

Effect of fenugreek seeds supplementation on growth performance, digestion coefficient, rumen fermentation and some blood metabolites of Awassi lambs

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Abstract

Twelve Awassi lambs with 5-6 month average 25.13 ± 0.33 kg body weight (BW) were used to investigate the effect of adding graded levels of fenugreek seeds (FS) to the diet on growth performance, nutrient digestibility, rumen fermentation, and some blood metabolites. The lambs were randomly assigned to four treatments (3 lambs per treatment) in a completely randomized design. Treatment diets were control diet without FS 0 g/head/day (FS₀), control diet plus 2.5gFS/head/day (FS₁), control diet plus 5gFS/head/day (FS₂), and control diet plus 7.5gFS/head/day (FS₃). Lambs were housed in individual pens and received 600g of concentrate diet once a day with *ad libitum* choice of rice straw as roughage. Body weight and feed intake were recorded. At the day 42, the digestibility trial was performed for all lambs with same diets. Rumen liquor and blood samples were withdrawn from all the lambs at the last day of digestibility trial. Results showed that increasing level of FS did not improve ($P > 0.05$) feed intake, live weight gain (LWG) and feed conversion ratio (FCR). Fenugreek seeds addition had no effect ($P > 0.05$) on diet digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Results also showed that fenugreek seeds administration did not affect ($P > 0.05$) ruminal pH, ammonia-nitrogen (NH₃-N) and total volatile fatty acids (TVFA's) concentration. The NH₃-N and TVFA's concentrations were tended ($P > 0.05$) to decrease as levels of FS increased in the diet. Serum glucose (SG), serum total protein (STP) and serum urea nitrogen (SUN) were not affected also by increasing levels of FS.

It can be concluded; supplementation of fenugreek seeds in the diets of fattening Awassi lambs did not improve growth performance, nutrient digestibility and ruminal fermentation without adverse effects on blood metabolites.

Keywords: Fenugreek seeds, growth performance, digestibility, rumen fermentation, blood metabolite, Awassi lambs

تأثير اضافة بذور الحلبة على الاداء الانتاجي، الهضم، تخمرات الكرش وبعض معايير الدم لدى الحملان العواسية

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المستخلص

تم استخدام اثني عشر حملا عواسيا بعمر 5-6 شهر؛ وبمعدل وزن جسم 25.13 ± 0.33 كغم لبيان تأثير إضافة مستويات تصاعديّة من بذور الحلبة في الزيادة الوزنيّة، معامل هضم العناصر الغذائيّة، متغيرات التخمر في الكرش وبعض صفات الدم. وتم توزيع الحملان عشوائيا على اربعة معاملات (3 حملان لكل معاملة) في تصميم تام التعشبية، وكانت العلائق عليقة قياسية بدون اي اضافات (FS_0)، عليقة قياسية مع اضافة 2.5 غم حلبة /راس/يوم (FS_1)، عليقة قياسية مع اضافة 5 غم حلبة /راس/يوم (FS_2)، عليقة قياسية مع اضافة 7.5 غم حلبة /راس/يوم (FS_3). ووضعت الحملان في اقفاص فردية وغذيت لمرة واحدة يوميا على 600غم من المركز وعلى تبن الرز المقطع بشكل حر. وتم تسجيل وزن الجسم والمنتاول من العلف وفي اليوم 42 تم اجراء تجربة هضم على جميع الحملان وباستخدام نفس العلائق، كما تم سحب عينات من سائل الكرش والدم في نهاية تجربة الهضم. اظهرت النتائج بان زيادة مستوى المعاملة ببذور الحلبة لم يؤدي الى حصول زيادة معنوية ($P > 0.05$) في المنتاول من العلف، الزيادة الوزنيّة او كفاءة التحويل الغذائي. كما ان معاملات الهضم للمادة الجافة والعضوية والبروتين الخام ومستخلص الالياف المتعادل ومستخلص الالياف الحامضي لم تتاثر معنويا ($P > 0.05$) بزيادة مستوى الاضافة ببذور الحلبة. كما ان الاس الهيدروجيني وتركيز نيتروجين الامونيا والاحماض الدهنية الطيارة في سائل لم تتاثر معنويا ($P > 0.05$) بزيادة مستوى الاضافة ببذور الحلبة، كما ان زيادة مستوى المعاملة ببذور الحلبة لم يؤدي الى حدوث تغير في تركيز الكلوكوز والبروتين الكلي ونيتروجين اليوريا مصد الدم. انه يمكن الاستنتاج بان اضافة بذور الحلبة الى علائق تسمين الحملان العواسية لم يؤدي الى تحسين الزيادة الوزنيّة، معامل الهضم للعناصر الغذائيّة و متغيرات التخمر في الكرش وليس لها تأثير سلبي على

