

Research Article

Reclamation of Astragalus By-Product through Dietary Inclusion in Ruminant Diets: Effects on Growth Performance, Nutrient Digestibility, Rumen Fermentation, Blood Biochemical Parameters, and Humoral Immune Response in Sheep

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This study was conducted to investigate the effects of Astragalus by-product (ABP) through dietary supplementation at different levels on performance, nutrient digestibility, rumen fermentation, blood metabolites, and immune response in sheep. Twenty-four Doper × Small Tail Han ewes (6-7 months of age; 29.07 ± 2.28 kg initial body weight) were randomly assigned to one of three treatments for a 47 d feeding period. Treatments consisted of the sheep diet supplemented with 0% ABP-control, 10% ABP, or 15% ABP of the diet (dry matter basis). Blood samples were collected on days 0, 15, 30, and 45 of the feeding period. APB supplementation did not affect growth performance and apparent digestibility of organic matter, crude protein, and acid detergent fibre ($P > 0.05$). However, ether extract digestibility was decreased in the 10% ABP group and increased in the 15% ABP group ($P < 0.001$), and both 10% ABP and 15% ABP decreased the neutral detergent fibre digestibility ($P = 0.005$). Feeding ABP increased rumen pH ($P < 0.001$) and ammonia N ($P < 0.001$) and decreased concentrations of acetate ($P = 0.007$) and propionate ($P = 0.001$) which resultantly increased the acetate-to-propionate ratio ($P < 0.001$) in ruminal fluid. There were no interaction effects between treatment and sampling time for plasma metabolites and immunity ($P > 0.05$). However, inclusion of dietary 10% ABP decreased concentrations of plasma cholesterol ($P = 0.043$). Also, plasma concentrations of low-density lipoprotein decreased on days 30 and 45 ($P = 0.017$) of the feeding period. Metabolite concentrations of total protein, albumin, globulin, blood urea N, glucose, triglyceride, and high-density lipoprotein cholesterol and humoral immune indicators were not affected ($P > 0.05$) by dietary ABP supplementation. The results suggest that ABP could be reclaimed through dietary inclusion in animal feed since it had beneficial effects on rumen fermentation patterns and lipid metabolism and had no adverse effects on performance and humoral immunity in sheep.

1. Introduction

Astragalus membranaceus, also known as Huangqi in Chinese and Radix Astragali in Latin, is a widely used immunomodulating herb mainly in traditional Chinese medicine. The root of *A. membranaceus* contains over 100 bioactive compounds prominent among which

include polysaccharides, flavonoids, amino acids, and saponins [1, 2]. Several studies have indicated the immunomodulatory, cardioprotective, antiviral, antioxidative, hepatoprotective, antitumor, antidiabetic, and anti-inflammatory properties of *A. membranaceus* mainly due to the activities of the bioactive compounds contained in them [1, 3, 4].

In China, the annual yield of herbal by-products including those of *A. membranaceus* is approximately 30 million tons and mostly disposed of through combustion, heaping in the open or sanitary burial, causing serious environmental pollution, especially in water quality [5–7]. For example, most methods used to extract *Astragalus* polysaccharides involve water extraction and alcohol precipitation. These methods not only leave many other bioactive constituents behind, but also produce a large amount of *Astragalus* by-products (ABP) [4, 8]. Several reports show that ABP still contains many nutrients such as crude protein, amino acids, crude fibre, crude fat, and lignin and also includes abundant medicinal components such as terpenoids, alkaloids, and saponins [4]. For instance, a polysaccharide extraction yield of 12.93% with a purity of 93.27% was realized when polysaccharide was extracted from ABP with water [9].

Several studies have attempted to reclaim herbal by-products as feed additives, preparation of activated carbon, papermaking, cultivation of edible fungi, preparation of ethanol, or fermentation for the purpose of limiting infections such as diarrhea [5, 10]. In an experiment to evaluate the therapeutic effects of the fermentation supernatant of herbal residue (composed of parts of *Pseudostellaria heterophylla*, *Dioscorea opposita*, *Hordeum vulgare*, *Crataegus pinnatifida*, *Citrus reticulata*, and Jianweixiaoshi), the fermentation supernatant scavenged 77.8% of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 78% of O₂•, 36.7% of •OH, 39% of Fe²⁺ chelation, and 716 mg/L reducing power. In the same experiment, the fermentation supernatant inhibited diarrhea at a high rate (56%, $p < 0.05$), significantly boosted the destruction of antibiotic-based bacterial diversity, and reestablished the prevalence of *Lactobacillus johnsonii* in the treatment of antibiotic-associated diarrhea in mice [10]. In another study, ABP improved immunity and promoted the digestion ability of white ducks resulting in a corresponding increase in body weight and weight of the ducks' immune organs [3].

