THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Effect of Dietary Supplementation of *Rhus coriaria* Grind Seeds and Exogenous Fibrolytic Enzymes on Some Blood Lipids and Ruminal Fermentation Parameters of Awassi Male Lambs

Lateef I Hadi*[1], Majid J Al-saadi[2]

[1]*Department of Anatomy and Histology, College of Veterinary Medicine, Thi-Qar University, Thi-Qar, Iraq,*
[2]*Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq*

***Correspondence**:
Latef.e@utq.edu.iq

Received: 10 March 2022
Accepted: 8 April 2022
Published: 28 June 2022

DOI:
https://doi.org/10.30539/ijvm.v46i1.1553

Cite:
Hadi LI, Al-saadi MJ. Effect of dietary supplementation of *Rhus coriaria* grind seeds and exogenous fibrolytic enzymes on some blood lipids and ruminal fermentation parameters of Awassi male lambs. Iraqi J. Vet. Med. 2022; 46(1): 30-38

A B S T R A C T

This study was conducted to assess the potential dietary effect of grind seeds of *Rhus coriaria* (gsRC) with and without exogenous fibrolytic enzymes (EFE) on some lipid profiles and ruminal fermentation characteristics of Awassi male lambs. A total of twenty-four Awassi male lambs weighing 21.56±0.77 kg at 3-4 months of age were randomly divided based on BW into four groups with six animals each. Dietary treatments were provided to lambs for 4 months as follows: control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet supplemented with 15 g/head dried grind seeds of *Rhus coriaria* daily with diet; EFE-5 group, fed the control group diet supplemented with 5 g/head of EFE daily with diet; gsRC-EFE group, fed on the control group diet supplemented with 15 g/headdried grind seeds of *Rhus coriaria* and 5 g/head of EFE daily with diet. The results showed that serum cholesterol and triglycerides significantly ($P<0.05$) reduced in the treated groups compared to the control group, particularly observed in 3rd and 4th months of the experiment. Comparable results were observed for lambs fed on EFE alone in the 4th month of the study. The treatment groups showed significantly ($P<0.05$) lower ruminal pH levels at the beginning, in the middle, and at the end of the experiment. At the middle and end of the study's last month, the volatile fatty acids (VFAs), ammonia nitrogen (NH_3-N), and microflora activity of the rumen were significantly ($P<0.05$) higher in the treated groups compared to the control group. It can be concluded that sumac seed powder and EFE can be used in combination or alone as an effective feed additive to improve lipid profiles and rumen fermentation parameters in Awassi male lambs.

Keywords: *Rhus coriaria*, sumac, exogenous fibrolytic enzymes, lipid profile, rumen, lamb

INTRODUCTION

Improved feed quality and livestock output are more desirable goals in animal agriculture, followed by mating selection, factual supervision, besides feed composition advancement. Food stuff effectiveness into ruminants is primarily determined by feed uniformity, rumen fermentation, and processes controlled by rumen microbial populations (1).

Some medicinal plants have antibacterial activity (2). Medical plants such as *Rhus coriaria*, also known as sumac, contain organic acids and have shown health-promoting attributes including antiviral, anti-inflammatory, antigastric, antioxidant, antibacterial, antidiarrheal, antispasmodic, astringent, antiulcer, fungicide, and lipoxygenase inhibitors due to their contents of flavones, phenolic acids as gallic acid, and tannic acid (3). Along with its antibacterial and inhibitory actions, sumac may be able

to reduce lipoprotein levels, particularly when combined with statins or other anti-hyperlipidemic medications (4). Moreover, (5) reported that 0.02% seed powder of sumac in the diet extensively elevated high-density lipoprotein (HDL) level and reduced the triglyceride, cholesterol, and low-density lipoprotein (LDL) level in broiler serum. In Japanese quail, it was reported that diet supplemented with sumac seed powder at 1.5% and 2% decreased serum cholesterol, triglycerides, and LDL levels and increased HDL (6, 7). In laying hens, (8) reported that serum levels of LDL decreased in all groups treated with sumac and ginger compared with the control group. Also, significant reduction in the systolic and diastolic blood pressure, total serum cholesterol, and LDL, was observed in the *Rhus coriaria* treated group compared to the placebo one (9, 10).

The incorporation of moderate concentrations of phenolic compounds (consumption less than 50 g/kg DM) may progress animal outputs, possibly because of improved nutritional operation, particularly protein, therefore increased obtainability of intestinal amino acids (11). Furthermore, there are conflicting findings in the literature on the relationship between phenolic compound concentration and animal productivity in ruminants, researchers have presented the capacity to further develop nitrogen use, diminish methane discharge and increment healthy fatty acids, by influencing ruminal digestion, which is mirror the incorporation of polyphenols in ruminant eating programs (12).

