

INFLUENCE OF ALFALFA SEED OIL SUPPLEMENTATION LEVELS ON GROWTH PERFORMANCE, BLOOD METABOLITES AND MUSCLE FATTY ACIDS IN BROILERS.

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SUMMARY

The objective of the present study was to investigate the effect of alfalfa seed oil (ASO) supplementation levels on growth performance, some blood metabolites and muscle fatty acids profile in broilers. A total of 600 one-day-old, unsexed Cobb broilers chicks were divided into four equal experimental groups (150 birds), each included three replicates of 50 chicks. The first treatment was control without any additives in feed; however, from second to fourth group were supplemented with 250, 500, 1000 g / ton feed of ASO. Body weight, body weight gain and mortality rate were not influenced by alfalfa seed oil supplementation. On the other hand, feed intake was significantly ($P=0.05$) decreased and thus, feed conversion ratio (FCR) was improved significantly ($P=0.03$). Supplementation of ASO in broiler diet did not affect the carcass, heart, liver, gizzard and breast and thigh muscle relative weights, while abdominal fat relative weight was decreased significantly ($P=0.03$) by added 1000 g ASO /ton feed. Plasma total protein, albumin, globulin, glucose and the activity of glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) were not affected by alfalfa seed oil supplementation levels, while plasma total cholesterol was significantly ($p=0.002$) decreased. Furthermore, the muscle contents of palmitic, oleic acid and liver MDA were not statistically ($P> 0.05$) affected by dietary alfalfa seed oil. However, the muscle contents of linolenic acid and vitamin E were significantly increased by increasing ASO supplementation level from 250 to 1000 g /ton feed, compared to the control group. Therefore, it could be concluded that alfalfa seed oil supplementation to broiler diets improved growth performance, modified plasma cholesterol and muscle fatty acids profile in broilers.

Keywords: *alfalfa seed oil, fatty acids, lipid peroxidation, cholesterol, broilers*

INTRODUCTION

Broiler chickens have great benefit as a source for human protein. Therefore, numerous studies focus on broiler's nutrition to maintaining sustainable broiler production to meet the human demand of protein. At the same time, recent trends in the field of poultry production are no longer focused only on quantity, but also began to pay attention in an unprecedented way to the quality of the meat produced and its health importance for the consumer. So that, in poultry industry the use of supplemental essential oils in broiler diets became a widespread practice to support high energy values required by chicken, as well as, improving the level of essential unsaturated fatty acids and natural antioxidants, which contributes to improving the quality of animal products (Elstaha *et al.*, 2019 and Saleh *et al.*, 2019).

Numerous studies tried to increase productive and reproductive performance of poultry by using essential oils (Bhatt and Swain, 2003; Mehmet *et al.*, 2010; Isabel and Santos, 2009; Chalghoumi *et al.*, 2013; Ghazanfari *et al.*, 2014; Khattak *et al.*, 2014; Mehr *et al.*, 2014 and Elstaha *et al.*, 2019), which extracted from medicinal and aromatic plants.

Alfalfa seed oil is one of essential oil was extracted from dried alfalfa seeds. Its unsaturated acid fraction contains representatives of three common types, linolenic (omega-3) and linoleic acids (omega-6), neither of which, apparently, is present there in isomeric form, and oleic acid (omega-9). It contains about 13.8% oleic acid (mono-unsaturated omega-9), 20.5 % linoleic acid (poly-unsaturated omega-6) and 20.7 % linolenic acid (poly-unsaturated omega-3) as published by (Schuette *et al.*, 1938; Jacobson and Holmes, 1916 and Rezvukin *et al.*, 1995), which used as a vegetable source of n-3, n-6 and n-9 UFA in broiler diets to improve productive performance and physiological status (Isabel and Santos, 2009; Mehmet *et al.*, 2010; Mukhtar, 2011 and Chalghoumi *et al.*, 2013).

Some authors studied the effect of essential oils such alfalfa seed oil or clove extract as feed additive on feed intake, feed conversion and mortality rate of broilers and they revealed that essential oil improved feed consumption, feed conversion ratio and mortality rate (Isabel and Santos, 2009; Cao *et al.*, 2010; Al-Mashhadani *et al.*, 2011; Ghazanfari *et al.*, 2014; Mukhtar, 2011 and Mehmet *et al.*, 2010).

From this point of view, the supplementation of alfalfa seed oil to the broiler chicken's diets may improve the level of essential unsaturated fatty acids and natural antioxidants, which contributes to improving the quality of animal products. Therefore, the current study was carried out to assess the impact of alfalfa seed oil supplementation levels on performance, blood metabolites and muscle fatty acids in broilers.

