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Effect of Grind Seeds of Rhus Coriaria and Exogenous Fibrolytic Enzymes Supplementation in Ration on Some Haemobiochemical Parameters of Awassi Male Lambs

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Abstract
Curent study desgned to assess the influence of grind seeds of *Rhus Coriaria* and exogenous fibrolytic enzymes (EFE) on some haemobiochemical parameters of Awassi male lambs. 24 Awassi male lambs weighing (21.50-60.77) Kg at 3–4 months of age, and they were equally divided into four groups based on body weight. G1 (control) was fed a concentrate diet at a rate of 2.5% body weight with alfalfa hay everyday and was regarded a control group. G2 was fed the same food as the control group and received 15 g/head of dried grind *Rhus Coriaria* seeds daily with feed. The same food as in G1 was offered to G3 together with 5 g/head of exogenous fibrolytic enzyme (EFE). The same diet as G1 was used to feed G4, which included 5 g of EFE and 15g of dry ground *Rhus Coriaria* seeds daily. The results of glucose concentration showed G2, G3 and G4 significance $p<0.05$ decrease from the 2nd month to the end of experiment compared with control group. Values of total protein showed G4 significance $p<0.05$ surpass at the 3rd month of trial. Results of Albumin exhibited G2, G3 and G4 significance $p<0.05$ exceed at the 1st, 2nd and 4th months of experiment. Values of Globulin showed G2 and G3 significance $p<0.05$ exceed G1 at the 3rd month of experiment. Values of GGT showed G2, G3 and G4 significance $p<0.05$ decrease from the 2nd month toward the end of experimentation. Urea blood concentration values indicated a significant decline in G2, G3, and G4 from the second month until the conclusion of the investigation. It can be established that grind seeds of *Rhus coriaria* and EFE can be used in combination or alone as an effective feed additive to improve some haemobiochemical parameters in Awassi male lambs
Key words : Rhus coriaria, Exogenous fibrolytic enzymes, Haemobiochemical parameters

الملخص
تم دراسة تأثير بذور السماق المطحونة والأنزيمات الخارجية المحللة للألياف المضافة للعلائق على بعض صفات الدم في ذكور الحملان العواسية، حيث تم استخدام 24 ذكر عواسي، بعمر3-4 شهور، ومعدل وزن (0.77 ± 21.56) كغم. قسمت هذه الحيوانات عشوائيا الى اربعة مجاميع (6 حيوان /مجموعة) وبصورة متساوية وبالنظر الى وزن الجسم الحي وعوملت المجاميع كالاتي: المجموعة الاولى وهي مجموعة السيطرة حيث تم تغذيت هذه المجموعة على العلف المركز وبنسبة 2.5% من وزن الجسم مع دريس الجت. المجموعة الثانية اعطيت العليقة نفسها كما في المجموعة الاولى وأضيف اليها 15غرام/رأس من بذور السماق الجافة المطحونة مخلوطا مع العليقة. اما المجموعة الثالثة غذيت على العليقة نفسها للمجموعة الأولى وأضيف اليها 5 غم لكل رأس يوميا من الأنزيمات المحللة للألياف مخلوطا مع العليقة. بينما المجموعة الرابعة غذيت على العليقة نفسها للمجموعة الاولى مضافا اليها 15غرام/رأس من بذور السماق الجافه المطحونه مع 5 غم لكل رأس يوميا من الأنزيمات المحلله للألياف EFE مخلوطا مع العليقة. اما في الشهر الثالث من التجربه. ناقل البتيد غاما غلوتاميل. اظهرت المجموعة الثانية والثالثة والرابعة انخفاضا معنويا من الشهر الثاني وحتى نهاية التجربة. اليوريا اظهرت المجموعة الثانية والثالثة والرابعة انخفاضا معنويا من الشهر الثاني وحتى نهاية التجربة. يمكن الأستنتاج ان أستخدام بذور السماق المطحونة والأنزيمات المحللة للألياف معا أومنفردا كأضافات علفية فعالة لتحسين بعض الصفات الكيموحيوية في ذكور الحملان العواسية.
الكلمات المفتاحيه: السماق، الأنزيمات الخارجية المحللة للألياف، الصفات الدمية

Introduction

More than half of all production costs are spent on feeding livestock. In recent years, pre- and probiotics, ionophores, enzymes, and fodder shrub and tree extracts have all been used in ruminant nutrition.[1]

