the taxa with total relative abundances greater than 0.1% at the phylum, class, and order levels.

Saccharibacteria, c-unidentified_Saccharibacteria, and o-unidentified_Saccharibacteria were significantly greater in the CK group than in the RAM. Within Firmicutes, the abundances of c-Erysipelotrichia and o-Erysipelotrichales were significantly greater in AM than in the other groups. Within Fibrobacteres, the abundances of p-Fibrobacteres, c-Fibrobacteria, and o-Fibrobacterales were significantly greater in RAM than in the other groups.

The predominant taxa at the family and genus levels are presented in Table 3. At the family level, the predominant shared taxa were Rikenellaceae, Ruminococcaceae, and Prevotellaceae.

Lachnospiraceae was dominant in all groups except AM, and Christensenellaceae was dominant in all groups except RAM. Bacteroidales_BS11_gut_group was predominant in AM and RAM. At the genus level, the predominant shared taxa were *Rikenellaceae_RC9_gut_group*, *Prevotella_1*, *Christensenellaceae_R-7_group*, and *Ruminococcaceae_NK4A214_group*. *Prevotellaceae_UCG-003* was predominant in all groups except PAM, whereas *Butyrivibrio_2* was predominant in only PAM.

The dominant taxa that significantly changed among treatments at the family and genus levels are also presented in Table 3. Within Bacteroidetes, the abundances of f-Prevotellaceae and

Table 3. Effects of supplementation with *A. mongolicum* Regel extracts on relative abundances of predominant taxa and dominant taxa (expressed as percentages) at the family and genus levels of sheep rumen microbiome

	Treatment ^{1, 2}					
Taxa	CK	PAM	AM	RAM	SEM	P-value
Predominant taxa ³						
f-Prevotellaceae	29.60 ^a	20.96 ^{ab}	15.73 ^b	16.10 ^b	2.07	0.0496
f-Rikenellaceae	14.34 ^b	17.09 ^{ab}	20.75 ^{ab}	23.87 ^a	1.27	0.0312
f-Ruminococcaceae	16.38	20.62	23.61	19.18	1.15	0.1619
f-Lachnospiraceae	13.42 ^{ab}	15.73 ^a	8.55 ^c	9.93 ^{bc}	0.94	0.0157
f-Christensenellaceae	9.33	8.48	9.93	8.72	0.83	0.9374
f-Bacteroidales_BS11_gut_group	5.44	6.50	9.52	10.50	0.80	0.0638
g-Rikenellaceae_RC9_gut_group	13.91 ^b	16.21 ^b	19.66 ^{ab}	23.28 ^a	1.25	0.0329
g-Prevotella_1	15.52	14.25	8.69	8.26	1.52	0.2113
g-Christensenellaceae_R-7_group	9.19	8.30	9.65	8.60	0.81	0.9451
g-Prevotellaceae_UCG-003	8.77	3.66	5.30	5.44	0.78	0.1208
g-Ruminococcaceae_NK4A214_group	5.16	7.29	10.57	7.35	0.73	0.0574
g-Butyrivibrio_2	3.48	4.88	1.94	2.48	0.44	0.0831
Dominant taxa ⁴						
f-Erysipelotrichaceae	1.33 ^b	1.16 ^b	1.91 ^a	1.01 ^b	0.10	0.0035
g-Prevotellaceae_UCG-001	3.07 ^a	1.47 ^b	0.88 ^b	1.44 ^b	0.27	0.0131
g-Lachnospiraceae_XPB1014_group	0.98 ^b	2.01 ^a	0.64 ^b	0.76 ^b	0.18	0.0139

^{a-c}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹Treatments: CK = basal diet; PAM = basal diet supplemented with 3.4 g·sheep⁻¹·d⁻¹ of *A. mongolicum* Regel powder extract; AM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder; RAM = basal diet supplemented with 10 g·sheep⁻¹·d⁻¹ *A. mongolicum* Regel extract residue.

²*A. mongolicum* Regel extracts = *A. mongolicum* Regel extract and residue.

³For each treatment, the taxa with relative abundances greater than 0.5% and within the top 5 relatively abundant taxa were defined as the predominant taxa at each taxonomic level f = family; g = genus.