الدم

معايير
الكلمات المفتاحية: بذور الحلبة، الاداء الانتاجي، الهضم، تخمرات الكرش، معايير الدم، حملان العواسي

Introduction:

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume, is extensively cultivated in most regions of the world for its medicinal value and it is one of the oldest known medicinal plant from ancient times (1). During last decade, great attentions towards using of medicinal plants as natural feed additive for animal nutrition not only public concern about the potential of growth promoters and antibiotic residue in meat and milk (2), but these natural feed additive contain many of secondary components such as galactomannans, diosgenin and free amino acid which were good sources of well-known medicinal values to both humans and animals (3,4). Fenugreek seeds contain a substantial amount of phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamins A, B1, B2, C, nicotinic acid, niacin, and many other functional elements as recently reviewed by Ahmed *et al.* (5).

Fenugreek seeds contain 10, 3.8, 27.3g/kg of total phenol, total tannin and saponins, respectively (6). Numerous studies focus on the positive effect of FS on enhancing lactation performance of ewes and doses (7, 8, 9). However, few studies have been conducted to ascertain the effect of FS on growth performance (10, 11, 12), rumen fermentation and nutrient digestibility (13) in lambs. Therefore, the objective of the present study was to investigate the effect of graded levels of FS addition on growth performance, nutrient digestibility, rumen fermentation and some blood metabolites of Awassi lambs.

Materials and Methods:

The study was conducted at Ruminant Animal Farm belong to Department of Animal Production, Faculty of Agriculture, University of Kufa, Al-Najaf, Iraq.

Animal, feeding and management

Twelve Awassi male lambs of 5-6 months of age with an average live initial body weight (BW) of 25.13 ± 0.33 kg were randomly assigned to four dietary treatments (3 lambs per treatment) in a completely randomized design. Lambs were housed in individual pens (1.5m \times 2m) and fed a standard diet consisting of concentrate and roughage. The experimental animals were received 600g concentrate diet once a day at 8.30 a.m. with *ad libitum* choice of chopped rice straw as roughage. Fresh drinking water was available all the time. Treatment diets were FS₀=Fenugreek seeds 0 g/head/day, FS₁=Fenugreek seeds 2.5 g/head/day, FS₂=Fenugreek seeds 5 g/head/day, FS₃=Fenugreek seeds 7.5 g/head/day. Fenugreek seeds were added to the concentrate diet and mixing daily just before feeding.

Feeding trail

An adaptation period of 7 days was allowed prior to the experimental period started. Feed offered and refused were recorded daily to measure feed intake and sampled weekly and stored for subsequent analysis. Lamb weights were recorded weekly before the morning feeding. Then, daily live weight gain (DLWG) was calculated by subtraction the initial BW (IBW) from the final BW (FBW) and then divided by the duration of the experiment (42 days).