Sheep is one of the most important sources of meat produced and consumed worldwide. About 70% of the world's population consumes sheep and goat meat as part of their regular diet and, in several countries, are the main products of traditional dishes [11]. So far, no attempt has been made to reuse ABP as dietary feed additives in sheep production. Therefore, in the present study, ABP was incorporated into sheep diet to evaluate its effect on growth performance, apparent nutrient digestibility, rumen fermentation, blood biochemical parameters, and immune response in Doper × Small Tail Han hybrid sheep and to exploit this dietary option as a potential means of ABP reclamation.

2. Materials and Methods

All experimental protocols involving animals were approved by the Institutional Committee for Animal use and Ethics of the College of Animal Nutrition and Feed Science of Jilin Agricultural University and also in agreement with the provincial rules and regulations.

2.1. *Astragalus* By-Product. The fresh by-product of *A. membranaceus* root used in this study was obtained from Xiuzheng Pharmaceutical Company Limited in Tonghua City, China. The by-product was air-dried and then pulverized to pass a 2 mm screen.

2.2. Experimental Design, Animals, Diets, and Housing. A total of 24 6- to 7-month-old crossbred Doper × Small Tailed Han ewes with an average initial body weight of 29.07 ± 2.28 kg were allocated to 3 dietary treatments with 8 repetitions (8 animals in each treatment) in a completely randomized design by stratified randomization based on their live body weights. Thus, animals were weighed at the beginning of the experiment and randomly assigned to treatments consisting of diets supplemented with various levels of ABP as follows: diets with 0% ABP-control, 10% ABP, and 15% ABP (Table 1). The animals were arranged in a covered area and individually allotted on a suspended slatted floor into individual pens with free access to food and drinking water in a feedlot system. The experiment lasted for 47 days and was preceded by a 30-day adaptation period (a total of 77 days), during which time ewes were weighed, identified, and treated for ecto- and endoparasites, and diet adaptation was performed.

The experimental diets were formulated to be iso-nitrogenous and isoenergetic and on the basis of the National Research Council [12] recommendations for an average daily weight gain of 200 g/day (Table 1). The diets were fed twice daily at 08:00 and 16:00 h and were offered as roughage first and then concentrate. The leftovers were weighed daily, and the amount of supplied feed was adjusted to allow for leftovers of 10 to 20% refusal. ABP and dietary samples were analyzed for dry matter (DM), crude protein (CP), and ether extract (EE) according to the method of the Association of Official Analytical Chemist [13], and acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to the method described by Van Soest et al. [14]. At the end of the feeding period, sheep were weighed after 3 h fasting and then transported to a commercial slaughterhouse for slaughtering according to acceptable standards.

2.3. Sampling and Measurements. The sheep were weighed before the morning feeding on the first and last days of the feeding period to calculate the average daily gain (ADG), and the daily feed supply and orts for each sheep were recorded to calculate the average daily feed intake (ADFI). The feed conversion ratio (FCR) was expressed as feed consumption per unit of body weight gain.

Nine days of apparent nutrient digestibility measurements was conducted from days 37 to 45 at the end of the feeding period, and 6 d sampling (total feces) was preceded by 3 d adaptation. Feed refusals and total fecal samples were collected before the morning feeding from each sheep and weighed. Then, 10 mL hydrochloric acid (2.877 mol/L) was added to 10% of the fresh feces from each sheep to prevent ammonia N volatilization. At the end of the digestibility trial period, the feed refusal and feces samples were composited, dried at 65°C for 72 h, weighed, and milled through a 1 mm

TABLE 1: Ingredients and chemical composition of the experimental diets.