The useful effect of exogenous fibrolytic enzymes (EFEs) in the diet of ruminants can be described as a pre-utilization effect through partly digested feed stuff or reduced the barriers of cell wall which restrict ruminal microbial digestion and lower sugars from feedstuffs before ingestion (13, 14). Ruminal impact involves ruminal microbes working together to boost feed digestion, as well as an increase in attachment and the quantity of cellobiose and glucose-using bacteria in the rumen (15). Exogenous fibrolytic enzymes' post-ruminal benefit is that they function in combination with bacteria in the small and large intestine (16). Diets supplemented with EFE showed a significant reduction in cholesterol levels in dairy cows (17) or in fattening lambs (18). Therefore, the goal of this study was to see how dietary supplementation with *Rhus coriaria* grind seeds and EFEs as feed additives affected the blood lipid profile and ruminal fermentation parameters in Awassi male lambs.

MATERIALS AND METHODS

Animals and Management

Experimental procedures in this study were performed with the approval of the local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq (Protocol Number: 849/P.G. dated 20 December 2020).

Twenty-four Awassi local breed male lambs weighting 21.56±0.77 kg with 3-4 months of age were purchased from a local market in Baghdad province, Iraq. The animals were transferred to the Animal Farm of the College of Veterinary Medicine, University of Baghdad. Before the experiment began, the lambs were gradually introduced to the diets over a 20-day period, beginning with wheat straw and progressing to concentrates (Table 1). The amount of concentrated diet offered for each lamb was monthly adjusted according to the body weight gain which was recorded monthly to confirm the ingestion would be around 2.5% of live body weight (LBW). Before starting of the experiment, concentrated diets were offered twice daily, and alfalfa hay *ad libitum*. The lambs were released after the morning feeding in a closed barn contains only a source of water for the sport until the noon meal.

Table 1. Ingredients of concentrate diet

Ingredients	% diet
Barley grain	44
Wheat bran	41
Soybean meal (45% crude protein)	12
Calcium carbonate	1.8
Salt (NaCl)	0.2
Mineral vitamin premix	1

Experimental Design

The lambs were randomly allocated to one of four dietary groups (6 animals for each group) and housed in pens sized 2 × 4 m. Each pen was provided with two feeders to be used for roughage and concentrate diets singly, and clean fresh water was offered *ad libitum*. Dietary treatments were provided to lambs for 4 months (from February 1 to June 1, 2021) as follows: control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed on the control group diet supplemented with 15 g/head dried grind seeds of sumac daily with diet; EFE-5 group, fed the control group diet supplemented with 5 g/head of EFE daily with diet; gsRC-EFE group, fed on the control group diet supplemented with 15 g/head dried grind seeds sumac and 5 g/head of EFE daily with diet.

Rhus coriaria and EFE

The *Rhus coriaria* seeds were purchased from a local herbal market in Baghdad province, Iraq and authenticated by the botanist at the Iraqi National Herbarium, Directorate for Seed Testing and Certification, Ministry of Agriculture, Abu-Ghraib, Baghdad, Iraq. Sumac seeds were ground into a powder before using and maintained at room temperature.

EFE powder (Safizym®, France), contains β-glucanase and xylanase, was obtained from a local store and fed at 5 g/head, mixed with a concentrated diet of EFE-5 group and gsRC-EFE group as an addition to the daily feeding.

Blood Sample

Blood samples were taken monthly from the jugular vein from all animals after sterilization the site. The blood was drawn by using disposable sterilized syringes. The samples were kept in sterilized tubes (10 mL), then separated to serum by centrifugation at 3000× *g* for 15 min, to estimate total cholesterol and triglycerides.

Serum Total Cholesterol and Triglyceride

Serum level of total cholesterol was measured by enzymatic colorimetric method according to (19) and (20) using a commercially available Total Cholesterol Assay Kit (ABIN2345054) according to the manufacturers' instructions. Serum level of total triglycerides was measured by enzymatic colorimetric method according to (20) and (21) using a commercially available kit (Human Gesellschaft, Germany) according to the manufacturers' instructions.

Ruminal Fermentation Characteristics

At the beginning, middle and the end of the last month of the experiment, ruminal fluid samples were taken from all animals after 3 h of feeding to investigate ruminal fermentation features by measuring ruminal pH, total volatile fatty acids (VFAs), and ammonia nitrogen (NH_3-N) (23). Using a smooth stomach tube attached to a specific vacuum, samples were taken from the identical lambs and immediately filtrated through 4 layers of cheesecloth. Then, after adding 0.2 mL of 50% sulfuric acid to stop bacterial action and enclose ammonia, 10 mL subsamples were conserved and stored at -20°C for further analysis according to (24) and determined in the Nutrition Laboratory, College of Agricultural Engineering Sciences, University of Baghdad.

