Astragalus membranaceus root supplementation improves average daily gain, rumen TVFA production and immunity and antioxidant factors of Tibetan sheep

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Abstract

Background: The use of antibiotics as supplements in animal feed is restricted due to possible health hazards associated with them. Consequently, there is increasing interest in exploiting natural products as antibiotics with no detrimental side effects. In this study, we examined the effect of *Astragalus membranaceus* root (AMT) supplementation on dry matter intake, growth performance, rumen fermentation and immunity of Tibetan sheep.

Materials and methods: Twenty-four male Tibetan sheep (31 ± 1.4 kg; 9 months old) were assigned randomly to one of four dietary treatments with different levels of AMT: 0 g/kg, 20 g/kg, 50 g/kg and 80 g/kg dry matter (A 0, A 2, A 5 and A 8, respectively) in addition to their basal diets. A 0 acted as a control group and measurements were recorded over a 56-d feeding period.

Results: Sheep fed with AMT had a higher average daily gain (ADG) and a lower feed:gain ratio (F:G) than controls ($P < 0.001$). Rumen concentrations of $\text{NH}_3\text{-N}$ ($P < 0.001$), total volatile fatty acids (TVFA) ($P = 0.028$), acetate ($P = 0.017$) and propionate ($P = 0.031$) in A 5 and A 8 were higher than in A 0. The addition of AMT in the feed significantly increased serum antioxidant and immunity factors of the sheep and increased the concentrations of serum interleukin, immunoglobulin and tumour necrosis factor- α (TNF- α) ($P = 0.010$).

Conclusions: We concluded that AMT can be used as a feed additive to improve growth performance and rumen fermentation and enhance the immunity of Tibetan sheep. Some responses exhibited a dose-dependent response, whereas other did not exhibit a pattern, with an increase in AMT. The addition of 50 g/kg and 80 g/kg AMT of total DMI showed the most promising results.

Background

The high-altitude Qinghai-Tibetan Plateau (QTP) is characterized by low air temperature, low air oxygen content, a short growing season and sparse vegetation of poor quality during the winter [1]. As one of the major livestock species on the QTP, numbering over 50 million head, Tibetan sheep are well adapted to the harsh environment and provide meat and income for most nomadic and semi-nomadic peoples in these regions [2].

Feeding antibiotics to livestock to increase production and improve livelihoods is a common practice in many countries [3]. However, the use of antibiotics as supplements in animal feed is often restricted due to residues and resistant strains of bacteria, and the rising awareness of hazards associated with antibiotics [4]. Consequently, there is increasing interest to exploit natural products that have no public health hazards.

Many Chinese herbs, with low toxicity, have been used as feed additives for livestock to improve growth performance and/or reduce mortality [5]. For example, *Angelica sinensis* (root), *Epimedium brevicornu* (full plant), *Schisandra chinensis* (fruit) and *Acanthopanax senticosus* (root) can enhance immune functions, *Isatis indigotica* (root), *Rheum palmatum* (stem and root) can reduce pathogenic microorganisms, and *Citrus reticulata* (peel) and *Crataegus pinnatifida* (fruit) can increase animal appetite and improve feed conversion rate [6].

Astragalus membranaceus, a legume, is widely distributed throughout the temperate regions of the world [7]. The dried root of *Astragalus membranaceus* (AMT) contains polysaccharides, saponins and other biological active substances, which have been used in Chinese traditional medicine for nearly 2000 years as a booster of the immune system [8, 9]. Pharmacological studies have shown that AMT possesses immuno-stimulant, tonic, hepato-protective, diuretic, antidiabetic, analgesic and sedative properties [10, 11]. In addition, it has been reported that AMT and its extracts can improve growth performance and immunity of chickens and pigs [12]. However, little is known on the effects of AMT on ruminants. The aim of this study is to fill this gap by determining the effects of dietary AMT supplementation on growth performance, rumen fermentation and immunity and antioxidant factors in Tibetan sheep.

Materials And Methods

Study site and preparation of *Astragalus membranaceus*

The study was conducted between October 20 and December 30, 2018, at the Haibei Demonstration Zone of Plateau Modern Ecological Animal Husbandry Science and Technology, Haibei, China ($\text{N}36^{\circ}55'$, $\text{E}100^{\circ}57'$, 3170 m a. s. l.). During the experimental

period, the average air temperature was -4.4°C . All procedures were approved by the Animal Ethics Committee of Lanzhou University, China.