| Item | Dietary treatments ¹ | | |
|---|---------------------------------|---------|---------|
| | Control | 10% ABP | 15% ABP |
| <i>Ingredient (g/kg of DM)</i> | | | |
| <i>Leymus chinensis</i> | 258 | 250 | 220 |
| Corn straw | 175 | 145 | 145 |
| Astragalus by-product | 0 | 100 | 150 |
| Molasses | 20 | 20 | 20 |
| Corn | 260 | 259 | 278 |
| Distillers dried grains with solubles | 140 | 69 | 40 |
| Corn gluten meal | 99 | 115 | 97 |
| Premix ² | 5 | 4 | 7 |
| Urea | 8 | 4 | 8 |
| Sodium chloride | 8 | 8 | 8 |
| Calcium carbonate | 15 | 15 | 15 |
| Dicalcium phosphate | 4 | 4 | 4 |
| Sodium bicarbonate | 8 | 8 | 8 |
| <i>Chemical composition (g/kg DM)³</i> | | | |
| Dry matter (g/kg fresh weight) | 887 | 887 | 887 |
| Crude protein | 124 | 124 | 124 |
| Neutral detergent fibre | 437 | 436 | 430 |
| Acid detergent fibre | 260 | 269 | 273 |
| Metabolizable energy (Mj/kg) | 10 | 10 | 10 |
| Calcium | 8 | 8 | 8 |
| Phosphorus | 5 | 4 | 4 |

¹ABP, Astragalus by-product; ²supplied per kg of diets: vitamin A, 15,000 IU; vitamin D, 2,000 IU; vitamin E, 55 IU; Fe, 50 mg; Co, 0.2 mg; Cu, 12.0 mg; Se, 0.5 mg; Mn, 50 mg; I, 0.55 mg; Zn, 25 mg; ³nutrient levels were all measured values except metabolizable energy.

sieve for chemical analysis. The samples were analyzed according to the Association of Official Analytical Chemists standard procedures [15] for crude protein (CP, Kjeldahl procedure; method 976.06), ether extract (EE, method 920.29), and crude ash (550°C in a furnace for 6 h; method 942.05). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by the method of Van Soest et al. [14] inclusive of residual ash, according to the filter bag technique with addition of amylase and sodium sulfite.

Ruminal fluid samples were collected from the rumen immediately after sheep were slaughtered. Ruminal pH was measured immediately using a portable type pH meter (Testo 205, Testo AG, Lenzkirch, Germany). Afterwards, the ruminal fluid was filtered through 4 layers of cheesecloth and the extracts were centrifuged at 10,000 × g for 15 min at 4°C. The supernatant of the ruminal fluid (15 ml) was transferred into tubes containing 0.3 ml of 500 mg/g sulfuric acid, mixed, and stored at -20°C for NH₃-N analysis by the phenol-hypochlorite method, as described by Broderick and Kang [16]. The remaining strained ruminal fluid samples, 10 ml, were mixed with 1 ml of 250 mg/g metaphosphoric acid and stored at -20°C for later determination of volatile fatty acid profiles by gas chromatography [17].

Before the morning feeding on days 0, 15, 30, and 45 of the feeding period, blood samples were collected from the jugular vein of each sheep into 5 ml heparinized collection tubes and centrifuged at 3000 × g for 15 min at 4°C. The

supernatant (plasma) was collected and frozen at -20°C pending further analysis. For blood biochemical parameters, total protein (TP), albumin (ALB), globulin (GLB), blood urea N (BUN), glucose (GLU), triglyceride (TG), total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-CH), and low-density lipoprotein cholesterol (LDL-CH) concentrations were determined using an automatic biochemistry analyzer (SYNCHRON CX5 PRO, Beckman Coulter, Fullerton, CA, USA). For immune response, immunoglobulins (IgG, IgA, and IgM) were determined using an ELISA test kit (Diagnostic Automation/Cortez Diagnostics, Inc., Los Angeles, CA, USA).