Digestibility trail

Digestibility trial was conducted at the end of feeding trial with same lambs and diets. Lambs were fitted with facial collection bags for 2 days as an adaptation period followed by 5 days for feces collection during which daily feed intake and refusal feed of each lamb was recorded before morning feeding. Representative samples (10%) of feces were collected over conclusive days, kept at -18°C. On the last day of the collection period, the composite feces samples were thawed and thoroughly mixed and subsamples of fecal were dried in

an oven at 60°C for 72 h and ground in a Wiley mill, to pass a 1 mm sieve screen and kept in airtight plastic bags pending analysis. Samples of feed offered and feed refused were also collected every day and subsampled at the end of digestibility trail.

Rumen fermentation characteristics

At the last day of digestibility trail, rumen liquor samples were withdrawn using stomach tube from all lambs before morning feeding (0 h), 3 and 6 h post feeding. Rumenal pH was measured immediately after obtaining sample using digital portable pH meter (HANNA instrument). Rumen liquor samples were stained through four layers of cheesecloth and preserved by adding of 2 ml of 0.2N HCl and kept frozen for later analysis. Ammonia-N (NH₃-N) concentration (mg/100ml) was determined following the procedure of AOAC (14), while total volatile fatty acids (TVFA's) concentration (Mm) was assayed according to procedure of Warner (15).

Blood sampling and Sera harvesting

Blood samples were withdrawn by the jugular vein puncture from all the lambs at the last day of digestibility trail (2h post feeding) in Vacutainer[®] tubes without anticoagulant allowed to clot for 2h before centrifugation at 3000 rpm for 20 minutes. Sera were harvested, labeled and stored at -18°C till biochemical analysis. Serum glucose (SG), serum total protein (STP), and serum urea nitrogen (SUN) concentrations were determined by an automated biochemical analyzer using the commercial kits (Biomagrib, Tunisia) according to the manufacture's instruction based on the methods of Trinder (16) Henry *et al.*(17), Patton and Crouch (18), for SC, STP and SUN respectively

Chemical analysis and calculations

Ingredient of the concentrate diet and Chemical analysis of concentrate diet, rice straw and fenugreek seeds used in this study are shown in Table 1. Fenugreek seeds (*Trigonella-foenum-graecum L.*), chopped

rice straw and concentrate feed ingredients were purchased from the local market in Al-Najaf. Feed and fecal samples were ground in a Wiley mill through a 1 mm screen and analysis for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and Ash according to standard methods of AOAC (14), Nitrogen free extract (NFE) was calculated by difference [NFE= OM-(CF + CP+ EE)]. Neutral detergent fiber (NDF)

and Acid detergent fiber (ADF) were completed according to Van Soest *et al.* (19) without sodium sulfate and α amylase for NDF analysis. Both NDF and ADF were expressed without residual ash. Metabolizable energy (ME) was calculated following the equation described by MAFF (20): $ME (MJ/kg DM) = 0.012CP + 0.031EE + 0.005CF + 0.014NFE$.

Table1. The ingredient and chemical composition of concentrate diet, rice straw and fenugreek seed used in this experiment on DM %basis

Item	Concentrate diet	Rice straw	Fenugreek seeds
Ingredient %			
Barley grain	44		
Corn grain	8		
Soybean meal	10		
Wheat bran	36		
Limestone	1		
Salt	1		
Chemical composition %			
Dry Matter (DM)	91.73	94.91	93.32
Organic Matter (OM)	94.18	90.08	96.37
Crude protein (CP)	15.35	2.86	26.64
Crude Fiber (CF)	7.88	35.06	8.42
Ether Extract (EE)	2.99	1.30	7.79
Nitrogen Free Extract (NFE)	67.96	50.86	53.52
Neutral Detergent Fiber (NDF)	31.94	72.92	35.45
Acid Detergent Fiber (ADF)	9.56	40.49	16.49
ME(MJ/Kg DM)*	12.68	9.62	13.53

* Metablizable energy (ME) $ME (MJ/kg DM) = 0.012CP + 0.031EE + 0.005CF + 0.014NFE$

Statistical Analysis:

Data were statistically analyzed as a completely randomized design (CRD) using ANOVA procedures of the statistical analysis system SAS (21) with the following model: $Y_{ij} = \mu + L_i + e_{ij}$, Where: Y_{ij} = the observation of the parameter; μ = overall means; L_i = is effect of the graded level of FS; e_{ij} = the random error. Significant

differences among means were detected using Duncan's multiple range test (22). Data were presented as mean \pm standard error (SE).