⁴Dominant taxa were defined as the taxa with total relative abundances greater than 0.5% at the family and genus levels.

g-Prevotellaceae_UCG-001 were significantly greater in the CK group than in the AM and RAM treatments, whereas the abundances of f-Rikenellaceae and g-Rikenellaceae_RC9_gut_group were significantly greater in the RAM group than in the CK treatment. The abundance of f-Lachnospiraceae was significantly greater in CK group than in AM group; the abundance of g-Lachnospiraceae_XPB1014_group was significantly greater in the PAM group than in the other groups; whereas the abundance of f-Erysipelotrichaceae was significantly greater in the AM than in the other groups within Firmicutes.

The taxa with low relative abundances that exhibited significant changes are presented in Table 4. Within Saccharibacteria, the abundance of f-unidentified_Saccharibacteria was significantly greater in CK group than in RAM. Within Proteobacteria, the abundance of o-Betaproteobacteria was significantly greater in CK than in the other groups. Within Firmicutes, the abundance of g-Roseburia was significantly greater in PAM than in AM and RAM,

whereas the abundance of g-Erysipelotrichaceae_UCG-009 was significantly greater in AM than in the other groups. Within Fibrobacteres, the abundances of f-Fibrobacteraceae and g-Fibrobacter were significantly greater in RAM than in the other groups. Within Mollicutes, the abundances of o-Anaeroplasmatales and f-Anaeroplasmataceae were significantly greater in RAM group than in CK and PAM. Within Chloroflexi, the abundances of p-Chloroflexi, c-Anaerolineae, o-Anaerolineales, and f-Anaerolineaceae were greater in AM than in CK. Within the kingdom Archaea, the abundances of c-Thermoplasmata, o-Thermoplasmatales, and f-Thermoplasmataceae were significantly greater in AM than in the other groups.

Spearman Correlation Coefficient Analysis between the Relative Abundances of the Taxa and ADG

Correlations between the taxa and ADG at the phylum, class, order, family, and genus levels are presented in Fig. 2. The Tenericutes abundance

Table 4. Effects of supplementation with *A. mongolicum* Regel extracts on taxa with low relative abundances of sheep rumen microbiome (expressed as percentages)

Taxa with low abundance ³	Treatment ^{1,2}				SEM	P-value
	CK	PAM	AM	RAM		
p-Chloroflexi	0.05 ^b	0.07 ^{ab}	0.12 ^a	0.02 ^b	0.01	0.0085
c-Anaerolineae	0.05 ^b	0.08 ^{ab}	0.12 ^a	0.02 ^b	0.01	0.0070
c-Thermoplasmata	<0.01 ^b	0.01 ^b	0.03 ^a	0.01 ^b	<0.01	0.0145
c-Betaproteobacteria	0.03 ^a	0.01 ^b	0.02 ^b	0.01 ^b	<0.01	0.0124
c-unidentified_Firmicutes	<0.01	<0.01	<0.01	<0.01	<0.01	0.4482
o-Anaerolineales	0.05 ^b	0.08 ^{ab}	0.12 ^a	0.02 ^b	0.01	0.0070
o-Anaeroplasmatales	0.01 ^b	0.03 ^b	0.06 ^{ab}	0.09 ^a	0.01	0.0098
o-Thermoplasmatales	<0.01 ^b	0.01 ^b	0.03 ^a	0.01 ^b	<0.01	0.0145
o-Neisseriales	0.01	0.01	<0.01	0.01	<0.01	0.1946
f-Fibrobacteraceae	0.04 ^b	0.04 ^b	0.06 ^b	0.38 ^a	0.05	0.0154
f-unidentified_Saccharibacteria	0.30 ^a	0.38 ^a	0.25 ^{ab}	0.09 ^b	0.04	0.0302
f-Anaerolineaceae	0.05 ^b	0.08 ^{ab}	0.12 ^a	0.02 ^b	0.01	0.0070
f-Anaeroplasmataceae	0.01 ^b	0.03 ^b	0.06 ^{ab}	0.09 ^a	0.01	0.0099
f-unidentified_Thermoplasmatales	<0.01 ^b	0.01 ^b	0.03 ^a	0.01 ^b	<0.01	0.0145
g-Roseburia	0.20 ^{ab}	0.38 ^a	0.07 ^b	0.11 ^b	0.04	0.0452
g-Erysipelotrichaceae_UCG-009	0.43 ^b	0.36 ^b	0.70 ^a	0.29 ^b	0.05	0.0060
g-Fibrobacter	0.04 ^b	0.04 ^b	0.06 ^b	0.38 ^a	0.05	0.0138

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹Treatments: CK = basal diet; PAM = basal diet supplemented with 3.4 g-sheep⁻¹·d⁻¹ of *A. mongolicum* Regel powder extract; AM = basal diet supplemented with 10 g-sheep⁻¹·d⁻¹ *A. mongolicum* Regel powder; RAM = basal diet supplemented with 10 g-sheep⁻¹·d⁻¹ *A. mongolicum* Regel extract residue.