MATERIALS AND METHODS

Design and diets

Cobb broiler chicks (n= 600), one-day old were divided into four treatments, each included three replicates of 50 chicks (floor pens; ten birds/m²). Chicks were raised in a windowed experimental farm. The experiment was started during February and brooding heat was provided and the temperature inside the barn was maintained at around 32 to 34°C from day 1 to day 5 post-hatch, and gradually decreased to 24°C at 21 d. The control diet was based on corn, soybean meal and corn gluten meal (Table 1). The first treatment was control without any additives in feed; however, from second to fourth group were supplemented with 250, 500 and 1000 g/ ton diet of ASO. Broilers were fed ad-libitum starter, grower and finisher diets (day 1 to 42 of age) and water was available freely.

Growth performance

Mortality was registered daily through experiment period, while pen body weight (BW) and feed consumption were registered every week. Feed conversion ratio (FCR) was considered by dividing feed intake to the weight gain.

Blood and liver sampling

At the day 42 of age, broiler chickens (n= 24) based on average BW were selected (6 birds/treatment). They were weighed individually, slaughtered, and dissected to collect visceral organs. Weights of hot carcass, breast muscle, liver, heart, gizzard and abdominal fat were recorded. Blood samples were collected into heparinized test tubes and centrifuged (3000x g) for 20 min) at 5 °C to separate plasma and it was aspirated by pipette and stored in Eppendorf tubes at -20 °C pending analysis.

Biochemical analysis

Total protein, albumin, globulin, total cholesterol, glucose, glutamic oxaloacetic transaminase (GOT), and glutamate pyruvate transaminase (GPT) were measured by using kits from (Diamond Diagnostics, Egypt).

Fatty Acids levels in Breast Muscle

Palmitic, oleic and linolenic acids contents in breast muscle were analysed according to (Ceylan and Aksu 2011). Ten-grams of breast meat and 40 ml of 0.1 N HCl were homogenized for 45 s at 4°C then centrifuged 15,000 × g for 50 min at 4 °C. The supernatants were filtered and were analyzed using (GC-4 CM-PFE, Shimadzu gas chromatograph, Tokyo, Japan) equipped with a flame ionization detector (FID). The values were expressed in grams of fatty acid per 100 grams of breast meat.

Assaying malondialdehyde

Lipid peroxidation was evaluated by assaying level of malondialdehyde (MDA) in the liver by using kits from Cell Biolabs Inc. (San Diego, CA, USA).

Economic efficiency

Economic efficiency was calculated from the following equation:

$$\text{Economic efficiency (\%)} = \frac{\text{Net revenue (LE)}}{\text{Total feed cost (LE)}} \times 100$$

Statistical analysis

The differences between the experimental treatments and the control were analyzed with a General Linear model using SAS 9 (Statistical Analysis System, released October 2001). Tukey's multiple comparison test was used to identify which treatment conditions were significantly different from each other at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The effect of alfalfa seed oil supplementation levels on performance traits of broiler chicks are tabulated in Table (2). Results showed that, at the end of the experimental period, there were no significant ($P > 0.05$) differences among all treatments for the final body weight (gram/42 day), body weight gain (gram/42 day) and mortality rate. On the other hand, feed intake (FI) and feed conversion ratio (FCR) are significantly ($P \leq 0.05$) affected by alfalfa seed oil (ASO) supplementation levels. Whereas feed intake of birds fed on diet supplemented with ASO at the level of 1000 g/ton feed was significantly ($P = 0.05$) decreased by 4.33% compared with the control group. At the same time, FI of other groups supplemented with 250 or 500 g ASO/ton feed was insignificantly decreased by 2.85 and 3.63 % compared with the control group. The same direction was observed for FCR, whereas feed conversion ratio of supplemented groups with different levels of ASO was significantly ($P = 0.03$) improved. Birds fed on diet supplemented with 1000 g ASO/ton feed significantly ($P = 0.03$) had the best FCR followed by those received 500 g/ton and then group treated with 250 g/ton by 6.66, 5.45 and 1.82 % respectively, when compared with the control group.

Some authors studied the effect of essential oils such alfalfa seed oil or clove extract as feed additive on feed intake, feed conversion and mortality rate of broilers and they revealed that essential oil improved feed consumption, feed conversion ratio and mortality rate (Isabel and Santos, 2009; Cao *et al.*, 2010; Al-Mashhadani *et al.*, 2011; Ghazanfari *et al.*, 2014; Mukhtar, 2011 and Mehmet *et al.*, 2010). Also, the present results are agreeing with Mehr *et al.* (2014), they found that birds fed diets supplemented with 150, 300 and 450 ppm clove essential oil achieved the better feed consumption, feed conversion ratio and mortality rate than control. Khattak *et al.* (2014) noted that feed consumption, feed conversion ratio and mortality rate were significantly improved in the groups fed diet supplemented with natural blend of essential oils from basil, laurel, caraway, lemon, sage, tea, oregano and thyme than the control one. On the other hand, Chalghoumi *et al.* (2013) studied the effect of a commercial blend of essential oils (clove and cinnamon essential oils) and probiotic on performance of broiler chicks. They found that there hasn't been any statistical difference detected in ration of feed conversion throughout the dietary treatments in the whole experimental period. However, birds which were fed on diet without additives (probiotic/EOB) have shown numerically the worse FCR.