Results

Growth performance and Feed intake

Statistical analysis revealed that final body weight (FBW), total body weight gain

(TBWG), daily body weight gain (DBWG) and feed conversion ratio (FCR) of Awassi lambs were not significantly ($P > 0.05$) affected by increasing levels of FS supplemented to the diet (Table 2). No

significant differences ($P > 0.05$) were observed in DM, OM, CP and ME intakes for lambs fed diet supplemented with graded levels of FS (Table 2).

Table 2. Growth performance and feed intake of Awassi lambs fed graded levels of FS (Means±SE)

Item	Fenugreek seeds dose (g/head/day)				Sign.
	FS ₀ (0)	FS ₁ (2.5)	FS ₂ (5)	FS ₃ (7.5)	
Performance					
IBW(kg)	25.48±0.68	24.47±0.57	24.5±1.30	25.06±0.82	NS
FBW(kg)	31.18±0.66	30.19±0.42	30.03±1.28	30.58±1.13	NS
TBWG (kg)	5.70±0.16	5.73±0.34	5.53±0.14	5.52±0.33	NS
DBWG (g)	135.79±1.75	136.35±2.16	131.75±1.25	131.43±1.40	NS
FCR(g/ g)	9.32±0.78	9.34±0.65	9.22±0.25	9.57±0.72	NS
Feed intake					
DM (g/day)	1263.56±17.03	1265.32±13.35	1212.95±10.27	1246.21±13.44	NS
OM (g/day)	1085.23±14.63	1085.14±20.06	1041.41±9.07	1030.08±11.54	NS
CP (g/day)	142.27±1.92	142.91±2.64	139.96±1.18	140.15±1.51	NS
ME(MJ/day)	12.72±0.18	12.73±0.25	12.16±0.11	12.53±0.15	NS

None of the difference reached significant levels, NS=Non-significant; SE=Standard error; IBW=Initial body weight; FBW=Final body weight; TBWG= Total body weight gain; DBWG= Daily body weight gain, FCR= Feed conversion ratio.

FS₀=Fenugreek seeds 0 g/head/day, FS₁=Fenugreek seeds 2.5 g/head/day, FS₂ =Fenugreek seeds 5 g/head/day, FS₃=Fenugreek seeds 7.5 g/head/day

Nutrient digestibility

The apparent nutrients digestibility of diet in Awassi lambs fed graded levels of FS are shown in Table 3. The digestibility of DM, OM, CP, NDF and ADF were not affected significantly ($P > 0.05$) by increasing levels of FS. There is a slight decrease ($P > 0.05$) in NDF and ADF digestibility as levels of FS increased in the diet.

Table 3. Nutrient digestibility of Awassi lambs fed graded levels of FS (Means±SE)

Digestibility %	Fenugreek seeds dose (g/head/day)				Sign
	FS ₀ (0)	FS ₁ (2.5)	FS ₂ (5)	FS ₃ (7.5)	
DM	70.69±2.92	70.92±2.02	72.79±2.57	71.86±3.85	NS
OM	73.50±3.03	72.85±1.79	74.05±1.83	72.90±4.46	NS
CP	76.36±2.22	76.10±1.42	76.27±1.15	75.28±2.42	NS
NDF	66.66±0.98	64.23±1.50	63.18±1.38	62.34±1.57	NS
ADF	45.23±2.38	43.48±1.76	42.94±0.98	41.71±0.76	NS

None of the difference reached significant levels NS = non-significant; SE=Standard error; DM=Dry matter, OM=Organic matter; CP=Crude protein; NDF= Neutral detergent fiber; ADF= Acid detergent fiber.