²*A. mongolicum* Regel extracts = *A. mongolicum* Regel extract and residue.

³Taxa with low relative abundances were defined as taxa with total relative abundances less than 0.1% at the phylum, class, and order levels or less than 0.5% at the family and genus levels p = phylum; c = class; o = order; f = family; g = genus.

($[\rho] = 0.5021$, $P = 0.0124$) was positively correlated with ADG, whereas the Saccharibacteria abundance ($[\rho] = -0.4762$, $P = 0.0187$) was negatively correlated with ADG.

At the class level, the Mollicutes abundance (within Tenericutes; $[\rho] = 0.5021$, $P = 0.0124$) was positively correlated with ADG, whereas the abundances of unidentified_Saccharibacteria ($[\rho] = -0.4739$, $P = 0.0193$), Betaproteobacteria ($[\rho] = -0.5669$, $P = 0.0039$), and unidentified_Firmicutes ($[\rho] = -0.4210$, $P = 0.0405$) were negatively correlated with ADG.

At the order level, the Anaeroplasmatales abundance (within Mollicutes) was positively correlated with ADG ($[\rho] = 0.5458$, $P = 0.0058$), whereas the abundances of unidentified_Saccharibacteria ($[\rho] = -0.4739$, $P = 0.0193$) and Neisseriales (within Betaproteobacteria; $[\rho] = -0.4057$, $P = 0.0492$) were negatively correlated with ADG.

At the family level, the abundances of Anaeroplasmataceae (within Mollicutes; $[\rho] = 0.5458$, $P = 0.0058$) and Rikenellaceae (within Bacteroidetes; $[\rho] = 0.4085$, $P = 0.0475$) were positively correlated with ADG, whereas the unidentified_Saccharibacteria abundance ($[\rho] = -0.4739$,

$P = 0.0193$) was negatively correlated with ADG. Whereas, at the genus level, the abundance of *Prevotellaceae_UCG.001* ($[\rho] = -0.4590$, $P = 0.0241$) was negatively correlated with ADG.

DISCUSSION

Effects of Supplementation with *A. mongolicum* Regel Extracts on ADG

In this study, the ADG was significantly greater in AM and RAM than in CK. This result was similar to the results obtained for *A. cepa* extracts in sheep, where, compared with untreated sheep, all treated animals exhibited considerably increased body weights (Mehlhorn et al., 2011; Jatzlau et al., 2014). *Allium sativum* extracts could be provided as supplements to calves for increased performance, leading to increased mean BWG values (Ghosh et al., 2011). Nevertheless, different results have been obtained among plants within the *Allium* genus. Treatment with *A. sativum* essential oil (Lai et al., 2014) and *Allium fistulosum* ethanolic and aqueous extracts led to decreased body weight and other obesity-related parameters in mice (Sung

Figure 2. Correlation patterns based on the slaughtered sheep ($n = 24$) showing associations between microbial taxa and ADG. (A) Correlation patterns of all the detected phyla. (B) Correlation patterns of the top 35 taxa at the class level. (C) Correlation patterns of the top 35 taxa at the order level. (D) Correlation patterns of the top 35 taxa at the family level. (E) Correlation patterns of the top 35 taxa at the genus level. Correlation analyses were conducted using Spearman's rank correlation coefficient analysis. Significant correlations ($P < 0.05$) are indicated by *, and extremely significant correlations ($P < 0.01$) are indicated by **. Red represents positive correlation coefficients, deep red represents the strong positive correlation coefficients, blue represents negative correlation coefficients, and deep blue represents strong negative correlation coefficients. The bar on the right with numbers shows the values of the correlation coefficients [p]. The greatest to lowest ranked relative abundances of the microbiome taxa based on all the samples are shown in the transverse direction from left to right; the ADG values are shown in the longitudinal direction.

et al., 2018). Therefore, *Allium* plants and their extracts can positively or negatively influence growth performance.