Determination of Ruminal pH

Ruminal fluid samples were filtered and immediately estimated for pH using a portable pH-meter (HI98103 Checker pH Tester, Hanna Instruments Inc., Romania) balanced by 4 and 9 normal pH moderatesolutions (25).

Total Volatile Fatty Acid

After the ruminal fluid sample was thawed at room temperature and shacked, the contents were placed into a glass tube and centrifuged at 4,000 × *g* for 20 min to obtain the supernatant, which was then tested using the Kjeldahl-Markham device for ruminal VFAs (27). Briefly, after the reaction tube and receptor flask were washed with distal water, 1 mL of sample was placed in the reaction tube. Then, 0.5 mL of red methyl was added, and The reaction was started by using water vapor to release the VFA. The vapor of the reaction was collected in the receptor flask until the volume arrived at 25-50 mL. Titration was done with NaOH (0.1 N), until the color just changed to purple (equilibrium state). Total VFAs was calculated as follows:

$$\text{VFAs (mmol/dL)} = \frac{\text{mL NaOH} - \text{Blank}}{\text{mL sample}} \times \text{NaOH concentration} \times 100$$

Determination of NH_3-N

Frozen ruminal fluid samples were thawed at room temperature and mixed, then the contents were transferred into glass tubes and centrifuged at 4,000 × *g* for 20 min. The supernatant was analyzed for ruminal NH_3-N using Kjeldahl device (28). Briefly, 1 mL of sample with 7 mL of magnesium oxide solution (as an assistant factor) was added into washed reaction tube. Then, 5 mL of protein buffer (boric acid) was placed in the receptor beaker, heated, and vapor (50 mL or 5 min) was collected in the receptor flask until the color changed to green-blue. Titration with HCl (0.1 N) was done until the color changed to rosy in the receptor flask. NH_3-N was calculated according to the following equation:

$$NH_3\text{-N (mg/dL)} = \frac{\text{mL HCl} - \text{Blank}}{\text{mL sample}} \times \text{HCl concentration} \times 0.014 \times 100$$

Ruminal Microflora Activity and Movement

After measuring the pH directly, a drop of ruminal fluid was taken on the glass plate and examined microscopically to observe the activity and movement of the rumen microflora. The observation was classified into three levels: Level 1 (+) indicating weak movement and low number, Level 2 (++) indicating active movement but low number, and Level 3 (+++) indicating more active movement and a higher number. This classification method was described by (26).

Statistical Analysis

To estimate significant differences among means, two-way ANOVA was used to examine the data, and the least significant differences (LSD) post hoc test was used. In terms of statistics $P \leq 0.05$ was considered significant (29).

RESULTS AND DISCUSSION

Total Cholesterol and Triacylglycerol Concentration

The results of cholesterol exhibited significant differences among the groups except in the second month while gsRC-EFE group significantly ($P<0.05$) increase in the first month compared with control group. While gsRC-EFE group and gsRC-15 group showed significantly ($P<0.05$) decrease in the third and fourth months compared with control group and then gsRC-EFE group significantly ($P<0.05$) decrease in the fourth months compared with gsRC-15 group. Moreover, EFE-5 group significantly ($P<0.05$) decrease in the fourth months compared with control group (Table 2). For triacylglycerol there was significantly ($P<0.05$) decrease of gsRC-EFE group in the first month compared with EFE-5 group, gsRC-

15 group and control group. In addition, EFE-5 group significantly ($P<0.05$) decrease compared with gsRC-15 group at the same time. But in the second month there were significantly ($P<0.05$) increase of EFE-5 group and gsRC-EFE group compared with control group. Although there were significantly ($P<0.05$) decrease of gsRC-15 group, EFE-5 group and gsRC-EFE group compared with control in the 3rd and 4th months, also gsRC-EFE group significantly ($P<0.05$) decrease at the 4th month compared with the EFE-5 group (Table 1).