Dried roots of *Astragalus membranaceus* were purchased from the Gansu Hebo Chinese Herbal Medicine (Long) Science and Technology Co., Ltd, China. The roots were washed, dried, ground and passed through a 1 mm sieve. The contents of AMT, determined by the ultraviolet branch photometer method, included 125.8 g/kg astragalus polysacharin, 0.12 g/kg calycosin-7-glucoside and 0.87 g/kg astragaloside IV. The main bioactive ingredients in AMT conforms to Pharmacopopoeia of the People's Republic of China [13].

Experimental design

Twenty-four 9-month-old male Tibetan sheep (31 ± 1.4 kg), with similar genetic backgrounds, were used in a completely randomized design. Each sheep was penned individually (each pen = 2.5×3.5 m) and had free access to fresh water. The sheep were offered 450 g concentrate (Menyuan Yongxing Ecological Agriculture and Animal Husbandry Development Co. LTD, Haibei, China) and ad libitum oat hay daily, at 07:00 and 17:00. The compositions of the feed are presented in Table 1. After a 2-week adaptation period, the sheep were assigned randomly to one of four treatments ($n = 6$ per treatment) that differed in the level of AMT: control – 0 g/kg (A_0), 20 g/kg (A_2), 50 g/kg (A_5) and 80 g/kg (A_8) dry matter intake (DMI) for 56 days. The AMT, mixed with 10 g concentrate, was fed separately to each sheep at 07:00, and the amount fed each sheep was based on DM intake from pre-trial feed intakes, when the sheep consumed approximately 800 g DM oat hay along with the 450 g concentrate, and the amount of AMT was then adjusted weekly.

Table 1
Composition (DM basis) of the concentrate, oat hay and *Astragalus membranaceus* root (AMT)

Ingredient ¹ (g/kg DM)	Concentrate	Oat hay	AMT
CP	201	84	194
Ash	78	85	50
EE	46	09.7	10.3
NDF	2	485	523
ADF	-	392	416
Ca	9.8	4.5	6.1
¹ CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; Ash, crude ash; GE, gross energy.			
² "-" not detected			

Data collection and sampling

The sheep were weighed at the beginning and end of the trial before morning feeding. Dry matter intake (DMI), which included the AMT, was recorded every day by weighing DM offered and DM refusals. Average daily gain (ADG) was calculated by the difference between initial and final BW and the feed:gain (F:G) ratio was calculated from the DMI and ADG. Feed samples and refusals (only oat hay) were collected every 2 weeks, mixed thoroughly and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA) before chemical analyses. Dry matter (DM) was determined by oven drying at 105°C for 48 hours. Crude protein (CP; method 984.13), ether extract (EE; method 920.29) and ash content (method 942.05) were measured according to AOAC (1990) [14]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of the feed samples were measured using fiber analyzer (ANKOM A2000i, NY, USA) following Van Soest et al. (1991) [15].

Jugular vein blood samples were collected into vacuum tubes before the morning feeding on days 14, 28 and 56. The tubes were centrifuged at 3000 g (4°C) for 15 min within 2 h of sampling, and the serum was stored at -80°C for subsequent analysis. Serum concentrations of tumour necrosis factor- α (TNF- α), interleukin (IL)-2, -4, -6 and -10, growth hormone (GH), insulin (INS) and soluble CD14 (sCD14) were measured using commercial ELISA kits (Beijing Sinoouk Institute of Biological Technology, Beijing, China) and

serum immunoglobulin (IgA, IgG and IgM), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) were measured using commercial colorimetric assay kits (Beijing Sinoouk Institute of Biological Technology, Beijing, China).

A rumen fluid sample was collected from each sheep every two weeks before morning feeding using rumen fluid collection tubes (Anscitech, Wuhan, China). Approximately 80 mL were collected from each sheep of which the first 30 mL were discarded to minimize contamination from saliva. After the pH was measured by a pH meter (M90; Corning Inc., Corning, NY, USA), the rumen fluid was strained through four layers of cheesecloth, and stored in 10 mL tubes at -80°C until further analysis. Volatile fatty acids (VFA) were determined using a chromatograph (BEIFEN SP-3420A, Beijing, China) following the method of Zhang et al. (2016) [16] and ammonia-N ($\text{NH}_3\text{-N}$) was determined following Hristov et al. (2001) [17].

Statistical analysis

Results are presented as means $\pm$ SEM. Two-week values (time) of dry matter intake, TVFAs, and immune factors for each sheep were analyzed by repeated measures ANOVA using the mixed procedure of SAS (Version 9.1; SAS Institute) with day as the independent variable. A Tukey's test was used to separate means where a treatment $\times$ period interaction was significant.