2.4. Statistical Analysis. The data of growth performance of sheep, apparent nutrient digestibility, and rumen fermentation parameters were analyzed using a general linear model, followed by Duncan's multiple range tests [18]. The data for plasma parameters of sheep were analyzed using the mixed model procedure [19] with a model consisting of treatment, sampling time, and treatment × time interaction as fixed effects and animal as the random effect. Measurements obtained from each sheep at different sampling times were treated as repeated measures. Polynomial analysis was conducted to determine the linear or quadratic response to the increasing ABP dosage in the diet. The means of each trait were compared by Turkey multiple comparisons and presented with the standard error of the mean. Differences were considered statistically significant if $P \leq 0.05$.

3. Results

3.1. Growth Performance and Nutrient Digestibility. Table 2 shows the effects of increasing levels of ABP on sheep growth performance attributes. Dietary supplementation of ABP marginally improved the ADG and ADFI of sheep even though the differences were not significant ($P = 0.771$; 0.253). Conversely, FCR among dietary treatments did not differ significantly ($P = 0.911$). Feeding ABP decreased the apparent digestibility of dietary NDF in a linear ($P = 0.008$) and quadratic ($P = 0.034$) manner. Moreover, dietary ABP supplementation linearly ($P = 0.013$) and quadratically ($P < 0.001$) increased the apparent digestibility of dietary EE ($P < 0.001$) in comparison with the control group. Dietary CP, CF, ADF, and ash were not affected by ABP supplementation ($P > 0.05$).

3.2. Rumen Fermentation Parameters. Rumen fermentation parameters were affected by the treatment diet (Table 3). The pH of the rumen liquid varied between 5.8 and 6.6 and had a linear ($P < 0.001$) and quadratic ($P = 0.055$) response to dietary ABP supplementation. The pH was greater ($P < 0.001$) for sheep in the 10% ABP and 15% ABP groups than for sheep in the control group. The molar proportions of acetate and propionate were significantly decreased ($P = 0.007$; $P = 0.001$) by ABP. The butyrate concentration varied between 5.6 mmol/L and 9 mmol/L and had a linear ($P = 0.023$) response to dietary ABP addition. The highest acetate-to-propionate ratio was observed in the 10% ABP

TABLE 2: Growth performance and apparent nutrient digestibility of sheep fed diets supplemented with different levels of dietary Astragalus by-product.

| Items ¹ | Dietary treatments ² | | | SEM | Diet | P value | |
|--|---------------------------------|--------------------|--------------------|-------|--------|---------|-----------|
| | Control | 10% ABP | 15% ABP | | | Linear | Quadratic |
| <i>Growth performance</i> | | | | | | | |
| ADG (kg/d) | 0.12 | 0.13 | 0.13 | 0.005 | 0.771 | 0.620 | 0.637 |
| ADFI (kg/d) | 1.48 | 1.56 | 1.57 | 0.024 | 0.253 | 0.162 | 0.423 |
| FCR | 12.41 | 11.95 | 12.47 | 0.500 | 0.911 | 0.969 | 0.674 |
| <i>Apparent nutrient digestibility (%)</i> | | | | | | | |
| OM | 49.62 | 37.73 | 40.92 | 2.392 | 0.086 | 0.124 | 0.111 |
| CP | 70.58 | 65.70 | 67.44 | 0.988 | 0.099 | 0.179 | 0.096 |
| EE | 55.56 ^b | 24.50 ^c | 72.32 ^a | 5.831 | <0.001 | 0.013 | <0.001 |
| ADF | 45.42 | 30.90 | 39.32 | 2.970 | 0.110 | 0.377 | 0.062 |
| NDF | 69.40 ^a | 54.78 ^b | 56.31 ^b | 2.370 | 0.005 | 0.008 | 0.034 |

^{a,b,c}Means within a row with different subscripts differ when $P < 0.05$; ¹ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; ²ABP, Astragalus by-product; SEM, standard error of the mean.