Table 2. Effect of grind seeds of *Rhus coriaria* and exogenous fibrolytic enzymes on total serum cholesterol and triglyceride (mg/dL) of local Awassi male lambs

	Cholesterol				Triglyceride			
	Period (month)							
Groups	First	Second	Third	Fourth	First	Second	Third	Fourth
Control	53.5±2.43 $^{b, A}$	50.7±4.00 $^{a, AB}$	44.7±0.21 $^{a, B}$	45.7±0.76 $^{a, B}$	75.0±2.16 $^{ab, A}$	55.5±2.74 $^{b, B}$	62.3±1.33 $^{a, B}$	62.2±3.26 $^{a, B}$
gsRC-15	59.3±3.20 $^{ab, A}$	52.2±2.86 $^{a, A}$	34.5±1.80 $^{b, B}$	33.8±1.27 $^{b, B}$	79.7±5.48 $^{a, A}$	63.3±5.78 $^{ab, B}$	36.3±1.49 $^{b, C}$	25.3±2.47 $^{bc, D}$
EFE-5	59.3±2.45 $^{ab, A}$	50.2±2.16 $^{a, B}$	39.3±2.27 $^{ab, C}$	33.2±1.13 $^{bc, C}$	65.7±2.81 $^{b, A}$	67.3±7.47 $^{a, A}$	33.3±4.04 $^{b, B}$	32.0±2.43 $^{b, B}$
gsRC-EFE	64.8±2.91 $^{a, A}$	48.8±1.64 $^{a, B}$	35.2±0.94 $^{b, C}$	27.0±1.36 $^{c, D}$	51.0±3.09 $^{c, B}$	73.2±4.83 $^{a, A}$	31.8±1.99 $^{b, C}$	21.3±1.74 $^{c, D}$
LSD	6.17				10.49			

Mean±SEM, n=6. $^{a-c}$Means within a column with no common superscript differ significantly at $P\leq0.05$ between groups. $^{A-B}$Means within a row with no common superscript differ significantly at $P\leq0.05$ within periods. Control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet+15 g/head dried grind seeds of *Rhus coriaria* (sumac) daily with diet; EFE-5 group, fed the control group diet+5 g/head of exogenous fibrolytic enzyme (EFE) daily with diet; gsRC-EFE group, fed the control group diet+15 g/head dried grind seeds sumac and 5 g/head EFE daily with diet

These results may be due to the common dealing of the rumen ecosystem with lipids in the diet which produced many fatty acids reaching the small intestine especially in the oily diet. Bacteria in the rumen degraded diet fat to free fatty acids, also rumen microflora produce fatty acid for their metabolism, these free fatty acids subjected to severe bio-hydrogenation producing saturated fatty acids which are absorbed in the small intestine. The amount of oil in plants usually low, except in the oilseed plant that provides a high amount of oil such as Sumac seed which contains a high ratio of oil especially linoleic and oleic acids and that increases the lipoprotein in the serum, this explanation agrees and confirms with (30), who refer to fats are found in small levels with most natural feedstuffs suitable for animal feeding, excluding oilseeds, and ruminant fat use is defined by processes in the rumen before they are absorbed in the gut.

Also, the results are subject to two possible explanations related to the bio-hydrogenation process in the rumen. First, the huge increase of fatty acid in the rumen due to sumac oil led to suppressing bio-hydrogenation in the rumen. Second, Sumac oil contains a high amount of the polyphenol, all that decrease bio-hydrogenation due to suppression the bio-hydrogenation responsible microflora, both two reasonsled to increase free reach of unsaturated fatty acids in the small intestine. This explanation agrees and confirms with (31) and (32) who found that oleic acid to be effective justas linoleic acid in reducing plasma cholesterol concentrations. The hypocholesterolemia effect of oleic acid was confirmed, although it was less successful than linoleic acid in lowering plasma cholesterol levels, despite both fatty acids producing a same decrease in Apo lipoprotein B (Apo B) levels. Apo lipoprotein B was the principal Apo lipoprotein of chylomicrons, VLDL, IDL, and LDL particles, and it was in responsible of transporting fat molecules (lipids), including cholesterol, around the body (inside the water outside cells) to all cells in all tissues. In addition, polyphenols, particularly tannins, have a selective function on the ruminal bacteria community, as evidenced by studies showing various impacts of a same tannins on different bacterial strains. Besides, the capacity of condensed tannins to suppress the last step of bio-hydrogenation of unsaturated fatty acids, namely the enzymatic reduction of vaccenic acid to stearic acid, has been confirmed. Furthermore, (7) found that dietary sumac oil also may increase cholesterol 7-α-hydroxylase which decreases cholesterol in the liver. Additionally, (33) and (34) reported that sumac contains a high ratio of unsaturated fatty acids, which decrease total cholesterol, due to their role in decreasing cholesterogenic enzyme and increase lipoprotein lipase therefore decrease free fatty acid ratio and changing the nature of LDL receptors in liver cell membrane. Omega-3 has also been reported to decrease liver cytokine levels, including tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), which in turn leads to an increase in α-cholesterol 7-Hydroxylase levels. Additionally, omega-3 may contribute to the oxidation of cholesterol into bile acids, which helps decrease cholesterol levels in the blood. Furthermore, sumac is known to contain a high level of anthocyanins, which can act as antioxidants for lipoproteins (35).