Medicinal plants have stimulatory effects on the digestive system and are often good sources of some minerals and vitamins. Medicinal plants like sumac (*Rhus coriaria*) contain organic acids such as malice, citric and tartaric acids, and have shown health-promoting attributes including antiviral, anti-inflammatory, anti-gastric, antioxidant, antibacterial, antidiarrhoeal, antispasmodic, astringent, hepatoprotective, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitors due to their contents of flavones, phenolic acids as gallic acid, myricetin, quercetin, isoquercitrin and tannic acid.[2, 3] Also type 2 of diabetic patient eating a flour of *Rhus coriaria* (3.0 g, daily for 3 months) found a considerable drop in the levels of serum glucose and an rise of whole function of antioxidants.[4]

Exogenous enzymes are commercially available and have the benefit of lowering anti-nutritional elements in dietary components to make better digestibility.[5] Dairy cows' dry material ingestion, in vivo fibre digestibility, and milk output were all improved when some enzyme formulations were used.[6] The validities of enzyme additions, on the other hand, vary depending on the type of animal (energy need and amount of feeding), feed conformation, enzyme design, dosing amount, and enzyme product provision mechanism.[7] There for such research targeted for assess the impact of the supplementation of Sumac grain powder and exogenous fibrolytic enzymes

(EFE) as feed additives on some haemobiochemical parameters of Awassi Male Lambs.

Material and methods:

Design of experiment:

The research study was conducted at the University of Baghdad's College of Veterinary Medicine's Animal's Farm and for the period from 1/2/2021 to 1/6/2021. Twenty-four Awassi local breed male lambs weighted (21.56±0.77) Kg with 3 - 4 months of age from a trusted and familiar source were used. Lambs were randomly separated into 4 groups, with 6 animals each. Nutritive dealing designed for 4 months by way of: G1 (control) was fed on concentrate diet at the rate of 2.5% body weight with alfalfa hay daily, G2 was fed on the same diet as control group and was given 15g/head dried grind seeds of Rhus Coriaria daily with diet, G3 was fed on the same diet that was given in G1 with 5g/head of exogenous fibrolytic enzyme (EFE), G4 was nourished on similar feed in G1 with a 15g/head dried grind seeds of Rhus Coriaria and 5g/head of EFE daily with diet.

Management and feeding system

Lambs were divided into 4 groups in pens sized 2 × 4 m for each group, at the same farm and each yards were provided with two containers to be utilized for roughage and concentrated feed separately. Clean freshwater was available at all times before starting of the experiment. The diets were gradually introduced to the lambs for 20 days preliminary period, starting with wheat straw and followed by concentrates. To ensure that the intake would be about 2.5% of the recorded live body weight (LBW), the amount of concentrated diets offered for each lamb was adjusted monthly in accordance with the body weight gain that was weighed on a monthly basis. Before starting of the experiment, concentrated diets were offered twice daily and alfalfa hay was offered *ad libitum*. The sheep were released after the morning feeding in a closed barn contains only a source of water for the sport until the noon meal.

***Rhus Coriaria* classifications:** *Rhus Coriaria* was purchased from the local market. The plant description was completed at the Directorate of Seed Testing and Certification (DSTC), Ministry of Agriculture, Abu Graib, Baghdad, with certificate number 2676 and data 16/12/2020.

Supplementation of exogenous fibrolytic enzyme (EFE): EFE powder (Safizym- France) was purchased from the local market and was given at 5 gm/head, mixed with a concentrated diet of G3 and G4 as an addition to the daily feeding.

Blood samples: From the jugular vein, blood samples were obtained once a month after sterilization the place of blood drawn by using one-use decontaminated needle. The specimens kept in sterilized (10 ml) tubes, then separated to serum by centrifuge (3000 rpm) for 15 minutes, to estimate glucose, total protein, albumin, glubulin, gamma glutamyl-transferase (GGT), urea.

Determination of glucose concentration: It was estimated according to [8]. (Glob), (g/dl). Methodology: Brocresolgreen assay kit (7D52 Glucose concentration BCG was supplied as a liquid, ready-to-use, single reagent kit). Using Glucose. Kit from (Abbott reagents Co.) Humalyzer 3500.

Determination of total serum protein concentration g/dl:

The modification of Weichselbaum serves as the foundation for this total Protein method. Proteins and polypeptides having at least two peptide links react with cupric ions in an alkaline solution to form a violet-colored complex. The concentration of protein in the sample is directly proportional to the complex's absorbance at 540/660 nm.[9]

Determination of serum albumin concentration g/dl:

By using, a neutral buffered solution of bromocresol green (BCG) as the dye binding indicator, Rodkey (1965) devised a quick and straightforward method for detecting albumin quantities in serum. In order to reduce turbidity and enhance linearity and sensitivity of the reaction by including a nonionic surfactant into the reagent. Using a new buffering system, this albumin approach modifies the Doumas and Rodkey processes. Albumin and bromocresol green combine at pH 4.2 to produce an intensely green complex. The albumin quantity in the sample is directly correlated with the bichromatically determined absorbance of the albumin-BCG complex (600/800nm).