TABLE 3: PH value and volatile fatty acid (VFA) concentration in ruminal fluid of sheep fed diets supplemented with different levels of dietary Astragalus by-product.

| Items | Dietary treatments ¹ | | | SEM | Diet | P value | |
|----------------------------|---------------------------------|--------------------|--------------------|-------|--------|---------|-----------|
| | Control | 10% ABP | 15% ABP | | | Linear | Quadratic |
| Rumen pH | 5.83 ^b | 6.47 ^a | 6.51 ^a | 0.114 | <0.001 | <0.001 | 0.055 |
| Acetic acid (mmol/L) | 59.29 ^a | 40.72 ^b | 36.7 ^b | 3.877 | 0.007 | 0.003 | 0.134 |
| Propionic acid (mmol/L) | 22.58 ^a | 11.2 ^b | 11.25 ^b | 1.999 | 0.001 | 0.001 | 0.011 |
| Butyric acid (mmol/L) | 8.91 | 6.81 | 5.67 | 0.606 | 0.059 | 0.023 | 0.627 |
| Acetic/propionic acid | 2.63 ^c | 3.64 ^a | 3.27 ^b | 0.151 | <0.001 | 0.001 | <0.001 |
| NH ₃ -N (mg/dL) | 3.51 ^c | 4.38 ^b | 7.13 ^a | 0.329 | <0.001 | <0.001 | 0.003 |

^{a,b,c}Means within a row with different subscripts differ when $P < 0.05$; ¹ABP, Astragalus by-product; SEM, standard error of the mean.

dietary group (3.64) and that of the 15% ABP group was significantly higher than for the control group ($P < 0.001$). Feeding ABP markedly increased the ammonia N concentration of the rumen linearly ($P < 0.001$) and quadratically ($P = 0.003$).

3.3. Blood Biochemical Parameters. There were no interaction effects between treatment and sampling time for plasma metabolites and humoral immunity ($P > 0.05$) among dietary treatments (Table 4). However, inclusion of dietary ABP significantly decreased ($P = 0.043$) concentrations of plasma cholesterol of sheep in the 10% ABP dietary group. Also, there was a time effect on LDL-CH as plasma concentrations decreased with time and significantly so on days 30 and 45 ($P = 0.017$) of the feeding period. Metabolite concentrations of TP, ALB, GLB, BUN, GLU, TG, and HDL-CH and humoral immune indicators IgG, IgM, and IgA were not affected ($P > 0.05$) by dietary supplementation on ABP.

4. Discussion

This study is among the first to investigate the effects of the by-products of Astragalus, a traditional Chinese herbal medicine as a feed supplement in the diet on growth performance, apparent nutrient digestibility, rumen fermentation, blood biochemical parameters, and humoral immune

response in sheep. The primary aim of this study was to provide evidence that it is more feasible to use ABP as a feed additive in animal production rather than piling and burning, which results in environmental pollution. This is also in sync with previous attempts that have been made to increase rumen development and weight gain of postweaned lambs by nutritional strategies instead of performance-enhancing drugs considering the high demand for organic animal products in the consumer markets [1]. In the present study, we found that dietary supplementation of ABP did not affect the growth performance indicators in sheep, even though there was a marginal improvement in ADG and ADFI. Similar to the present observations, Zhong et al. [1] had found that dietary supplementation of *A. membranaceus* root did not affect dry matter intake, ADG, and FCR in lambs. Furthermore, a study on the effects of dietary *A. membranaceus* root powder addition at various levels on the growth performance of broiler chicks showed that dietary ADFI was not affected [20]. This is an indication that dietary inclusion of ABP does not affect the palatability of feed. In contrast to our results, previous studies with nonruminants showed that dietary *A. membranaceus* supplementation increased dry matter intake and ADG in pigs [21] and improved the ADG and FCR in broilers [20]. The observed differences may have been due to the different microenvironments in the digestive tract between ruminants and nonruminants, as well as the different dose levels of Astragalus.

TABLE 4: Blood biochemical parameters and humoral immune response of sheep fed diets supplemented with different levels of dietary Astragalus by-product.