The combination of EFE and Sumac maybe produce an active mediator in the rumen, due to the Sumac working as nutrients source for the EFE and microflora, the significant of EFE may become from their ability to manipulating the rumen ecosystem, especially bacteria, EFE metabolic activity could support a certain bacterial strain on other which may be decreased methane release and repress bio-hydrogenation, also it maybe can protect unsaturated fatty acid from conjunction. This explanation agrees and confirms with (36) who found some of the key factors in enhancing feed efficiency and digestion in response to EFE

supplementation were synergism with ruminal microorganisms, stimulation of bacterial colonization, ruminal microbial populations, bacterial adhesion, and enhancement in ruminal hydrolytic capacity.

Ruminal Fluid pH

The pH value of the rumen environment is managed by an auto control mechanism, which maintains acidity in the usual range for microflora to perform their metabolic functions. If the acidity rises, it causes acidosis and suppresses rumen function.

From the result in the Table 3, the fluid pH significantly ($P<0.05$) decreased in the treatment groups related with the control group. *Rhus coriaria* may have a vital role in pH control, maybe by acting as a correction factor for acidity due to contains elements to keep the pH in the regular range. This could be done by increasing rumination and inducing saliva secretion, this explanationagrees and confirms with the data from the chemical composition of *Rhus coriaria* according to (37, 38) who recognized that carbohydrate accounts up 70% of dry matter. High carbohydrate fermentation efficiency in the rumen causes a drop in pH and an aggregation of VFAs and lactate. *Rhus coriaria* also has lower pH (values reached between 3.02 to 3.7) due to their high content of organic acids mainly malic, citric and tartaric acids. However,at the same time, neutral detergent fiber (NDF), which accounts for 62% of the dry matter, keeps ruminal pH by preserving digesta in the rumen, which supplies the buffering capacity innate in feedstuffs, improves salivary buffer fluid motion through rumination, and rises the concentration grade through ruminal motility stimulation (39).

Table 3. Effect of grind seeds of *Rhus coriaria* and exogenous fibrolytic enzymes on ruminal fluid pH between of local Awassi male lambs

	Fourth month		
Groups	**Beginning**	**Middle**	**Last**
Control	6.40±0.07 $^{a, A}$	6.50±0.14 $^{a, A}$	6.37±0.11 $^{a, A}$
gsRC-15	5.65±0.08 $^{b, A}$	5.80±0.04 $^{b, A}$	5.77±0.07 $^{b, A}$
EFE-5	5.70±0.09 $^{b, A}$	5.70±0.09 $^{b, A}$	5.80±0.04 $^{b, A}$
gsRC-EFE	5.72±0.04 $^{b, A}$	5.70±0.10 $^{b, A}$	5.45±0.06 $^{b, A}$
LSD		0.24	

Mean±SEM, n=6. $^{a-c}$Means within a column with no common superscript differ significantly at $P\leq0.05$ between groups. $^{A-B}$Means within a row with no common superscript differ significantly at $P\leq0.05$ within periods. Control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet+15 g/head dried grind seeds of *Rhus coriaria* (sumac) daily with diet; EFE-5 group, fed the control group diet+5 g/head of exogenous fibrolytic enzyme (EFE) daily with diet; gsRC-EFE group, fed the control group diet+15 g/head dried grind seeds sumac and 5 g/head EFE daily with diet

The exogenous fibrolytic enzymes are thought to excess fiber digestion by raising the level of ruminal digestion of the potentially digestible NDF fraction (40), modifications in ruminal fermentation (15) and/or improved colonization and connection to the cell wall of plant by ruminal microorganisms (41, 42) and/or by synergistic effect with enzymes in rumen fluid (43). This result disagrees with (44) who found that supplementation of lambs' ration with EFE significantly increased rumen fluid pH at 0, 3, and 6 h after feeding compared with the control group (not supplemented), perhaps due to increase ruminating process and saliva excretion which enhance the buffering influence through decreasing ruminal lactate concentration and resulted in increase the pH value.