Orthogonal contrasts were used to evaluate differences due to level of AMT and test the linear, quadratic, and cubic effect of an increasing proportion of AMT. The results are presented as least square means. Correlation analysis of variables was done using bivariate correlation analysis (SPSS correlation analysis). Statistical significance was accepted at $P < 0.05$.

Results

Effect of AMT on dry matter intake and average daily gain

Body weight (BW) did not differ among groups ($P = 0.973$) at the beginning of the study (day 0), but was higher ($P < 0.001$) in sheep supplemented with AMT on day 56 than in controls (Table 2). The addition of AMT increased the average DMI of sheep ($P = 0.001$). All the supplemented concentrate was consumed by the sheep in each treatment, therefore the differences in total DMI reflected mainly the differences in oat hay intake. The groups consuming AMT all had higher ADG than controls ($P < 0.001$) and there was a linear increase ($P < 0.001$) with an increase in AMT. The ADG of groups A_8 , A_2 and A_5 were 79%, 21% and 25%, higher, respectively, than group A_0 . The highest feed:gain (F:G) ratio occurred in the A_0 group, and the lowest ratio in the A_8 group ($P < 0.001$), and there was a linear decrease ($P < 0.001$) with an increase in AMT.

Table 2

The effect of Astragalus membranaceus root (AMT) on body weight (BW), average daily gain (ADG) and feed:gain (F:G) ratio and dry matter intake (DMI) of Tibetan sheep

Items ¹	Treatment ²				SEM	P-value ³			
	A ₀	A ₂	A ₅	A ₈		L	Q	C	Treatment (T)
BW, initial (kg)	32.0	31.8	31.5	31.2	0.59	0.644	0.998	0.975	0.975
BW, final (kg)	35.6 ^a	36.9 ^b	37.2 ^b	37.8 ^b	0.25	0.137	0.689	0.713	0.005
DMI (kg/day)	1.23 ^a	1.28 ^b	1.27 ^b	1.31 ^b	0.040	0.198	0.766	0.096	0.001
ADG (g/day)	64.3 ^a	91.1 ^b	101.8 ^b	117.9 ^c	5.55	< 0.001	< 0.001	< 0.001	< 0.001
F:G ratio (g:g)	18.0 ^c	13.8 ^b	12.6 ^b	11.0 ^a	0.751	< 0.001	0.182	0.026	< 0.001
a-c, means within a row followed by different lower-case letters differ significantly (P < 0.05).									
¹ BW = body weight; DMI = dry matter intake; ADG = average daily gain; F:G ratio = feed to gain ratio.									
² A ₀ contains 0 g/kg AMT in diet (dry matter basis); A ₂ contains 20 g/kg AMT in diet (dry matter basis); A ₅ contains 50 g/kg AMT in diet (dry matter basis); A ₈ contains 80 g/kg AMT in diet (dry matter basis).									
³ L = Linear response of AMT; Q = Quadratic response of AMT; C = Compound response of AMT.									

Effect of AMT on rumen pH and volatile fatty acids

Rumen pH decreased ($P = 0.016$) on days 28 and 56 in the AMT fed groups when compared to controls (Table 3) and was lower on day 56 than on days 14 and 28 in all groups ($P = 0.003$). Rumen acetate concentration was higher ($P = 0.017$) in sheep consuming AMT than in controls on days 28 and 56 and acetate was also higher on day 56 than day 14 in all four treatment groups ($P = 0.022$). Generally, AMT increased rumen propionate concentration ($P = 0.031$) and there was a linear increase ($P < 0.001$) with an increase in AMT. There was no consistent trend in rumen butyrate concentration in relation to the level of AMT ($P = 0.059$). The concentration of TVFA in the rumen was higher in the AMT fed sheep than controls ($P = 0.028$), was higher on day 56 than days 14 and 28 ($P = 0.041$) and increased linearly ($P < 0.007$) with an increase in AMT. The A:P ratio was lower in A₂, A₅ and A₈ groups than in A₀ on day 56 ($P < 0.001$), with the lowest value in the A₈ group. The addition of AMT increased ($P < 0.001$) rumen NH₃-N concentration on days 28 and 56, the NH₃-N concentration increased significantly ($P < 0.001$) with days and the treatment x day interaction was significant ($P = 0.041$) (Table 3).