| Items ¹ | Dietary treatments ² | | | SEM | Time ³ | | | | | P value | | | |
|-------------------------------------|---------------------------------|-------------------|--------------------|-------|-------------------|--------------------|-------------------|-------------------|-------|---------|-------|-------|-------|
| | Control | 10% ABP | 15% ABP | | D0 | D15 | D30 | D45 | DT | T | DT×T | L | Q |
| <i>Blood biochemical parameters</i> | | | | | | | | | | | | | |
| TP (g/L) | 66.86 | 59.63 | 64.58 | 0.491 | 65.49 | 61.34 | 63.72 | 64.21 | 0.071 | 0.535 | 0.621 | 0.726 | 0.428 |
| ALB (g/L) | 35.58 | 32.81 | 35.51 | 0.669 | 35.09 | 33.09 | 34.67 | 35.68 | 0.184 | 0.437 | 0.449 | 0.437 | 0.245 |
| GLB (g/L) | 31.29 | 26.82 | 29.08 | 0.734 | 30.41 | 28.25 | 29.05 | 28.54 | 0.111 | 0.620 | 0.862 | 0.703 | 0.785 |
| BUN (mmol/L) | 6.17 | 5.42 | 6.48 | 0.231 | 5.99 | 6.22 | 6.76 | 5.11 | 0.125 | 0.077 | 0.206 | 0.145 | 0.627 |
| GLU (mmol/L) | 4.07 | 3.76 | 4.09 | 0.080 | 4.18 | 3.71 | 4.00 | 4.02 | 0.255 | 0.225 | 0.507 | 0.318 | 0.514 |
| TG (mmol/L) | 0.39 | 0.33 | 0.41 | 0.017 | 0.37 | 0.36 | 0.45 | 0.33 | 0.357 | 0.052 | 0.734 | 0.953 | 0.579 |
| CHOL (mmol/L) | 1.86 ^a | 1.41 ^b | 1.76 ^{ab} | 0.059 | 1.93 | 1.51 | 1.57 | 1.69 | 0.043 | 0.052 | 0.709 | 0.704 | 0.611 |
| HDL-CH (mmol/L) | 1.20 | 1.07 | 1.33 | 0.040 | 1.24 | 1.19 | 1.22 | 1.15 | 0.156 | 0.642 | 0.405 | 0.217 | 0.166 |
| LDL-CH (mmol/L) | 0.57 | 0.44 | 0.45 | 0.022 | 0.58 ^a | 0.45 ^{ab} | 0.43 ^b | 0.49 ^b | 0.285 | 0.017 | 0.375 | 0.143 | 0.286 |
| <i>Immune response</i> | | | | | | | | | | | | | |
| IgG (µg/mL) | 126.72 | 127.25 | 122.69 | 5.287 | 138.95 | 115.70 | 113.25 | 134.32 | 0.904 | 0.390 | 0.960 | 0.772 | 0.765 |
| IgM (µg/mL) | 364.69 | 363.40 | 361.72 | 9.664 | 405.67 | 347.71 | 342.74 | 356.97 | 0.988 | 0.301 | 0.649 | 0.103 | 0.278 |
| IgA (µg/mL) | 54.54 | 52.06 | 54.41 | 1.812 | 56.33 | 54.60 | 51.52 | 52.23 | 0.682 | 0.760 | 0.941 | 0.992 | 0.574 |

^{a,b}Means within a row with different subscripts differ when $P < 0.05$; ¹TP, total protein; ALB, albumin; GLB, globulin; BUN, blood urea nitrogen; GLU, glucose; TG, triglyceride; CHOL, cholesterol; HDL-CH, high-density lipoprotein cholesterol; LDL-CH, low-density lipoprotein cholesterol; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; ²ABP, Astragalus by-product; ³D, day; DT, dietary treatment; T, time; SEM, standard error of the mean.

Several studies have shown that phyto-genic-based feed additives could increase villi length and decrease crypt depth in the jejunum and colon [22, 23]. Apparent digestibility of protein may be increased by improving the digestive capability of the prececum since the dominant proportion of total fecal protein is bacterial proteins. An improved prececal digestive capability decreases the flux of fermentable material into the hindgut and consequently decreasing postileal microbial growth and fecal bacterial growth [24]. Also, most medicinal plants have been reported to contain essential oils that may improve nutrients digestibility in animals [25]. However, we found that dietary ABP supplementation did not affect the apparent digestibility of CP, ADF, and ash in the present experiment. This result is consistent with that of Zhong et al. [1] who reported that dietary inclusion of 50 g/kg *Astragalus membranaceus* root had no effect on apparent nutrient digestibility in growing lambs. Furthermore, Qiao et al. [26] reported that extracts of *Fructus Ligustri Lucidi*, a traditional herbal medicine supplied at 100, 300, and 500 mg/kg dry matter, had no effect on CP, ADF, and NDF digestibility in sheep. In contrast, El-Ashry et al. [25] found that dietary addition of three different medicinal plants in the diet improved nutrient digestibility at 8 months of the feeding trial. This variation may have been due to the different total feeding time periods in the two experiments. Dietary EE and NDF affect digestion, metabolism, and microbial activity in animals [27]. In the present study, the inclusion of dietary ABP resulted in a decreased apparent NDF digestibility in the 10% ABP (from 69.40% to 54.78%) and 15% ABP (from 69.40% to 56.31%) dietary groups and increased apparent ether extract digestibility in the 15% ABP (from 55.56% to 72.32%) dietary group. Similar results were observed by Ghasemi et al. [27] who reported that replacement of lucerne hay with pistachio by-product either partially or fully decreased the apparent NDF and increased ether extract digestibility in sheep. It is worth