Ruminal Fluid VFAs

VFAs are one of the main end-products of the carbohydrate metabolism through anaerobic digestion processes. The results showed that there was a significant ($P<0.05$) increase in VFAs concentration in gsRC-EFE compared with control at the begin, middle, and end of last month of the study (Table 4). In addition, the control group showed the lowest VFAs concentrations compared with other groups in the midst and the finale of study. This result may be attributed to the *Rhus coriaria* supplementation, may be the major reason for these results, *Rhus coriaria* contain solved carbohydrate, free fatty acid, which was immediately changed to volatile fatty acids this explanation agrees and confirms with Chemical analysis of *Rhus coriaria* according to (45) who showed that carbohydrate ratio reach 63.8% in the dried *Rhus coriaria*. Also, this result agrees with (46) who found improving and higher molarity of the ruminal VFAs description while not giving unfavorable influences on the performance of animals and this could be associated to the fact that hydrolyzable tannins found in sumac, metabolized to gallic and ellagic acids in the rumen, which can convert to acetic and butyric acids. This result is inconsistency with (47) and (48) who found that a decline in the VFAs amount in the existence of tannins, which could be due to the ability of tannins to attach to fiber fractions of the feed which may lead to the decrease of VFAs.

Table 4. Effect of grind seeds of *Rhus coriaria* and exogenous fibrolytic enzymes on volatile fatty acids concentration (mmol/dL) between different collections of local Awassi male lambs

	Fourth month		
Groups	**Beginning**	**Middle**	**Last**
Control	2.15±0.18 $^{b, A}$	1.19±0.21 $^{b, A}$	1.90±0.23 $^{b, A}$
gsRC-15	2.31±0.31 $^{ab, A}$	2.87±0.31 $^{a, A}$	2.83±0.24 $^{a, A}$
EFE-5	2.86±0.34 $^{ab, A}$	3.17±0.26 $^{a, A}$	3.04±0.34 $^{a, A}$
gsRC-EFE	3.03±0.19 $^{a, A}$	3.54±0.07 $^{a, A}$	3.00±0.26 $^{a, A}$
LSD		0.74	

Mean±SEM, n=6. $^{a-c}$Means within a column with no common superscript differ significantly at $P\leq0.05$ between groups. $^{A-B}$Means within a row with no common superscript differ significantly at $P\leq0.05$ within periods. Control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet+15 g/head dried grind seeds of *Rhus coriaria* (sumac) daily with diet; EFE-5 group, fed the control group diet+5 g/head of exogenous fibrolytic enzyme (EFE) daily with diet; gsRC-EFE group, fed the control group diet+15 g/head dried grind seeds sumac and 5 g/head EFE daily with diet

EFEs have been linked to various variations in rumen activity, changed in ruminal pH, enhanced of carbohydrate usage, and increased production of VFAs, this explanation agrees and confirms with the (49). Furthermore, there was a disagreement between researcher on the influence of the

EFEs on VFAs output as well as other results, which could be due to some causes, included the composition of the diet, the type of formulation, activity, the quantity, and stability of the EFE, that have been responsible for the inconsistency in the results and various responses to the exogenous fibrolytic enzymes (50).

NH_3-N Concentration in Ruminal Fluid

Results showed that there was significant ($P<0.05$) increase in the NH_3-N, in treatment groups compared with the control group in the all period of the last month of experiment (Table 5). Also, G2 significantly ($P<0.05$) increased G3 in the beginning of the last month. while G4 significant ($P<0.05$) increased G2 in the midst of last month. This result might be a marker for the excessive action of microflora, which was doing an excellent utilizer for nutrients this agree with (51) who demonstrated that protein was transformed into microbial protein using ruminal ammonia, thus providing extra protein to the host animals. This rapid breakdown to NH_3 can happen more quicker than rumen bacteria can use it, resulting in NH_3 buildup and migration from the rumen, despite the logical mechanism of polyphenol plants such as *Rhus coriaria* binding protein and protecting it from rumen degradation, which resulted in protein depilation in the rumen, this result and explanation were obtained, and this agrees with the (52) who found that sumac tannins attached to nutritional proteins and reduce the ruminal fermentation and the NH_3 release, as a result enhancing nitrogen absorption, this is because of reduced protein breakdown, leads in more digestion of protein in the small intestine, which enhances N use. However, the rumen environment seems to have an unknown mechanism, and in an effort to describe this consequence, *Rhus coriaria* may play an equalizer role in the rumen. When sumac polyphenol binding with a ratio of protein, sumac supplied rumen with protein because it has a respectable amount of protein in its content, which increases protein usage, this explanation confirms with the data from the chemical composition of *Rhus coriaria* according to (45), which was show that *Rhus coriaria* contains 2.3-2.6% protein from dry matter which can influence on metabolism ecosystem of the rumen.