Table 3
The effect of AMT on rumen pH and VFA on days 14, 28 and 56 in Tibetan sheep

Items ¹	Day	Treatment ²				SEM	P-value ³					
		A ₀	A ₂	A ₅	A ₈		L	Q	C	Day (D)	Treatment (T)	T × D
pH	14	6.63 ^B	6.6 ^B	6.65 ^B	6.62 ^B	0.011	0.542	0.638	0.781	0.003	0.016	0.266
	28	6.63 ^{bB}	6.57 ^{aB}	6.57 ^{aB}	6.56 ^{aB}	0.011						
	56	6.52 ^{bA}	6.45 ^{aA}	6.42 ^{aA}	6.45 ^{aA}	0.013						
Acetate (mmol/L)	14	32.4 ^A	31.9 ^A	32.5 ^A	34.5 ^A	0.347	0.282	0.779	0.677	0.022	0.017	0.923
	28	33.7 ^{aA}	35.4 ^{bB}	34.2 ^{bA}	37.4 ^{cB}	0.455						
	56	36.4 ^{aB}	37.9 ^{bC}	38.0 ^{bB}	38.1 ^{bB}	0.271						
Propionate (mmol/L)	14	6.57 ^a	6.94 ^a	7.54 ^b	8.08 ^c	0.192	0.002	0.811	0.528	0.227	0.031	0.960
	28	6.85 ^a	7.62 ^b	7.49 ^b	8.51 ^c	0.183						
	56	6.87 ^a	8.18 ^b	8.48 ^b	8.96 ^b	0.276						
Butyrate (mmol/L)	14	6.00	4.40	5.53	4.76	0.193	0.900	0.423	0.667	0.344	0.200	0.692
	28	4.52	5.97	4.52	5.54	0.214						
	56	5.20	7.39	6.57	5.43	0.322						
TVFA (mmol/L)	14	47.0 ^{aA}	48.9 ^{aA}	50.4 ^{bA}	52.2 ^{cA}	0.540	0.007	0.8061	0.835	0.041	0.028	0.971
	28	48.1 ^{aA}	51.3 ^{bB}	52.1 ^{bA}	54.5 ^{cB}	0.682						
	56	52.8 ^{aB}	55.2 ^{aC}	61.0 ^{bB}	60.6 ^{bC}	1.342						
A/P	14	4.20 ^A	4.08	4.40	4.30	0.052	0.431	0.961	0.404	< 0.001	0.014	0.144
	28	4.55 ^A	4.62	4.70	4.58	0.054						
	56	5.30 ^{cB}	4.67 ^b	4.79 ^b	4.27 ^a	0.093						
NH ₃ -N (mg/L)	14	20.9 ^A	22.0 ^A	21.6 ^A	21.7 ^A	0.230	0.543	0.885	0.803	< 0.001	< 0.001	0.041
	28	24.6 ^{aB}	24.4 ^{aB}	28.4 ^{bB}	29.1 ^{bB}	0.540						
	56	31.5 ^{aC}	33.0 ^{bC}	35.9 ^{bC}	34.3 ^{bC}	0.480						
a-c, means within a row followed by different lower-case letters differ significantly (P < 0.05). A-C, means within a column followed by different capital letters differ significantly (P < 0.05).												
¹ TVFA = total volatile fatty acids; A/P = Acetate/Propionate; NH ₃ -N = ammonia-N.												
² A ₀ contains 0 g/kg AMT in diet (dry matter basis); A ₂ contains 20 g/kg AMT in diet (dry matter basis); A ₅ contains 50 g/kg AMT in diet (dry matter basis); A ₈ contains 80 g/kg AMT in diet (dry matter basis).												
³ L = Linear response of AMT; Q = Quadratic response of AMT; C = Compound response of AMT.												

Effect of AMT on serum immune factors

The serum concentrations of IgA (P = 0.016) and IgG (P = 0.045) were higher in A₅ and A₈ than in A₀ and A₂ on day 56 (Table 4). In addition, serum concentrations of IgA (P = 0.004), IgG (P = 0.006) and IgM (P = 0.020) in the AMT fed lambs were higher on day 56

than on 14. The interaction between treatment x day was significant for IgA ($P = 0.028$) and IgM ($P = 0.014$). The serum concentration of IL-2 (groups A_2 , A_5 and A_8) was higher in the AMT fed lambs than in controls, and the treatment x day interaction was significant ($P = 0.029$), with the highest concentration in group A_8 ($P = 0.014$). With days, serum IL-2 concentration decreased in all groups ($P = 0.029$), except for the A_8 group ($P = 0.146$), and IL-4 concentration increased in A_0 , A_2 and A_5 groups ($P = 0.022$), but not in the A_8 group ($P = 0.273$), and increased linearly ($P < 0.034$) with an increase in AMT. There was no significant treatment effect on serum IL-6 concentration ($P = 0.093$), but its concentration was significantly lower on day 56 than on days 14 and 28 in all four groups ($P = 0.032$); while, in contrast, the highest IL-10 concentration occurred on day 56 in all four groups ($P = 0.028$). No significant treatment of sampling date was found for serum sCD14 concentration ($P = 0.366$). The concentration of serum TNF- α in A_8 group was higher ($P = 0.010$) than in the other three groups on days 14, 28 and 56, was lower ($P = 0.044$) on day 56 than on day 14 and 28 in the four treatments and increased linearly ($P < 0.047$) with an increase in AMT.