FS₀=Fenugreek seeds 0 g/head/day, FS₁=Fenugreek seeds 2.5 g/head/day, FS₂ =Fenugreek seeds 5 g/head/day, FS₃=Fenugreek seeds 7.5 g/head/day

Rumen fermentation characteristics

Mean value of Ruminal pH, NH₃-N and TVFA’s concentrations in the rumen liquor of Awassi lambs fed graded levels of FS are shown in Table 4 and Figures 1, 2, and 3. The lowest pH value and peak TVFA’s concentrations were recorded at 3h post-feeding ,while NH₃-N concentrations reached peak at 3h post-feeding and then decreased at 6h post feeding for all diets. Regards effect of graded levels of FS supplementation, the concentrations of NH₃-N, TVFA’s and ruminal liquor pH were similar among treatments (P>0.05). Data also showed that NH₃-N and TVFA’s concentrations were tended (P>0.05) to decrease as levels of FS increased.

Table 4. Rumen fermentation characteristics of Awassi lambs fed graded levels of fenugreek seeds (Means±SE)

Rumen fermentation	Fenugreek seeds dose (g/head/day)				Sign.
	FS ₀ (0)	FS ₁ (2.5)	FS ₂ (5)	FS ₃ (7.5)	
Ruminal pH	6.23±0.17	6.29±0.20	6.29±0.07	6.56±0.08	NS
NH ₃ -N (mg/100ml)	18.88±0.40	18.52±2.19	18.25±1.23	18.16±1.17	NS
TVFA’s (Mm)	117.74±0.53	117.59±1.41	116.65±0.51	114.12±0.33	NS

SE=standard error; NS=No-significant; NH₃-N= Ammonia nitrogen; TVFA’s= Total volatile fatty acids.

FS₀=Fenugreek seeds 0 g/head/day, FS₁=Fenugreek seeds 2.5 g/head/day, FS₂ =Fenugreek seeds 5 g/head/day, FS₃=Fenugreek seeds 7.5 g/head/day

