



Evaluation of using some medical herbs seeds as feed supplementation and their effects on the activity of some enzymes and hormones on male Awassi lambs

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Abstract

Twenty four male Awassi lambs aged 3.5-4 months were assigned into four main treatment groups .The 1st treatment was fed on control ration only, while 2nd, 3rd and 4th treatments were fed *ad libitum* on control ration and supplemented with 600 mg/kg.B.W. /day of black cumin or fenugreek or rocket seeds respectively, for four months. The blood samples were collected to measurement the activities of ALT, AST and ALP enzymes and Triiodothyronine, Thyroxin and Testosterone hormones. The Results indicated that activities of ALT and AST enzymes were increased significantly ($P \leq 0.05$) in T2 and T3, respectively, while the activity of ALP enzyme was increased significantly ($P \leq 0.05$) in T4 as compared to those in T1. The activities of Triiodothyronine and Thyroxin hormones were increased significantly ($P \leq 0.05$) in T2, T3 and T4, respectively, also the activity of Testosterone hormone was increased significantly ($P \leq 0.05$) in T2 and T4, respectively, as compared to those in T1. Also the activities of ALT, ALP and Testosterone were increased significantly ($P \leq 0.05$), while the activities of Triiodothyronine and Thyroxin was decreased significantly ($P \leq 0.05$) as lambs get older. The present study was carried out to determine the effect of supplementation of black cumin or fenugreek or rocket seeds to the rations on the activity of some enzymes and hormones in blood of male Awassi lambs.

Keywords: male Awassi lambs, black cumin seeds, fenugreek seeds, rocket seeds, lambs age, activity of ALT, AST, ALP, triiodothyronine, thyroxin and testosterone.

تقييم استخدام بذور بعض الأعشاب الطبية كإضافات علفية وتأثيرها في فعالية بعض

الأنزيمات والهرمونات في مصل دم الحملان الذكورية العواسية

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الخلاصة:

استخدمت في هذه الدراسة 24 حمل ذكر عواسي، تراوحت أعمارها بين 3.5-4 شهرا تم تقسيمها إلى أربعة مجاميع. غذيت المجموعة الأولى على العليقة القياسية فقط، بينما غذيت المجاميع الثانية والثالثة والرابعة بصورة حرة على العليقة القياسية مضافا إليها 600 ملغم/كغم وزن حي/اليوم من بذور الحبة السوداء أو الحلبة أو الجرجير على التوالي ولمدة أربعة أشهر. جمعت عينات الدم لدراسة فعالية أنزيمات ALT و AST و ALP و هرمونات Thyroxine و Triiodothyronine و Testosterone. أشارت النتائج أن فعالية أنزيمي ALT و AST ارتفعت معنويا ($P \leq 0.05$) في المعاملتين الثانية والثالثة على التوالي، في حين فعالية أنزيم ALP ارتفع معنويا ($P \leq 0.05$) في المعاملة الرابعة فقط مقارنة بالمعاملة الأولى. كذلك فعالية هرموني Thyroxine و Triiodothyronine ارتفع معنويا ($P \leq 0.05$) في المعاملات الثانية والثالثة والرابعة على التوالي، في حين ارتفع معنويا ($P \leq 0.05$) فعالية هرمون Testosterone في المعاملتين الثانية والرابعة على التوالي مقارنة بالمعاملة الأولى. كما ارتفعت معنويا ($P \leq 0.05$) فعالية أنزيمي ALT و ALP و هرمون Testosterone وانخفضت معنويا ($P \leq 0.05$) فعالية وهرموني Thyroxine و Triiodothyronine بتقدم الحملان بالعمر. أجريت الدراسة الحالية لمعرفة تأثير إضافة بذور الحبة السوداء أو الحلبة أو الجرجير في فعالية بعض الأنزيمات والهرمونات في مصل دم ذكور الحملان العواسية.