In spite of the effect of exogenous fibrolytic enzymes in the rumen environment, which provides an appropriate place for microflora, there was a disagreement among researchers about the role of EFE in ammonia production, with results ranging from an increase, no effect, and a decrease in NH_3-N, this result showed by (53) who found that EFE caused an increase in NH_3-N of rumen fluid. In contrast, (54) found that EFE significantly decreased in NH_3-N values. (55) showed no influence of EFE on NH_3-N concentration. Environmental conditions, feed type, feed provision method, and enzyme mixture type may all contribute to this variation (56).

Table 5. Effect of grind seeds of *Rhus coriaria* and exogenous fibrolytic enzymes on NH_3-N concentration (mg/dL) of local Awassi male lambs

	Fourth month		
Groups	**Beginning**	**Middle**	**Last**
Control	6.56±0.83 $^{c, A}$	7.35±0.58 $^{c, A}$	7.22±0.43 $^{b, A}$
gsRC-15	10.3±0.44 $^{a, AB}$	9.70±0.33 $^{b, B}$	11.3±0.53 $^{a, A}$
EFE-5	8.92±0.22 $^{b, B}$	10.9±0.59 $^{ab, A}$	12.2±0.42 $^{a, A}$
gsRC-EFE	9.95±0.23 $^{ab, B}$	11.9±0.44 $^{a, A}$	12.5±0.74 $^{a, A}$
LSD		1.48	

Mean±SEM, n=6. $^{a-c}$Means within a column with no common superscript differ significantly at $P\leq0.05$ between groups. $^{A-B}$Means within a row with no common superscript differ significantly at $P\leq0.05$ within periods. Control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet+15 g/head dried grind seeds of *Rhus coriaria* (sumac) daily with diet; EFE-5 group, fed the control group diet+5 g/head of exogenous fibrolytic enzyme (EFE) daily with diet; gsRC-EFE group, fed the control group diet+15 g/head dried grind seeds sumac and 5 g/head EFE daily with diet

The Activity of Rumen Microflora

Microflora in the rumen requires a variety of nutrients for development and reproduction, which must be provided by the feed ingredients, such as carbohydrates, protein, and ruminal pH. The association of microorganisms with other bacteria and with rumen conditions, such as strain conflict on the amounts and times of consuming water, may significantly improve or limit microflora proliferation. The results of Table 6 revealed a significant increase ($P<0.05$) in the activity of rumen microflora in the experiment groups when compared to the control. This result could be attributed to the *Rhus coriaria*, which may support microflora growth if it supplements in the optimal quantitative, and provides carbohydrates, protein, minerals, and fibers, all of which microflora require. In addition, *Rhus coriaria* contains polyphenols which suppress some pathogenic bacteria species and support the beneficial, this explanation agrees and confirms with a chemical composition of *Rhus coriaria* (57), also this explanation agrees with (58) and (59) who observed that various polyphenols can modify the formation of animal microflora by inhibiting pathogenic microorganisms' development, promoting beneficial bacteria growth, and increasing gut biodiversity.

Table 6. Effect of grind seeds of *Rhus coriaria* and exogenous fibrolytic enzymes on the activity of rumen microflora of local Awassi male lambs

	Fourth month		
Groups	**Beginning**	**Middle**	**Last**
Control	2.00±0.23 $^{b, A}$	2.00±0.15 $^{b, A}$	2.00±0.22 $^{b, A}$
gsRC-15	2.50±0.28 $^{a, B}$	2.75±0.25 $^{a, AB}$	3.00±0.00 $^{a, A}$
EFE-5	2.25±0.25 $^{ab, B}$	2.75±0.25 $^{a, A}$	3.00±0.00 $^{a, A}$
gsRC-EFE	2.25±0.25 $^{ab, B}$	3.00±0.00 $^{a, A}$	3.00±0.00 $^{a, A}$
LSD		1.48	

Mean±SEM, n=6. $^{a-c}$Means within a column with no common superscript differ significantly at $P\leq0.05$ between groups. $^{A-B}$Means within a row with no common superscript differ significantly at $P\leq0.05$ within periods. Control group, fed on a concentrate diet at the rate of 2.5% BW with alfalfa hay daily; gsRC-15 group, fed the control group diet+15 g/head dried grind seeds of *Rhus coriaria* (sumac) daily with diet; EFE-5 group, fed the control group diet+5 g/head of exogenous fibrolytic enzyme (EFE) daily with diet; gsRC-EFE group, fed the control group diet+15 g/head dried grind seeds sumac and 5 g/head EFE daily with diet