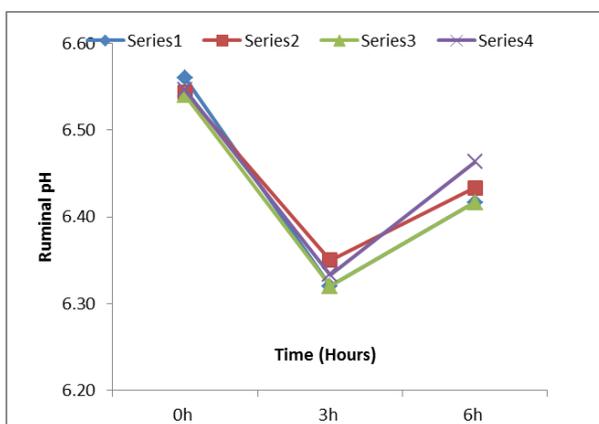


Figure 1. Diurnal pattern of Ruminal pH

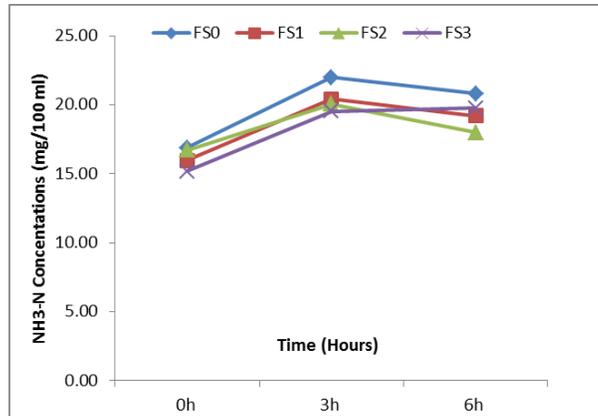


Figure 1. Diurnal pattern of NH3-N concentration

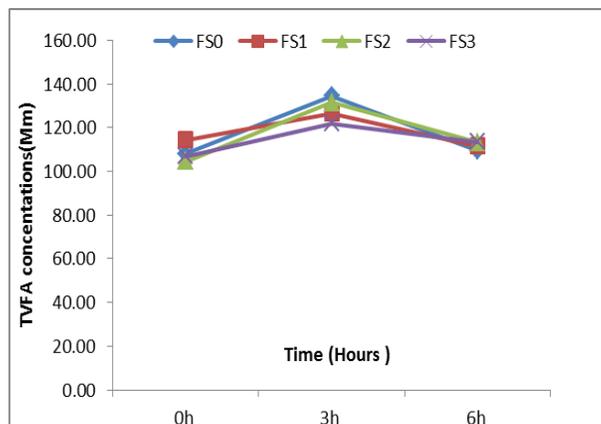


Figure 1. Diurnal pattern of TVFA's concentration

Serum metabolites

Serum metabolites such as SG, STP and SUN concentrations of Awassi lambs fed graded levels of FS are illustrated in Table 5. Results showed no significant difference (P>0.05) among these constitutions except for SUN concentrations.

Table 5. Serum metabolites of Awassi lambs fed graded levels of fenugreek seeds (Means±SE)

Serum metabolites	Fenugreek seeds dose (g/head/day)				Sign.
	FS ₀ (0)	FS ₁ (2.5)	FS ₂ (5)	FS ₃ (7.5)	
SG(mg/100ml)	67.32±5.33	66.49±2.10	65.44±3.33	59.60±5.74	NS
STP (g/100ml)	6.95 ±0.12	7.18±0.20	7.32±0.05	7.66±0.07	NS
SUN(mg/100ml)	42.35±0.78	41.05±0.80	41.43±0.68	40.23±0.08	NS

SE = standard error; NS= non-significant;

SG= serum glucose; STP= serum total protein; SUN= serum urea nitrogen;

FS₀=Fenugreek seeds 0 g/head/day, FS₁=Fenugreek seeds 2.5 g/head/day, FS₂

=Fenugreek seeds 5 g/head/day, FS₃=Fenugreek seeds 7.5 g/head/day.

Discussion

The results obtained in the current study were agreed with results reported by Sahin *et al.* (10) who found that feed intake, body weight gain, daily gain weight and FCR were not affected when inclusion FS at 0, 2, 4 and 8% in the diet of growing Awassi lambs. Al-Isawi (11) and Dosky and Taher (12) also observed similar results in Hamadani and Karadi lambs with no different in feed intake, total gain, average daily gain and feed conversion ratio. However, some studies reported increasing gain due to the addition of FS to diet of lambs (13, 23, 24) male kids (25). In addition, Hassan *et al.* (26) reported that oral administration of 2.5 g FS/kg BW led to increases in body weight gain, while 5 g FS/kg BW did not change body weight gain of Sudanese ewes. Atta Elmnan *et al.* (27) reported that the intake of DM and CP were increased in Nubian goats fed on diet supplemented with 5, 10 and 15% of FS as compared with those fed control diet without FS. Al-Sherwany (9) reported that DMI were significantly increased in Awassi ewes fed 1.2gFS/Kg BW as compared with those fed 0 and 0.6gFS/kg BW, they attributed this improvement in feed intake due to the presence of saponins in FS which stimulate eating center in hypothalamus similar like in non ruminant animal (28). Moreover, Saleh (23) found that DM intake of Barki rams (52.6 kg) increased with 20g FS/head/day and decrease with 40g FS/head/day. However, Mir and Kumar (29) also reported that DMI and CPI were not affected by supplementation 3% FS in goats diet. Similar results reported diary goats (30). The inconsistency of these results may be due to diet composition, levels of FS in the diet, level of feeding and plan of nutrition. Feed intake could not largely be affected by dietary inclusion of saponins as reviewed by Patra and Saxena (31). In addition, Aazami *et al.* (32) found that DMI and growth performance of small ruminants did not

improve by graded levels of saponins in the diets.