الكلمات الافتتاحية: حمل ذكر عواسي، بذور الحبة السوداء، بذور الحلبة، بذور الجرجير، عمر الحيوان، أنزيمات ALT و AST و ALP و هرمونات Triiodothyronine و Testosterone

Introduction:

There are many herbaceous plant in local market in Iraq are used in folk medicine and its named as medical herbs (1). There are about 360-370 herbs kinds in local market, such as black cumin seed (*Nigella Sativa Linn*) belong to botanical family Ranunculacea, fenugreek seed (*Trigonella foenum graecum L*) belong to botanical family Leguminosae and rocket seed (*Eruca sativa mill*) belong botanical family Brassicaceae. These seeds has some substance that can be used to elevated the production efficiency for different animals farms, and this substance can play important role in human and animal health because its antibacterial and antifungal (2&3). The black cumin seed can used as antibacterial because it contain substance thymoquinine (4). Also (5) found that fenugreek seed can used for cure debates and high cholesterol. While (6) mentioned that rocket seed contain materials like glucosinolates, flavonoides and carotenoids are used as antioxidant.

Also (3) found that some of these substances has active as ant parasites and it can be used for cure some inflammation, beside that rocket seed contain high percentage of carotenes (6) and vitamin C (7), which have antibacterial growth and glucopsinolates were found to have several biological activities including anti-carcinogenic, antifungal, anti-bacterial plus its antioxidant action (8). These seeds have many effect on the activity of enzymes (9) and hormones (10).

The objective of this study was to evaluate the effects of black cumin or fenugreek or rocket seeds as feed supplementation to the rations and lambs age of Awassi male lambs on the activity of some liver enzymes (ALT, AST and ALP) and some hormones (triiodothyronine, thyroxine and testosterone).

Materials and methods:

The present study was carried out in sheep farm of animal production department in the technical agriculture college, Mosul/Iraq, from 15/2 to 15/6/2013 (4 months). In this respect study, twenty four male Awassi lambs aged 3.5-4 months and 21-22 kg. body

Table 1: Feedstuffs (gm./kg.feed) and chemical analysis (%) of experimental ration and some medical herbs seeds.

Feed stuffs		Chemical analysis%				
Ingredients	gm./kg.feed	items	Control ration	Black cumin	Fenugreek	Rocket
Barley grain	420	Dry matter% *	93.1	93.67	93.51	93.11
Wheat bran	420	Crude protein%*	15.26	26.64	27.88	29.94
Soy bean meal	70	Ether extract%*	2.58	10.67	.896	9.64
Yellow corn	70	** %Crude fiber	6.21	8.12	9.92	4.38
Nacl (Salt	10	Ash %*	5.44	4.53	4.42	6.89
Limestone(Caco ₃)	10	** %NFE	63.61	43.71	44.40	42.26
Metabolism energy (MJ/Kg.feed) ***			11.85	13.03	12.19	12.72

*Determined on dry matter base according to (12)

**Calculated from chemical analysis tables for Iraqi feed stuffs (13).

***Calculated according to equation of (14).

weight were divided into four comparable main groups of 6 lambs each according to their live weight and each main group was divided into two subgroups (3 lambs /sub group). The first group (T1) was fed on control ration (basal ration) (table 1) according to (11), the other three groups were fed the same basal ration and supplemented daily with either 600 mg. / kg. B.W./day of black cumin seeds (T2) or fenugreek seeds (T3) or rocket seeds (T4), respectively. All rations were fed *ad libitum* twice daily at 8 a.m. and 5 p.m. for four months, while straw (2-3 cm) was offered at 1% of their body weight for the experimental animals. Fresh water and mineral blocks were freely available at all times. All lambs were free from diseases and parasites and housed in semi-shaded well-ventilated pen.

Blood samples (5ml) were taken from the all experimental lambs from jugular vein and drawn into heparinized syringe before morning feeding at age 4 (start), 6 (middle) and 8 months (end) the experiment, blood samples were centrifugation at 4000 rpm /15 minutes, was stored at -20°C⁰ until chemical analysis that included Aspartate amino transferase (AST) and Alanine amino transferase (ALT) enzyme activities

according to (15), and Alkaline Phosphates (ALP) was measured according to (16). Testosterone levels were determined by using French kits (Immunotech/ISO) according to (17), Triiodothyronine and Thyroxin levels were assayed by using immulite kits, USA, with modification described by (18) by using Radioimmunoassay technique (RIA).