Determination of serum globulin g/dl: The globulin was estimated by subtraction of albumin from total proteins.[10]

Determination of gamma glutamyl-transferase U/L activity: Kinetic colorimetric technique to measure serum GGT production. The gamma-glutamyl group is transferred from the colorless substrate, gamma-glutamyl-p-nitroaniline, to the acceptor, glycylglycine, in the reaction, which results in the creation of the colorful product, p-nitroaniline. Over a predetermined period of time, the system tracks the rate of change in absorbance at 410 nm. The activity of GGT in the sample is exactly related to the rate of change in absorbance.[11]

Determination of urea mg/dl: Ammonia and carbon dioxide are produced when urea is digested by urease1, 2. In the presence of sodium nitroprusside as

a coupling agent, the produced ammonia combines with sodium salicylate and alkaline hypochlorite to produce a green cromophore. According on the amount of urea present in the sample, the color's intensity increases.[12, 13]

Statistical analysis: Two-way ANOVA was used to analyze the data, and the least significant differences (LSD) post hoc test was used to determine whether there were significant differences between the means. Statistical significance was defined as P <0.05.[14]

Results and discussion

Glucose concentration:

The result of glucose concentration showed that treatment groups significantly (P<0.05) decrease compared with the control group and there were significant (P<0.05) decrease between the treatment groups itself table (1). These findings support the findings of Mohammadi *et al*. (2010), who believed that the antihyperglycemic effects of R. *coriaria* fruits might be caused by variation of insulin (INS) discharge or excitement because there has been no change in the expression of the INS and glucose transportation type-4 (GLUT-4) genes, or by a reduction of carbohydrate breakdown or the absorption process. The in-vitro hypoglycemic action of R. *coriaria* fruit ethyl acetate extract has been linked to amylase inhibition in the past.[15] Gallic acid, methyl gallate, kaempferol, and quercetin are only among the many of the phenol acids and flavonoids that are found in sumac[16] suggesting any or all of them might be to blame for sumac's hypoglycemic effects.[17] Subsequently is known that quercetin lowers high blood serum levels of cholesterol and low density lipoproteins, which are present in diabetes, and encourages correction of the level of glycemia.[18] This result was most likely caused by the GLUT-2 restricting glucose from the gut intake.[19] In contrast[20] showed a significance increase in the levels of blood sugar after orally administration of sumac to the adult Wistar male albino rats. From the result the Sumac and EFE have many advantages for ruminant feeding, it has been shown that the addition of EFE causes the cell wall components to partially dissolve, releasing reducing sugars that serve as a fast source of nutrients for the microbial species for rapid multiplication and this explanation agrees with[21, 22] who showed that the incorporation of EFE helps to increase the numbers of rumen bacteria, particularly fibrolytic bacteria, and shortens the period needed for bacterial growth before taking effect by releasing soluble sugars from cell wall components and rumen bacteria.

Different little letters in the same column indicate substantially different means (P< 0.05). Means in the identical row containing various capital letters are substantially distinct (P< 0.05).

Total serum protein, albumin, globulin:

The overall significant (P<0.05) increase in total protein, albumin, and globulin in the treatment groups compared to the control, as well as the increase in the control in some periods, tables (2, 3, 4) respectively could be described by the animals' good body health, which could lead to an increase in protein utilization, but the significant increase in total protein in the serum in the treatment group (G4) is likely due to the effect of Sumac supplementation with exogenous fibrolytic enzymes.