Table 4

The effect of Astragalus membranaceus root (AMT) on serum immune factors on days 14, 28 and 56 in Tibetan sheep

Items ¹	Day	Treatment ²				SEM	P-value ³					
		A ₀	A ₂	A ₅	A ₈		L	Q	C	Day (D)	Treatment (T)	T × D
IgA (g/L)	14	0.60	0.55 ^A	0.53 ^A	0.60 ^A	0.017	0.258	0.212	0.564	0.004	0.016	0.028
	28	0.65	0.56 ^A	0.66 ^B	0.66 ^A	0.023						
	56	0.62 ^a	0.62a ^B	0.68b ^B	0.76c ^B	0.018						
IgG (g/L)	14	14.52	14.07 ^A	14.46 ^A	14.90 ^A	0.377	0.144	0.686	0.373	0.006	0.045	0.087
	28	14.70	13.15 ^A	16.50 ^B	15.88 ^A	0.632						
	56	15.66 ^a	15.98 ^{aB}	18.99 ^{bC}	20.41 ^{bB}	0.762						
IgM (g/L)	14	0.87	0.80 ^A	0.85 ^A	0.84 ^A	0.036	0.359	0.183	0.114	0.020	0.366	0.014
	28	0.79	0.79 ^A	1.26 ^B	0.78 ^A	0.069						
	56	0.85	1.01 ^B	1.19 ^B	1.04 ^B	0.072						
IL-2 (pg/mL)	14	295 ^C	286 ^B	290 ^C	287	21.85	0.667	0.346	0.213	0.029	0.014	0.029
	28	274 ^B	285 ^B	273 ^B	281	12.6						
	56	236 ^{aA}	258 ^{bA}	259 ^{bA}	300 ^c	16.3						
IL-4 (pg/mL)	14	3.97 ^A	6.45 ^A	6.08 ^A	9.05	0.754	0.034	0.759	0.501	0.022	0.067	0.617
	28	5.32 ^B	7.12 ^A	7.27 ^B	9.40	0.533						
	56	9.58 ^C	9.31 ^B	9.12 ^C	10.04	0.212						
IL-6 (pg/mL)	14	145 ^B	142 ^B	143 ^B	146 ^B	1.60	0.852	0.858	0.966	0.032	0.093	0.099
	28	147 ^B	146 ^B	145 ^B	147 ^B	3.0						
	56	128 ^A	127 ^A	124 ^A	122 ^A	1.07						
IL-10 (pg/mL)	14	11.26 ^{aA}	12.36 ^{aA}	12.25 ^{aA}	18.49 ^{bB}	1.156	0.431	0.643	0.856	0.028	0.035	0.724
	28	10.58 ^{aA}	10.27 ^{aA}	10.79 ^{aA}	14.04 ^{bA}	0.556						
	56	23.15 ^{aB}	23.36 ^{aB}	23.83 ^{aB}	26.92 ^{bC}	0.586						
sCD14 (ng/mL)	14	1.49	1.48	1.39	1.34	0.045	0.915	0.733	0.817	0.366	0.518	0.652
	28	1.66	1.59	1.61	1.62	0.034						
a-c, means within a row followed by different lower-case letters differ significantly (P < 0.05). A-C, means within a column followed by different capital letters differ significantly (P < 0.05).												
¹ Ig = immunoglobulin; IL = interleukin; sCD14 = soluble CD14; TNF-α = tumour necrosis factor-α.												
² A ₀ contains 0 g/kg AMT in diet (dry matter basis); A ₂ contains 20 g/kg AMT in diet (dry matter basis); A ₅ contains 50 g/kg AMT in diet (dry matter basis); A ₈ contains 80 g/kg AMT in diet (dry matter basis).												
³ L = Linear response of AMT; Q = Quadratic response of AMT; C = Compound response of AMT.												