Statistical Analysis:

Data was statistically analyzed using completely randomized design model (CRD) procedure by (19). Duncan's multiple range test was used to determine the significance of differences between treatments means (20). Analysis of variance was carried out on all data. The treatment was partitioned into main effects and their interaction (21).

Result:

Data in table (2) clearly indicated that activities of AST and ALT enzymes were significantly ($P \leq 0.05$) increased, but the activity of ALP enzyme was significantly ($P \leq 0.05$) decreased in T2 and T3 that supplemented by 600 mg./ kg.B.W./day of black cumin or fenugreek seeds respectively, as compared to the T1 (control ration). While the activities of ALP

and ALT enzyme were significantly ($P \leq 0.05$) increased but the activity of AST enzyme was significantly ($P \leq 0.05$) decreased in T4 that supplemented by rocket seeds as compared to the T1 (control ration). Also the data in table(2) indicate that activities of ALT and ALP enzymes were significantly increased ($P \leq 0.05$), while the activity of AST was decreased mathematically as lambs get older. The interaction between medical herbs seeds supplementation and lambs age had a significant ($P \leq 0.05$) effect the activities of ALT, AST and ALP enzymes.

Data in table (3) revealed that the activity of triiodothyronine and thyroxin hormones were increased significantly ($P \leq 0.05$) in T2, T3 and T4, that supplemented by 600 mg. /kg. B.W / day of

black cumin or fenugreek or rocket seeds respectively as compared to the T1 (control ration). Also the data in table(3) revealed that activity of testosterone was increased significantly ($P \leq 0.05$) in T2 and T4 that fed 600 mg. /kg.B. W./day of black cumin or rocket seeds respectively, but the activity of testosterone was decreased significantly ($P \leq 0.05$) in T3 that fed fenugreek as compared to the T2 & T4, while no significant differences in the activity of testosterone was found between T3 and T1. Also the data in table (3) revealed that activity of testosterone was significantly ($P \leq 0.05$) increased, but the activity of triiodothyronine and thyroxin were significantly ($P \leq 0.05$) decreased as lambs get older.

Table 2: Effect of adding medical herbs seed and lamb age in concentration of some enzymes (Mean \pm S.E. \pm litters)

groups	parameter	AST(Units/ml)	ALT(Units/ml)	ALP(Units/ml)
Effect of adding some medical herbs seed (600 mg/ kg.B.W/day.)				
Control ration only(T1).		83.64 \pm 2.98 b	27.29 \pm 1.48 b	68.83 \pm 2.75 b
Control ration +black cumin seed (T2)		94.75 \pm 4.12 a	32.24 \pm 3.23 a	62.04 \pm 2.14 c
Control ration + fenugreek seed (T3).		95.31 \pm 4.54 a	31.97 \pm 3.17 a	62.92 \pm 2.31 c
Control ration + rocket seed (T4).		72.96 \pm 2.14 c	31.85 \pm 1.22 a	75.36 \pm 3.58 a
Effect of lamb age				
Four month(A1).		\pm 3.14 a 88.21	\pm 1.48 c24.93	\pm 2.31 c56.68
Six month(A2).		86.43 \pm 2.73 a	\pm 3.35 b30.88	68.21 \pm 2.75 b
Eight month(A3).		85.36 \pm 2.78 a	\pm 3.16 a36.65	\pm 3.64 a76.97
Effect of interaction between adding some medical herbs seeds and lamb age				
control ration only) \times (4 month)		86.02 \pm 2.64 b	\pm 1.48 c21.19	\pm 2.95 e58.21
(control ration+black cumin) \times (4 month)		\pm 3.91 a95.61	\pm 2.33 bc 26.23	\pm 2.31 f51.39
(control ration + fenugreek) \times (4 month)		96.81 \pm 4.77 a	\pm 1.31 bc 26.17	\pm 2.78 f52.97
(control ration + rocket) \times (4 month)		74.40 c \pm 2.14	\pm 2.31 bc 26.13	\pm 3.64 d64.17
(control ration only) \times (6 month)		83.41 \pm 2.31 b	\pm 3.85 bc 27.27	69.64 \pm 3.75 cd
(control ration+black cumin) \times (6 month)		94.54 \pm 4.12 a	\pm 3.78 ab 32.49	62.78 \pm 3.12 d
(control ration + fenugreek) \times (6 month)		95.58 \pm 3.78 a	\pm 3.69 ab31.96	63.81 \pm 3.35 d
(control ration + rocket) \times (6 month)		72.21 \pm 1.98 c	\pm 3.51 b31.80	76.61 \pm 4.53 bc
(control ration only) \times (8 month)		82.02 \pm 2.25 b	\pm 3.92 ab 33.15	\pm 4.64 b78.64
(control ration+black cumin) \times (8 month)		94.10 \pm 3.54 a	\pm 4.57 a38.02	\pm 4.12 c71.95