Rhus Coriaria may have a vital role in protein use by supplying protein to the body, as well as acting as a rumen ecosystem enhancer by increasing the escape of protein and protein-related materials from rumen breakdown, this demonstration confirms with the result from [23] who found that dried sumac contains crude protein (2.3-2.6%) which was a valued amount that maybe can affect rumen metabolism. Also this explanation agrees and confirms with [24, 25] who found that plants rich in polyphenols, the tannins in particular, affect strongly metabolism processes in the ruminant. Intense variations in protein, carbohydrate, and lipid metabolism result from a combination of direct effect of nutrients and interaction with enzymes and the ruminal microbial population. Understanding these mechanisms allows for better product performance such as meat and dairy product quality. For example, tannin protects hay proteins from rumen degradation and improves lamb growth performance, while anthocyanins, another type of sumac polyphenol, can precipitate proteins and reduce fermentation. Furthermore, the interaction of polyphenols and protein can improve the flow of nitrogen from therumen to the gut, resulting in better protein utilization (EFE) have the ability to play a major role in the rumen ecosystem, it may be act as manipulating factor in protein metabolism. This explanation agrees and confirms with[26] who found that supplementation the ration of lambs with (EFE) significantly improved crude protein and numerically in plasma total protein concentration compared to lambs fed ration not supplemented, this may be attributed to EFE supplementation which play important role in the improving metabolic process as a response to increase nutrients digestibility specially, crude protein and organic matter as well as increase flow of microbial protein from the rumen.[27] On the other hand, Microbes expend less energy to reduce sugars to tiny units comparing to starch or structural carbohydrates[28] this means that rumen

microorganisms can utilize glucose more quickly, allowing them to grow faster and breakdown more fiber. In some cases, increasing the amount of carbohydrate fermentation might be helpful since it can result in better rumen degradable protein (RDP) uptake and more metabolizable protein supply (MP).[29]

Gamma glutamyl-transferase:

Gamma glutamyl-transferase (GGT) concentration was the most commonly used for the evaluation of liver disease.[30] Treatments groups significantly show a low level of (GGT) comparing with the control group table (5), those results might be owing to the dietary *Rhus Coriaria* to protect hepatocytes from inflammatory and harmful materials such as oxygen free radicals, and enhance liver function, this explanation agrees and confirms with[31, 32] who found that Rhus Coriaria can protect the hepatocytes from injuries, improved the liver functions of the treated animal by reducing liver enzyme levels which indicated hepatoprotective activity. Rhus Coriaria contained gallic acid (GA) that can inhibit the apoptosis and damage cells that produced by oxygen free radicals. Also the anti-inflammatory and antioxidant properties of gallic acid (GA) may be associated with elevated liver superoxide dismutase, liver catalase activities and vitamin C levels and reduction of liver tumor necrosis factor-α (TNF-α) expression, liver Malondialdehyde (MDA), and serum protein carbonyl.[33] liver efficiency associated with the common health, addition of the (EFE) enhance feed consumption, which provides the body with nutrients and increased productive performance, this explanation agrees and confirms with[34, 35] who discovered that the inclusion of (EFE) increases lamb growth rate as a consequence of boosting feed intake and promoting dietary fiber digestibility, with no negative impacts on the welfare of animals, and who also found that animal liver tissues were functioning normally without any negative effects on the liver's functioning.

Blood urea nitrogen:

The results revealed that the urea aggregation significantly decrease in treatment groups these findings concur and confirm with[36] who also discovered that adding (EFE) to lambs' ration presented numerically increased in serum total protein concentration in comparison to lambs fed ration without addition. The groups supplemented with EFE recorded significant reductions in urea concentration compared to control group. This might be as a result of (EFE) supplementation, which enhances metabolic function in response to increased nutrient digestibility, particularly for crude protein and organic matter, as well as increased flux of microbial protein from the rumen. ompared to control group table (6), started from second months to the end of experiment, this result was may due to *R. coriaria* plant which is known as an abundant source of tannins (condensed and hydrolysable), phenolic acids, anthocyanins, gallic acid derivatives, flavonoid glycosides, organic acids.[36] Plant tannins have a significant protein affinity and reported to bind dietary proteins, which has been successfully used in ruminant nutrition to reduce protein breakdown in the rumen and decrease their rumen fermentation and NH_3 production, then improving protein use by increase the amount of dietary protein reaching small intestine for absorption and improve N utilization and animal production efficiency,[1] or because of the conversion of ammonia by the liver to urea and discarded with urine.[5, 6] However, the rumen ecosystem still has an unknown mechanism, and in an effort to explain this result, Sumac polyphenol binding with a ratio of protein, sumac provided rumen with protein because it has a reasonable amount of protein in its content, which increases protein availability. This explanation agrees with chemical analysis of R. coriaria, which showed that it contains (2.3-2.6%) protein from dry matter[] which can affect rumen environmental metabolism. From the result showed these findings concur and confirm with[9, 12] who also discovered that adding (EFE) to lambs' ration presented numerically increased in serum total protein concentration in comparison to lambs fed ration without addition. The groups supplemented with EFE recorded significant reductions in urea concentration compared to control group. This might be as a result of (EFE) supplementation, which enhances metabolic function in response to increased nutrient digestibility, particularly for crude protein and organic matter, as well as increased flux of microbial protein from the rumen.[16]

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Table (1): Effect of Grind Seeds of *Rhus Coriaria* and Exogenous Fibrolytic Enzymes on blood glucose (mg/dl) of lambs (M±SE).