noting that decreased dietary NDF to 22.9% of DM did not affect cellulolytic bacteria in the rumen of lactating dairy cows [28]. In the present study, the levels of NDF in the treatment groups were higher than this amount. The decreased NDF digestibility may have been due to the increase in fibre content as the content of ABP increased. These results suggest that dietary ABP inclusion does not have adverse effects on nutrient digestibility in sheep.

One of the most important indicators of the microbial ecosystem of the rumen is the ruminal pH. A more acidic microbial ecosystem poses a threat to the establishment of a balanced microbial population and also decreases microbial attachment which affects the digestion of fibre [1, 29, 30]. In the present experiment, dietary inclusion of ABP significantly increased the pH of ruminal fluid from 5.83 in the control group to 6.51 in the 15% ABP group. This pH falls within the optimal pH range stipulated for the rumen environment and rumen fermentation [31] in sheep. This indicates that dietary ABP inclusion has the potential to regulate and stabilize the microbial ecosystem in the rumen.

Ammonia nitrogen, $\text{NH}_3\text{-N}$, is an essential indicator for the synthesis of microbial protein in the rumen since a bulk of the rumen bacteria use NH_3 as a source of N [32, 33]. The minimum concentration of $\text{NH}_3\text{-N}$ required for maintaining an optimum microbial activity in the rumen is 5 mg/dl. As such, a higher concentration results from a more rapid ruminal degradation of proteins and/or reduced transport through the rumen wall [33]. In the current study, increasing dietary ABP addition correspondingly increased $\text{NH}_3\text{-N}$ in the rumen of sheep, suggesting that protein degradation increased with increasing ABP supplementation. However, this trend was not observed in the current experiment as CP digestibility was unaffected. In contrast, ruminal $\text{NH}_3\text{-N}$ concentration was unaffected when dietary *Astragalus polysaccharide* and *Astragalus root* were added to lambs' diet [1]. Also, Soltan et al. [34] observed that dietary

inclusion of 25 and 50 g/kg DM of *Moringa oleifera* root bark, a herbal medicine, in growing lambs' diet did not affect the $\text{NH}_3\text{-N}$ concentration in the rumen. The increased $\text{NH}_3\text{-N}$ levels in the present study are probably as a result of lower contents of saponins (major active compounds in *Astragalus*) in the by-product as saponins have been suggested to decrease ammonia levels in the rumen [35].

In this study, dietary ABP inclusion significantly decreased the acetate and propionate concentrations and significantly increased the acetate-to-propionate ratio in the rumen while butyrate concentration remained unaffected even though a marginal decline was recorded. Several studies using herbal medicines as feed additives in animal production showed changes in VFA formation. For example, the molar proportion of acetate in ruminal fluid increased and that of butyrate decreased while those of total VFA and propionate as well as acetate:propionate proportion were unaffected when 500 g/kg *Urtica cannabina* was mixed with 500 g/kg concentrate and fed to growing lambs [36], indicating inconsistency with the current study. In another study, ruminal molar concentrations of total VFA, acetate, and acetate:propionate proportion increased while proportions of propionate, butyrate, isobutyrate, valerate, and isovalerate were unaffected when *Moringa oleifera* root bark was fed to lambs [34]. However, slightly consistent with the present experiment is that of Zhong et al. [1] who found that dietary inclusion of *Astragalus* root did not affect ruminal propionate and butyrate proportions although they had a decreasing tendency. Moreover, Ghasemi et al. [27] observed lower molar concentrations of ruminal acetate and propionate + isobutyrate, and no effect on butyrate when high and low levels of pistachio by-products were added to sheep diets, as was observed in the present experiment. The lower VFA concentrations in the present study were probably due to lower microbial activity in the rumen [27].