(control ration + fenugreek) ×(8 month)	93.55 ±3.22 a	±4.41 ab 37.80	±4.14 c 71.98
(control ration + rocket)×(8 month)	71.80 ±2.14 c	±4.28 ab 37.63	±4.92 a 85.30

*The small litters vertically refer to significant difference between groups at(P≤0.05).

Table 3:Effect of adding medical herbs seed and lamb age in the concentration of some hormones(Mean ±S.E. ± litters)

parameters groups↓	Triiodothyronine g./ml.)(μ	Thyroxin ng./ml.)(Testosterone ng./ml.)(
Effect of adding some medical herbs seed (600 mg/ kg.B.W/day.)			
Control ration only(T1)	1.38 ±0.01 b	3.11 ±0.01 c	1.58 ±0.01 b
Control ration +black cumin seed(T2)	2.29 ±0.02 a	6.82 ±0.04 a	2.71 ±0.02 a
Control ration + fenugreek seed (T3)	2.17 ±0.02 a	4.81 ±0.02 b	1.61 ±0.01 b
Control ration + rocket seed (T4)	1.98 ±0.01 a	6.38 ±0.04 a	2.42 ±0.02 a
Effect of lamb age			
Four month(A1).	2.72 ±0.01 a	±0.04 a 6.13	1.14 ±0.01 c
Six month(A2).	1.85 ±0.01 b	±0.03 b 5.21	1.79 ±0.01 b
Eight month(A3).	±0.02 c 1.29	±0.02 c 4.50	3.31 ±0.03 a
Interaction between adding medical herbs seeds and lamb age			
control ration only)×(4 month)(1.88 ±0.01 d	±0.04 cd 3.53	0.89 ±0.01 c
(control ration+black cumin)×(4month)	3.22 ±0.03 a	±0.05 a 7.83	1.52 ±0.01 c
(control ration + fenugreek) ×(4 month)	3.08 ±0.03 b	±0.03 bc 5.71	0.91 ±0.01 c
(control ration + rocket)×(4 month)	2.71 ±0.03 b	±0.05 a 7.45	1.24 ±0.01 c
(control ration only)×(6 month)	1.27±0.01 de	±0.01 cd 3.12	1.41 ±0.01 c
(control ration+black cumin)×(6 month)	2.13 ±0.02 c	±0.04 ab 6.73	2.31 ±0.02 b
(control ration + fenugreek) ×(6 month)	2.09 ±0.02 c	±0.02 bc 4.60	1.42 ±0.01 c
(control ration + rocket)×(6 month)	1.93 ±0.01 c	±0.04 ab 6.39	2.02 ±0.04 b
(control ration only) ×(8 month)	±0.01 e 1.01	±0.01 d 2.67	2.44 ±0.02 b
(control ration+black cumin)×(8 month)	±0.01 d 1.52	±0.03 b 5.89	4.29 ±0.04 a
(control ration + fenugreek) ×(8 month)	±0.01 de 1.33	±0.02 c 4.13	2.50 ±0.02 b

(control ration + rocket)×(8 month)	±0.01 de1.30	±0.03 bc 5.31	4.01 ±0.04 a
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*The small letters vertically refer to significant difference between groups at(P≤0.05).

The interaction between medical herb supplementation and lambs age had a significantly(P≤0.05) effect the activities of triiodothyronine, thyroxin and testosterone hormones.