The available literatures on the effect of FS supplementation on nutrients digestibility are also variable. Results of the current study revealed insignificant difference in the digestibility of DM and other nutrient (OM, CP, NDF and ADF) among diets. These results were agreed with results obtained in calves steer (33), lambs (13), and goats (30). In contrast, others reported significant increase in the digestibility of DM, OM and CP in goats (25, 27, 29). In the current study, NDF and ADF digestibility were numerically decreased as levels of FS increased in the diet of Awassi lambs. Saleh (22) noted a linear decrease of CF digestibility in sheep receiving FS (20-40gFS/head /day). Atta Elmnan *et al.* (27) obtained similar results in goats. In contrast, Abbas *et al.* (13) and Mir and Kumar (29) reported increase NDF and ADF digestibility in the diet of sheep and goats. In calves steer the administration of FS has no effect on NDF and ADF digestibility (33). A number of studies have demonstrated that saponins containing plants or saponins extracts did not affect digestibility (3, 32, 34) and the digestibility response of saponins seems to be dose dependent (31). The inconsistency of these results may be due to diet composition, levels of FS in the diet.

The finding obtained in the current study on rumen fermentations are supported by Abo-Donia *et al.* (33) who found that ruminal pH value, NH₃-N and TVFA's concentration were in beef steers fed 3% FS were similar to the value of control group. Mir and Kumar (29) also noted that FS supplementation did not affect on ruminal pH in goats. In contact, Atta Elmnan *et al.* (27) and Salama *et al.* (25) reported that increasing levels of FS in the diet of goats lead to significant increase in NH₃-N and TVFA's concentrations. Saponins, contained

in fenugreek seeds, have antimicrobial properties, and suppress the growth of ciliate protozoa, peptidase-producing bacteria, and cellulolytic bacteria in the rumen (36). The non significant reduction in $\text{NH}_3\text{-N}$ concentration obtained in the current study may be due to antiprotozoal effect of saponins in FS at their high dose (37). According to Makkar *et al.* (35) saponins alter rumen fermentation by increasing digestibility (either increase or not affect) and increase the microbial protein synthesis. In the current study the addition of FS did not affect the apparent digestibility of lambs, which might be due that FS in the current study not alert rumen fermentation.

The results concerning SG concentrations obtained in the current study are in accordance with results previously reported by several authors (8, 9, 11, 13, 22) who reported that increasing levels of FS in the diet did not affect on SG concentrations in sheep and goats. Similar results reported by Abo-Donia *et al.* (33) in calves steer. Others reported that supplemented FS led to decrease SG concentration in ruminant animal (7, 38), they attributed that to hypoglycemia effect of FS and stimulate pancreatic insulin or may be that FS stimulate pancreatic insulin secretion. The insignificant differences in STP concentrations obtained in the current study are in accordance with results reported by (9, 13, 22). Others reported that FS increased STP concentrations in ruminant (38). The non significant difference in SUN concentration in the current study are in accordance with results previously reported by several authors (13, 22), kids (29, 30) who reported that increasing levels of FS in the diet did not affect on SUN concentrations in lambs and kids. The SUN concentrations could be useful as an indicator of protein status within a group of sheep and could help to point out potential problem with a feeding regime (39). In addition, Aazami *et al.* (32) found that SG

and SUN concentration of sheep and goats did not alerted by graded levels of saponins in the diets. Moreover, most serum metabolites values estimated in the present study are within the normal ranges of healthy Awassi male lambs published by (40).

It can be concluded; supplementation of fenugreek seeds in the diets of fattening Awassi lambs did not improve growth performance, nutrient digestibility and ruminal fermentation without adverse effects on blood metabolites.

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