Concentrations of TP, ALB, BUN, and GLU are generally suggested to be determinants of protein synthesis and metabolism which are relevant for growth performance [35, 37]. Also, blood GLU concentration is generally regarded as the energy supply indicator in animals [38]. Kim et al. [39] suggested that increased TP and ALB concentrations indicate that more proteins are synthesized and absorbed, and decreased BUN indicate that more proteins are synthesized with amino acids since BUN is a product of protein degradation. In the present study, TP, ALB, BUN, and GLU concentrations were not affected by dietary ABP addition at the end of the feeding trial, indicating that ABP did not affect the synthesis and metabolism of protein. This is also reflected in the growth performance indicators in the present experiment as growth performance was not affected by dietary ABP addition. This is consistent with the findings of Zhong et al. [1] who reported that dietary inclusion of *Astragalus* root in lambs' diet did not affect TP, ALB, and BUN concentrations in the blood. Concentrations of LDL-CH and HDL-CH are related to lipid N metabolism [1, 40]. Higher plasma concentrations of CHOL and TG are considered risk factors related to

coronary heart diseases and atherosclerosis in humans [37, 41]. Moreover, the key function of plasma lipoproteins is the transportation of lipids from secreting organs like the intestine and liver to peripheral tissues [42, 43]. HDL-CH transports lipids from tissues to the liver and thus known as "good cholesterol," whereas LDL-CH is largely related to the low plasma cholesteryl esters transfer activity and thus known as "bad cholesterol" [42]. For example, Cornier et al. [44] found that elevated levels of cholesterol, especially of LDL-CH, increased the risk of cardiovascular diseases. Several traditional Chinese herbal medicines have been suggested to affect lipid metabolism and consequently inhibit blood lipid-related diseases. For example, dietary *Astragalus* root extract supplementation decreased serum and hepatic TG and CHOL concentrations in rat liver [45]. Also, *Astragalus* and *Lycium barbarum* polysaccharides have been reported to lower total CHOL, LDL-CH, and very LDL-CH in rats [46, 47]. In accordance with the previous studies, dietary ABP supplementation decreased the plasma CHOL concentrations of sheep in the present experiment. Concentrations of TG and LDL-CH were also decreased in the 10% ABP group even though the differences were not significant from the control group. This indicates that dietary ABP could effectively metabolize lipids and thus prevent lipid-related diseases.

An important indicator of humoral immunity in animals is the level of plasma antibodies which mainly include immunoglobulins G (IgG), M (IgM), and A (IgA). They defend the extravascular compartment against pathogenic viruses and microorganisms [37, 48]. Several flavonoid-containing herbal medicines including *A. membranaceus* have been reported to enhance immune function [1, 24]. Furthermore, previous research has indicated that *Astragalus* polysaccharides could enhance humoral immunity in chicks [49–51]. In the present study, dietary ABP inclusion did not affect the humoral immune response in sheep. A possible reason could be that ABP levels in the present study were suboptimal. Similar findings were reported by Che et al. [52] who found that humoral immunity indicators IgG, IgM, and IgA were not affected when 2.5, 5.0, and 7.5% pulverized *A. membranaceus* stem and leaf fibre were fed to weaned pigs. This result suggests that ABP supplementation has no adverse effect on the immune integrity of sheep.

5. Conclusions

The findings of the current experiment suggest that dietary inclusion of *Astragalus membranaceus* by-products in diet had beneficial effects on rumen fermentation patterns and lipid metabolism and had the potential to improve growth performance in sheep. Additionally, no adverse effects on the production performance and immunity traits in sheep were recorded according to the results of the present study. It can be concluded that it is more useful to use ABP as a novel natural dietary feed additive in animal production systems similar to those of the present study, as opposed to disposal by piling and/or burning which causes serious environmental pollution.

Data Availability

No data were used to support this study.

Conflicts of Interest

No potential conflicts of interest were reported by the authors.

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