	56	1.3	1.37	1.37	1.55	0.039						
TNF- α (pg/mL)	14	75.9 ^{aB}	74.7 ^{aB}	75.5 ^{aB}	82.1 ^{bB}	1.659	0.047	0.740	0.617	0.044	0.010	0.117
	28	71.4 ^{aB}	80.9 ^{bC}	79.6 ^{bB}	86.2 ^{cC}	1.823						
	56	64.2 ^{aA}	66.2 ^{aA}	65.7 ^{aA}	71.2 ^{bA}	1.230						
a-c, means within a row followed by different lower-case letters differ significantly (P < 0.05). A-C, means within a column followed by different capital letters differ significantly (P < 0.05).												
¹ Ig = immunoglobulin; IL = interleukin; sCD14 = soluble CD14; TNF- α = tumour necrosis factor- α .												
² A ₀ contains 0 g/kg AMT in diet (dry matter basis); A ₂ contains 20 g/kg AMT in diet (dry matter basis); A ₅ contains 50 g/kg AMT in diet (dry matter basis); A ₈ contains 80 g/kg AMT in diet (dry matter basis).												
³ L = Linear response of AMT; Q = Quadratic response of AMT; C = Compound response of AMT.												

Effect of AMT on serum hormones and antioxidant properties

AMT fed to sheep did not show an effect on serum INS (P = 0.06) and SOD (P = 0.997) concentrations (Table 5). The concentration of serum GH was significantly higher (P = 0.015) in the A₈ group than the other three groups on days 28 and 56. The concentrations of T-AOC in the A₅ and A₈ groups were higher than in the A₀ and A₂ groups on day 56. Concentrations of SOD did not differ among treatments (P = 0.997), but the interaction between treatment x day was significant (P < 0.010), Serum concentrations of INS (P = 0.011), GH (P = 0.049), T-AOC (P = 0.030) and SOD (P < 0.001) increased with time in all four groups.

Table 5

The effect of *Astragalus membranaceus* root (AMT) supplementation on serum hormones and antioxidant factors on days 14, 28 and 56 in Tibetan sheep

Items ¹	Day	Treatment ²				SEM	P-value ³					
		A ₀	A ₂	A ₅	A ₈		L	Q	C	Day (D)	Treatment (T)	T × D
INS (uIU/mL)	14	8.64 ^A	11.24 ^A	9.94 ^A	9.35 ^A	0.440	0.805	0.707	0.512	0.011	0.060	0.099
	28	10.61 ^A	15.62 ^B	12.95 ^B	13.92 ^B	0.806						
	56	17.05 ^B	16.07 ^B	15.13 ^C	16.74 ^C	0.340						
GH (ng/mL)	14	3.49 ^A	3.90 ^B	3.90 ^{AB}	3.73 ^A	0.135	0.051	0.219	0.512	0.049	0.015	0.050
	28	3.69 ^{aA}	3.77 ^{aA}	3.68 ^{aA}	5.49 ^{bB}	0.286						
	56	4.39 ^{aB}	4.24 ^{aC}	4.25 ^{aB}	6.22 ^{bC}	0.255						
T-AOC (U/mL)	14	6.68 ^A	6.84 ^A	7.64 ^B	7.44 ^A	0.204	0.345	0.598	0.757	0.030	0.227	0.138
	28	8.04 ^B	7.11 ^A	7.19 ^A	9.14 ^B	0.352						
	56	8.78 ^B	8.86 ^B	9.62 ^C	9.32 ^B	0.110						
SOD (U/mL)	14	68.9 ^A	73.6 ^A	76.8 ^A	66.1 ^A	2.506	0.792	0.592	0.969	< 0.001	0.997	0.010
	28	76.6 ^B	83.9 ^B	83.6 ^B	86.1 ^B	3.052						
	56	94.0 ^C	95.9 ^C	96.5 ^C	94.8 ^C	1.654						
a-c, means within a row followed by different lower-case letters differ significantly (P < 0.05). A-C, means within a column followed by different capital letters differ significantly (P < 0.05).												
¹ INS = insulin; GH = growth hormone; T-AOC = total antioxidant capacity; SOD = superoxide dismutase.												
² A ₀ contains 0 g/kg AMT in diet (dry matter basis); A ₂ contains 20 g/kg AMT in diet (dry matter basis); A ₅ contains 50 g/kg AMT in diet (dry matter basis); A ₈ contains 80 g/kg AMT in diet (dry matter basis).												
³ L = Linear response of AMT; Q = Quadratic response of AMT; C = Compound response of AMT.												