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	A65.50±1.28a	A63.00±0.81a	B55.66±1.25a	C43.00±0.81a
Sumac (G2)	A64.66±0.66a	B53.00±0.57b	C46.16±1.24b	D34.66±0.95b
Enzyme (G3)	A65.00±0.96a	C29.16±1.99c	B33.50±1.40c	BC32.00±1.29b
Sumac+Enzyme(G4)	A63.16±0.60a	BC24.66±1.11d	B26.83±0.87d	C23.33±1.47c
LSD	3.21			

Table (2): Effect of Grind Seeds of Rhus Coriaria and Exogenous Fibrolytic Enzymes on Total serum protein (g/dl) of lambs (M±SE**).**

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	A6.28±0.07a	A6.41±0.06b	A6.47±0.08b	A6.62±0.08a
Sumac (G2)	A5.68±0.29b	A6.93±0.19ab	A6.99±0.13ab	A7.13±0.11a
Enzyme (G3)	B4.94±0.33c	A6.80±0.12ab	A6.96±0.25ab	A6.98±0.14a
Sumac+Enzyme(G4)	B5.36±0.19bc	A7.11±0.26a	A7.04±0.22a	A7.10±0.15a
LSD	0.53			

Table (3): Effect of Grind Seeds of *Rhus Coriaria* and Exogenous Fibrolytic Enzymes on Albumin concentration (g/dl) of lambs (M±SE).

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	B47.66±1.96a	A58.01±1.74a	AB55.81±2.32a	AB54.43±1.84a
Sumac (G2)	A50.80±2.24a	B43.16±5.00b	B38.48±1.08b	C28.65±2.10bc
Enzyme (G3)	A50.70±2.58a	A49.38±5.05b	B36.61±1.36b	B30.56±2.13b
Sumac+Enzyme(G4)	A50.61±1.95a	AB44.85±2.58b	B38.88±2.08b	C22.83±0.73c
LSD	7.22			

Table (4): Effect of Grind Seeds of *Rhus Coriaria* and Exogenous Fibrolytic Enzymes on Globulin concentration (g/dl) of lambs (M±SE).

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	B2.79±0.13b	A3.50±0.12b	A3.14±0.07a	A3.47±0.05b
Sumac (G2)	B3.01±0.13ab	A3.87±0.07a	B3.28±0.14a	A3.81±0.12a
Enzyme (G3)	B3.09±0.05ab	A3.88±0.04a	B3.13±0.12a	A3.67±0.03ab
Sumac+Enzyme(G4)	B3.11±0.07a	A4.01±0.22a	B3.29±0.09a	A3.80±0.07a
LSD	0.30			

Table (5): Effect of Grind Seeds of *Rhus Coriaria* and Exogenous Fibrolytic Enzymes on Gamma glutamyl-transferase (GGT) U/L of lambs (M±SE).

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	A3.48±0.16a	B2.90±0.13a	AB3.16±0.15b	AB2.98±0.16a
Sumac (G2)	C2.67±0.28b	BC3.05±0.20a	A3.71±0.13a	AB3.31±0.15a
Enzyme (G3)	C1.84±0.31c	B2.91±0.16a	A3.83±0.24a	AB3.31±0.16a
Sumac+Enzyme(G4)	B2.24±0.17bc	A3.10±0.09a	A3.58±0.24ab	A3.29±0.14a
LSD	0.54			

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Table (6): Effect of Grind Seeds of Rhus Coriaria and Exogenous Fibrolytic Enzymes on blood urea (mg/dl) of lambs (M±SE).

Groups	1st Month	2nd Month	3rd Month	4th Month
Control (G1)	B47.66±1.96a	A58.01±1.74a	AB55.81±2.32a	AB54.43±1.84a
Sumac (G2)	A50.80±2.24a	B43.16±5.00b	B38.48±1.08b	C28.65±2.10bc
Enzyme (G3)	A50.70±2.58a	A49.38±5.05b	B36.61±1.36b	B30.56±2.13b
Sumac+Enzyme(G4)	A50.61±1.95a	AB44.85±2.58b	B38.88±2.08b	C22.83±0.73c
LSD	7.22			