Shared Predominant Taxa in the Sheep Rumen Microbiome

In this study, the shared predominant phyla among the 4 treatments, namely, Bacteroidetes, Firmicutes, and Proteobacteria, were consistent with those reported in other studies on sheep (Lopes et al., 2015), cattle (Jami et al., 2014), and humans (Arumugam et al., 2011).

In this study, the predominant shared taxa at the family level were Ruminococcaceae, Prevotellaceae, and Rikenellaceae. Ruminococcaceae is a type of cellulolytic bacterial lineage whose presence has been consistently reported (Comtet-Marre et al., 2017). However, according to the literature, there are inconsistencies in the reporting of Prevotellaceae, whether amylolytic or cellulolytic. On the one hand, some studies have suggested that these bacteria might play an important role in the breakdown of proteins and carbohydrates and might be considered as amylolytic bacteria (Liu et al., 2016). Ruminants affected by subacute rumen acidosis exhibit increased relative abundance of the genus *Prevotella* (McCann et al., 2016), indicating that these bacteria may be one of the causes of acidosis in ruminants. On the other hand, Prevotellaceae not only could cooperate with other cellulolytic organisms that are involved in ruminal fibrolytic activity (Naas et al., 2014) but also could be considered cellulolytic bacteria themselves (Ozbyram et al., 2018).

Association between the Rumen Microbiome and ADG

Some low-abundance rumen microbes may play key roles for ruminants (Morgavi et al., 2013). The results of this study also demonstrated that the taxa with low relative abundances were also correlated with ADG, including c-Betaproteobacteria, c-unidentified_Firmicutes, o-Anaeroplasmatales, o-Neisseriales, f-Anaeroplasmataceae, and f-unidentified_Saccharibacteria (Table 4).

Main Taxa Positively Correlated with ADG

Although its relative abundance did not significantly differ among treatments, the abundance of Mollicutes was positively correlated with ADG. Within Mollicutes, the abundances of Anaeroplasmatales and Anaeroplasmataceae

were positively correlated with ADG, and the relative abundances were significantly greater in RAM group than in CK in the present study. Interestingly, several other studies on Mollicutes have examined BWG or diet-induced obesity in mice. Mice fed Western diets unexpectedly exhibited considerable enrichment of the Mollicutes lineage, which reached, on average, 70% of the gut microbiome; this phenomenon was accompanied by an overall decrease in diversity, especially in the abundances of Mycoplasmataceae (Carmody et al., 2015; Turnbaugh, 2017). Nevertheless, human BWG was associated with bacteria assigned to Lachnospiraceae (Menni et al., 2017) and Christensenellaceae (Goodrich et al., 2014). The ADG-associated microbes were Veillonellaceae and Lachnospiraceae in steer (Myer et al., 2015), whereas Victivallaceae and Prevotellaceae were associated with ADG in heifer (Paz et al., 2018).

Although other studies have shown that methanogenic Archaea may also contribute to altered metabolism and weight gain in the host (Million et al., 2013), the current investigation has not found a correlation between Methanobacteria and ADG within the kingdom Archaea. Fibrobacteres at the phylum, class, order, family, and genus levels also were not correlated with ADG in our study, yet they were significantly greater in the RAM than in the other groups. They are consistently described as crucial cellulolytic bacterial lineages (Ransom-Jones et al., 2012).

Main Taxa Negatively Correlated with ADG

The main taxon that was negatively correlated with ADG at the phylum, class, order, and family levels was Saccharibacteria, and the relative abundances were significantly greater in CK group than in RAM in this study. A few Saccharibacteria species are considered to be cellulose utilizers or digesters of plant structural polysaccharides (Opdahl et al., 2018).

In the present study, the abundance of Betaproteobacteria was also negatively correlated with ADG, and the relative abundance of these bacteria was significantly greater in CK group than in AM and RAM. The phylum Proteobacteria has 5 kinds of lineages on the class level: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria (Joanne et al., 2011). In dairy cows, the abundance of Deltaproteobacteria was the most negatively correlated parameter with weekly average milk production (Lima et al., 2015).

CONCLUSIONS

In general, supplementation with *A. mongolicum* Regel powder and extracts could influence the rumen microbiome and increase the ADG. Therefore, it is recommended to supplement *A. mongolicum* Regel powder or extracts for growth improvement in sheep. This study also demonstrated that the main positively correlated microbes with ADG were members of Mollicutes, whereas the negatively correlated microbes were members of Betaproteobacteria and Saccharibacteria.

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