Although the effect of tannin on bacteria may appear to be suspect, the researchers discovered that the bacteria could adapt to tannin. This explanation agrees with (60) who showed that Tannins may have a negative or positive impact onmicroflora and fermentation of the rumen. The effect differs based on the source and amount of tannins, as well as diets that contain tannins. The rumen microbial community is dynamically adaptive, selectively enhancing or decreasing microbial populations in response to the impact of tannin in the diet. Dietary tannins have a favorable effect on some rumen microbes, and rumen microbial species react dynamically to these effects. Supplementing ruminant diets with exogenous fibrolytic enzymes may help microorganisms thrive by establishing a favorable environment due to preventing lactate accumulation, therefore reducing acidosis, and also aiding specific microorganisms, this explanations agree and confirms with (44) and (48) who recorded that EFE supplementation lead to increase the pH by decreasing lactate accumulation and then, increasing in the number ofruminal micro-organisms through enhancement of the activity of lactate fermenting bacteria such as *Selenomonas ruminantium* and *Megasphaera elsdenii* in the rumen, this may be related to an increase in rumination and saliva secretion, which improves buffering management and raises pH values.

ACKNOWLEDGEMENTS

N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تأثير المكملات الغذائية لبذور السماق المطحونة والأنزيمات المحللة للألياف في بعض صفات الدهون الدمية ومعايير تخمرات الكرش في ذكور الحملان العواسية

لطيف عيسى هادي[١]، ماجد جودة الساعدي[٢]

[١]فرع التشريح والانسجة، كلية الطب البيطري، جامعة ذي قار، ذي قار، العراق، [٢]فرع الصحة العامة، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

أجريت هذه التجربة في الحقل الحيواني التابع الى كلية الطب البيطري/جامعة بغداد لتقييم تأثير بذور السماق المطحونة والأنزيمات الخارجية المحللة للألياف على بعض صفات دهون الدم ومعايير تخمرات الكرش للحملان الذكور العواسية للمده من ٢٠٢١/٢/١ ولغاية ٢٠٢١/٦/١٥ استخدمت ٢٤ حملا من الذكور العواسية، وبعمر ٢–٣ شهر، وبمعدل وزن (٢١,٥٦±٠,٧٧) كغم. قسمت الحيوانات عشوائيا الى اربعة مجاميع (٦حيوان /مجموعة) وبصورة متساوية مع الاخذ بنظر الاعتبار وزن الجسم الحي وعوملت المجاميع كالاتي:المجموعة الاولى وهي مجموعة السيطرة / غذيت هذه المجموعة على العلف المركز وبنسبة 2.5% من وزن الجسم مع دريس الجت. المجموعة الثانية (gsRC–15) غذيت على العليقة نفسها كما في المجموعة الاولى وأضيف اليها ١٥غرام/رأس من بذور السماق الجافه المطحونة مخلوطا مع العليقة. المجموعة الثالثة (EFE–5) /غذيت على العليقة نفسها للمجموعة الأولى وأضيف اليها ٥ غم لكل رأس يوميا من الأنزيمات المحللة للألياف EFE مخلوطا مع العليقة. المجموعة الرابعة (gsRC–EFE)/غذيت على العليقة نفسها للمجموعة الاولى مضافا اليها ١٥غرام/رأس من بذور السماق الجافه المطحونة مع ٥ غم لكل رأس يوميا من الأنزيمات المحللة للألياف EFE مخلوطا مع العليقة. كشفت النتائج ما يلي: أظهرت قيم الكوليسترول الكليوالدهون الثلاثية انخفاض معنوي في المجموعة الرابعة والثالثه و الثانية خصوصا في الشهر الثالث وحتى نهاية التجربة مقارنة بالمجموعة الأولى .اما بالنسبهلمعايير تخمرات الكرش اظهرتقيم الأس الحامضي انخفاضا معنويا في المجموعة الرابعة والثالثة والثانية في بداية ووسط ونهاية الشهر الأخير من التجربة مقارنة بمجموعة السيطرة.بينما اظهرت نتائج الحوامض الدهنية الطيارة والأمونيا و نشاط مايكرو فلورا الكرش تفوق المجموعة الرابعة والثالثه والثانيه معنويا في وسط ونهاية الشهر الأخير من التجربة بالمقارنة مع المجموعة الأولى. يمكن الأستنتاج ان أستحدام بذور السماق المطحونه والأنزيمات المحلله للألياف معا أومنفردا كأضافات علفيه فعاله لتحسين بعض صفات الدهن الدميه ومعايير تخمرات الكرش في ذكور الحملان العواسية.

الكلمات المفتاحية: السماق، الأنزيمات الخارجية المحلله للألياف، صفات دهون الدم، معايير تخمرات الكرش، الحملان الذكرية