Discussion:

The results revealed that activities of ALT, AST and ALP in serum(table 2) found in the present study were fall within the normal range reported for different Iraqi sheep breeds (22 &23).The significant(P≤0.05) increased in the activity of ALT and AST enzymes in T2 (table 2), may be due to that black cumin seeds contains active compound like thymoquinone that stimulated the digestive system functions through increased the production of enzymes through stimulated the liver function (24). The obtained results are in accordance with those reported by (25), who reported a significant increased in the activities of ALT and AST enzymes in blood of local ewes that supplemented by 5 gm./head/day of black cumin. While the significant (P≤0.05) increased in the activity of ALT and AST enzymes in T3, may be due that fenugreek seeds stimulated the liver function (26).The present results are in agreement with (27), they reported a significant increased in the activity of ALT and AST enzymes by using 200 mg.fenugreek seeds / buffalo / day . But the activity of ALP enzymes was significantly (P≤0.05) increased in T4 that supplemented by rocket seeds ,may be due to progressively increased in skeleton growth because some of ALP formed from muscle bones (28),or may be due to that rocket seed contain higher percentage of vitamin C (6) ,or may be due that have a highly significant a positive relation between the activity of ascorbic acid and the activity of ALP and there are relative relation between ascorbic acid and bone metabolism

(29).The obtained results are in accordance with those reported by (30) who reported that a significant increased in the activity of ALP with 5% level of rocket seed as compared to control group. While the significant (P≤0.05) decreased in the activity of AST enzyme in T4 that supplemented by rocket seeds, may be due to that rocket seeds contains carotenes especially β- carotene which play as antioxidant for lipids(31). The present results are in agreement with (32) they reported that a significant decreased in the activity of AST enzymes in *white Hy-Line breeder* roosters groups supplemented by 1 or 2 or 3 kg. rocket seeds/ton of feed as compared to control group.The activity of ALT and ALP enzymes were significantly (P≤0.05) increased , while the activity of AST was decreased mathematically as lambs get older (table 2), this may be due to the stress which caused by consuming the experimental medical herbs seeds (black cumin or fenugreek or rocket) ,or may be due that the activity of ALT increased as lambs get older because of fast growth of lambs and significantly increased in daily feed intake as animal get older(33),as the results for increasing the nutritional requirements for maintains and growth (11) , which result increasing the digestion and metabolism that happen in animal stomach which result finally increased the activity of serum ALT (34) ,beside that there is apposite relation between digestion and metabolism operation and the activity of ALT (35).The present results are in accordance with those obtained by (36)on Egyptian Barki lambs and (23)on Iraqi Awassi lambs, they found that activity of ALT enzyme was increased significantly as lambs get older. While the reason for significantly increasing the activity of ALP as lambs get older, this may be due to the fast

growth of skeleton of lambs and some part of ALP formed from bone tissues (28). The present results are in agreement with (37), who reported that a significant increased in the activity of ALP enzyme on short horn calves as get older. While the activity of ALT was decreased mathematically as lambs get older. The present results are in accordance with those obtained by (23&38) on Iraqi Awassi lambs and Pakistani Punjab lambs, respectively, they found that animal age did not affected the activity of AST.

The significant ($P \leq 0.05$) increased in the activity of triiodothyronine and thyroxin hormones in T2 (table 3), may be due that black cumin seeds increased significantly ($P \leq 0.05$) the digestibility of carbohydrates, proteins and lipids which caused a positive reflect to increased the coefficient digestibility of carbohydrates, proteins and lipids (39), or may be due to have appositive correlation between energy intake and the activity of triiodothyronine and thyroxin (40). The present results are in agreement with (10 & 41) they found that activity of triiodothyronine and thyroxin were increased significantly in Egyptian Ossami lambs groups that fed ration supplemented with 100 mg. or 100 and 200 mg. black cumin seeds powder/kg.B.W./day, respectively as compared to control group. While the significant increased in the activity of triiodothyronine and thyroxin hormones in T4 may be due that rocket seeds contain vitamin C and carotene which are sulfa for vitamin A (6) and vitamin A increasing the activity of thyroid gland through increased the activity of pituitary gland in thyroid stimulating hormones (TSH) which caused increased the secretion of the thyroxin gland through the triiodothyronine and thyroxin hormones (42). While the caused for a significant ($P \leq 0.05$) increased in mean values of thyroxin and a significant reduction of triiodothyronine in T3 that feed fenugreek, this may be explained by