Discussion

Effect of AMT on dry matter intake and average daily gain

Supplementation of AMT in the current study resulted in a higher ADG, a better feed conversion and an increase in DMI in the Tibetan sheep, which is in contrast with the findings of Zhong et al. (2012) [18], who reported no change in DMI, ADG and feed conversion in Ujumqin male lambs receiving AMT at 50 g/kg DMI for 30 days. The increase in DMI in the present study did not increase linearly with an increase in AMT, but increased only at 80 g/kg AMT, while ADG and feed conversion improved linearly with an increase in AMT intake. These different patterns illustrated the importance of testing different levels of AMT on the responses of sheep. Our results, however, were consistent with reports in monogastrics in which ADG and feed conversion in early weaned piglets [12] and ADG and DMI in chickens [19] increased with AMT. In these studies, the improvements were attributed mainly to astragalus polysaccharide, which ranged between 2.4 and 9.6 mg/kg AMI in the three groups of the present study. It was reported that astragalus polysaccharide accelerates muscle growth and increases carcass weight through the expressions of related genes and proteins [20]. In addition, an in vitro study with rumen fluid showed that supplementary AMT can improve feed digestibility in steers [3].

Effect of AMT supplementation on rumen fermentation

Optimal fibrolytic bacteria growth was found to be at a pH between 6.2 and 7.2 [21] and its activity was inhibited at a pH below 6.0 [22]. Although the pH of the rumen fluid decreased with the addition of AMT, the pH of all groups fell within the optimal range for sheep. The pH was negatively correlated with the concentration of TVFA, as was reported previously for ruminants [23].

The TVFA produced in the rumen provides about 75 percent of the ruminants' energy [24]. In the current study, the concentration of rumen TVFA and propionic and acetic acids increased with AMT supplementation, indicating that AMT enhanced rumen fermentation. The increase in propionic acid was linear with an increase in AMT, but acetic acid increased with time but did not show a pattern of increase with an increase in AMT and as a result, the A:P ratio was reduced with AMT supplement. Similar results were shown in an in vitro study by Deng et al. (2007) who reported that AMT extract stimulated ruminal fermentation and modified the pattern of rumen fermentation by increasing rumen TVFA production and the molar proportion of propionate [3]. Zhong et al. (2012) also reported an increase in propionic acid, and as a consequence, a reduction in the A:P ratio, with supplementary AMT at 50 g/kg DMI [18].

Francis et al. (2002) [25] reported that saponins in plants have an inhibitory effect on rumen protozoa, while Wallace et al. (1994) [26] demonstrated that saponins have antimicrobial properties that can inhibit the activity of bacteria, which are normally acetate producers. Consequently, saponin or saponin-like substances in AMT may inhibit the activity of rumen protozoa and bacteria, resulting in an accumulation of propionate and reduction in the molar proportion of acetate in the rumen [3], which could explain the decrease in the A:P ratio in the Tibetan sheep with the addition of AMT in the current study. In addition, a number of plant extracts and secondary plant metabolites were shown to improve rumen fermentation characteristics by influencing the composition and activity of ruminal microorganisms [27].

NH₃-N is an intermediate product of protein metabolism and one of the main components for microbial protein synthesis in the rumen [26]. The increase of rumen NH₃-N concentration with the addition of AMT in this study was also reported in a previous report by Zhong et al. (2012) [18], which indicated that AMT can play a role in promoting dietary protein degradation and microbial protein synthesis.

Effect of AMT supplementation on immune factors in the serum

A number of herbs are immunologically active, functioning as excellent immuno-modulating agents. Astragalus polysaccharides and saponins stimulate macrophages and B cell activation [28], promote antibody formation, activate complement and increase T lymphocyte proliferation [29], while astragaloside increases T, B lymphocyte proliferation and promotes antibody production [30]. Bioactive fractions, isolated from AMT, were found to be the most potent with respect to its mitogenicity on murine splenocytes [8]. The addition of 50 and 80 g/kg DMI AMT in this study showed the strongest effect in increasing serum IgA, IgG and IgM concentrations, especially toward the latter stages of the study; IgA, IgG and IgM are important indicators of humoral immunity. Immunoglobulin is an antibody-active animal protein secreted by plasma cells and plays an important role in both specific and non-specific immunity [31, 32]. Immune enhancement was also observed in lambs with a supplementation of 50 g/kg DMI AMT [18].

Interleukin (IL) plays an important role in transmitting information, activating and regulating immune cells and mediating T and B cell activation, proliferation and differentiation. In the present study, the concentration of serum interleukin (IL-2, IL-4, IL-10) generally increased with an increase in the level of AMT. The increase in IL-4 was linear with an increase in AMT, whereas a definite pattern of increase was not detected for IL-2 and IL-10. A study by Hou et al. (2015) showed that AMT increased the percentage of IL-4-expressing T helper (Th) cells in the blood and prevented the decline in IL-6 mRNA expressions in spleen of mice [33]. The cytokine IL-2 is produced by activated T cells, and stimulates both the innate and acquired immune responses, including the release of secondary cytokines such as TNF, IL-1 and IL-6 [34]. IL-4 and IL-10 enhances the B-cell response and alternative activation of macrophages to promote tissue repair [35]; whereas, IL-6 functions as a differentiation factor on B cells and an activation factor on T cells [36]. Kurashige et al. (1999) observed that AMT protects against the reduction of IL-2 production in mice lymphocytes treated with a carcinogen [37]. Astragalus polysaccharides have important immuno-stimulating effects by promoting IL-2 production and splenocyte proliferation in vitro [38], while saponins have been shown to have high IL-2 inducing activity [34].

Serum TNF- α , which plays an important role in antigen enhancing and inflammatory regulation of macrophages [11], increased in concentration linearly with an increase in AMT consumed. TNF- α is induced mainly by activated peritoneal macrophages, which are important cells of the innate immune response that can eliminate invading microorganisms through phagocytosis [18, 39]. In vitro studies reported an increased production of TNF- α by peritoneal macrophages of C3H/HeN mice when cells were cultured with an

aqueous extract of AMT [40]. Bedir et al. (2000) [41] found that astragaloside enhanced mRNA expression of IL-1 β and TNF- α , while Wei et al. (2016) [42] reported that astragalus polysaccharide increased gene expression and production of NO, TNF- α , and IL-6 in RAW264.7 cells. Consequently, the current results demonstrated that adding AMT to Tibetan sheep diet can booster the immune system as it increases serum immunoglobulins and regulates the secretion of a number of cytokines.

Effect of AMT on serum hormones and antioxidant properties

In the present study, the addition of 80 g/kg DMI AMT increased serum GH concentration in the sheep, as was also observed in broilers offered AMT [43]. However, this response was not found in lambs fed AMT at 50 g/kg DMI [18]. Growth hormone (GH) promotes metabolism and growth by regulating the secretion of insulin-like growth factor to stimulate the entry of amino acids into cells and to accelerate the synthesis of DNA and RNA, thereby promoting the synthesis of proteins [44].

Oxidative stress is believed to be a primary factor contributing to animal diseases [45]. Astragalus polysaccharides have been shown to enhance antioxidant status and to scavenge free radicals [46] and at a dietary concentration of 5 g/kg DMI enhanced serum antioxidant enzymatic activity and improved antioxidant status of broilers [47]. In addition, flavonoids and saponins extracted from AMT possessed antioxidative activities [48]. The main parameters for assessing oxidative status in animals are T-AOC and T-SOD [49]. In this study, the addition of 80 g/kg DM AMT increased T-AOC concentration on day 56. In other studies, the addition of 50 g/kg AMT to lamb diet [18] and 10 g/kg AMT to broiler diet [50] increased liver T-AOC concentration. The addition of AMT did not affect SOD in the present study but there was an increase in concentration with time for every treatment.

Conclusions

This study demonstrated that supplementary AMT increased DMI and improved ADG and feed conversion rates in Tibetan sheep. AMT had beneficial effects on rumen fermentation and improved the immunity and antioxidant activities of Tibetan sheep. Some responses were dose dependent and increased with an increase in AMT, while other responses increased without a pattern. Based on the present study, we concluded that best results for Tibetan sheep could be obtained at 50 g/kg to 80 g/kg DMI of AMT.

Abbreviations

AMT: Astragalus membranaceus root; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; GE: gross energy; BW: body weight; DMI: dry matter intake; ADG: average daily gain; F:G ratio: feed:gain ratio; TVFA: total volatile fatty acids; A/P: Acetate/Propionate; NH₃-N: ammonia-N; Ig: immunoglobulin; IL: interleukin; sCD14: soluble CD14; TNF- α : tumour necrosis factor- α ; INS: insulin; GH: growth hormone; T-AOC: total antioxidant capacity; SOD: superoxide dismutase

Declarations

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Authors' contributions

LD conceived and designed the experiment. XW and HW performed the experiment; XW, HW, CJ, QY, CH GJ and YZ collected samples; XW performed laboratory analysis and analyzed the data; XW and LD wrote the manuscript; and ZH and AAD revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed are available from the corresponding author on request.

Ethics approval and consent to participate

The research protocol was approved by the Animal Ethics committee of Lanzhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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