the stimulatory the effects of fenugreek on the thyroid gland lead to lower the active thyroid hormone and triiodothyronine eventually will exert a negative feedback mechanism on the hypothalamus and pituitary gland which in turn increased the thyroid stimulating hormones of thyroxin (43). The present results are in agreement with (44) in Sudanese desert sheep, found that activity of triiodothyronine was increased significantly, while activity of thyroxin was decreased significantly in Sudanese desert sheep that fed ration supplemented with 5 or 25 g fenugreek seed /kg.B.W./day./day, as compared to control group, also similar results was reported by (45) in Nubian goat that fed rations contained 10 or 15% fenugreek seed respectively.

While the cause of a significant increased in the mean value of testosterone in T2 that fed black cumin seeds, this may be due to black cumin seeds that increased thyroid gland hormones activity (table 3), which may be lead to stimulant the activity of testes to increased production of testosterone and there are appositive relationship between thyroid gland activity and the gonad activity (40), or may be due that black cumin seeds rich in oil (26.6%). linoleic and archidonic acids are essential fatty acids, which considered as a precursor for the biosynthesis of prostaglandin (46), and it increases circulating of gonadotropin hormone and stimulated the steroid hormone production (testosterone) that is essential for normal reproductive function of male animals (46), or also due to that black cumin seeds contain considerable amount (0.32-1.8 ng./ml) of testosterone hormones (47), or may be due to that black cumin seeds contains some chemical compounds like phenols and alkaloids that have stimulate the secretion of testosterone hormones (48). The present results are in agreement with (10 & 41), they found that activity of testosterone was increased significantly in Egyptian Ossami lambs groups that fed

ration supplemented with 100 mg. or 100 and 200 mg. black cumin seeds powder /kg.B.W./ day, respectively as compared to control group. While the cause of a significant ($P \leq 0.05$) increased in the mean value of testosterone hormones in T4 that fed rocket seeds, may be due to that rocket seeds contain some nutritional compounds like vitamins E (49) and C (50) both play apposite effect on the activity of testosterone. The present results are in accordance with (30) who reported that a significant increased in the activity of testosterone when Iraqi Awassi lambs was fed ration contained 5% of rocket seed as compared to those on control ration. The activity of testosterone was decreased significantly ($P \leq 0.05$) in lambs in T3 that fed fenugreek seeds, this may be due to that fenugreek seeds contained some chemical compounds like diosgenin (51) and phytoestrogens (45) that compounds had a similar role to play as female sex hormones like estrogen, which may be caused in reduction of testosterone activity, or also may be due that fenugreek seeds contained saponin and diosgenin fractions which are precursor of progesterone and testosterone reducer which caused decreased the activity of testosterone (52).

The significant ($P \leq 0.05$) decreased in the activity of triiodothyronine and thyroxin as lambs get older (table 3), this may be due to that thyroxin gland

hormones has a relation with the environmental condition that surrounded animals, so the level of triiodothyronine and thyroxin will be elevated in cooled climate and declined in hot climate (53), or may be due to that animal to be liable to high temperature which caused significantly declined in the activity of thyroid gland (54), beside that our experiment was begin in cooler climate (January) and end in hot climate (June), or may be due to that thyroxin has deiodination operation to triiodothyronine, so the concentration of the thyroxin was decreased in blood. While the activity of testosterone was increased significantly ($P \leq 0.05$) as lamb get older (table 3), this may be due that there was apposite relationship between thyroid gland activity and the gonad activity (40), which finally reversal on increased the activity of testosterone as lamb get older. The present results are in agreement with (10) they found that activity of testosterone hormones was increased significantly in Egyptian Ossami lambs as lambs get older (4 and 8 months).

It can be concluded that supplementation with 600 mg. /kg.B.W./day of black cumin or fenugreek or rocket seeds to the rations of Awassi lambs without any adverse effect on the activity of some liver enzymes and hormones, however further studies are needed in this aspect.

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