Data of carcass characteristics that influenced by dietary alfalfa seed oil supplementation levels are summarized in Tables (3). The current results reflected that; abdominal fat relative weight was significantly ($p = 0.03$) decreased by increasing ASO supplementation level from 250 to 1000 g/ton feed compared to the control one. However, carcass, heart, liver, gizzard, breast muscle and thigh muscle relative weights were not affected by supplementation of ASO to broiler diet. These results are in line with the results observed by (Lee *et al.*, 2004; Mehmet *et al.*, 2010; Cao *et al.*, 2010; Al-Mashhadani *et al.*, 2011; Khaksar *et al.*, 2012; Mukhtar, 2011 and Ghazanfari *et al.*, 2014). They found that abdominal fat relative weight was decreased significantly with increasing essential oil supplementation levels in the diet.

Table (4) shows the effect of alfalfa seed oil (ASO) supplementation levels on some blood biochemical parameters of broilers. Plasma total protein, albumin, globulin, glucose and the activity of liver enzymes (GOT and GPT) were not statistically ($P > 0.05$) affected by dietary alfalfa seed oil. However, plasma total cholesterol was significantly ($P = 0.002$) reduced by 11.95, 15.72 and 20.12% for groups supplemented with 250, 500 and 1000 g ASO/ton feed respectively, compared to the control group.

These modifications in plasma total cholesterol might be connected with a low statistical feed intake ($P = 0.05$) (Table 2). Additionally, the decreasing in plasma cholesterol values may be due to phospholipids and poly unsaturated fatty acids PUFA; oleic, linoleic and linolenic in alfalfa seed oil that inhibits accumulation of lipid peroxidation product (Rezvukin *et al.*, 1995 and Wang *et al.*, 2009). The dietary monounsaturated fatty acids (e.g., oleic acid) were very effective in lowering blood cholesterol concentration and may be important in preventing coronary heart disease (Hornstra, 1980). The current

results of biochemical traits are compatible with the finding of (Elstiha *et al.*, 2019) that showed rabbits fed diet supplemented with the essential oil (sunflower oil) have shown remarkable positive decrease on total cholesterol. Also, Khaksar *et al.* (2012) investigate the influences of thyme essential oil (TEO) and showed that, serum total cholesterol was decreased by dietary TEO of Japanese quail.

The data illustrated in Table 5 shows the effect of ASO supplementation levels on the muscle content of fatty acids, and liver MDA and vitamin E content. The muscle contents of Palmitic, oleic acid and liver MDA were not statistically ($P > 0.05$) affected by dietary alfalfa seed oil. However, the muscle contents of linolenic acid and vitamin E were significantly increased by increasing ASO supplementation level from 250 to 1000 g /ton feed, compared to the control group. The muscle content of linolenic acid was significantly ($P=0.02$) increased by 3.06, 34.66 and 36.93% for groups supplemented with 250, 500 and 1000 g ASO/ton feed respectively, compared to the control group. The same trend was observed for the muscle content of vitamin E, which significantly ($P=0.047$) increased by 12.24, 20.40 and 24.48% for groups supplemented with 250, 500 and 1000 g ASO/ton feed respectively, compared to the control group.

Levels of fatty acids (FA) in the animals' meat, including broiler chickens, rely to a great extent on their diet levels; also, FA Levels of in products (eggs, meat, milk) represent a composition of biosynthesis and FA digested lipids. It has also been concluded that fatty acid which is linolenic and linoleic acids are categorized as substantial, a matter that implies that the organism cannot generate them, so they must be included in the feed. It has also been proposed possible directions for future research has to be focused on the developing broiler chicken hybrids including more preferable levels of n-3 FA and n-6 FA within meat and fat , also it becomes foreseeable that chickens' meat may be become efficient ground of n-3 FA for the diet of humans (Suchy *et al.*, 2016). These results may attribute due to phospholipids and poly unsaturated fatty acids PUFA; oleic, linoleic and linolenic in alfalfa seed oil that inhibits accumulation of lipid peroxidation product and improved the muscle contents of PUFA and antioxidants indices (Rezvukin *et al.*, 1995 and Wang *et al.*, 2009).

Table (6) showed economic efficiency of broilers chicks fed test diets for 42 days. Results showed that, economic efficiency was increased by increasing alfalfa seed oil supplementation level up to 1000 g/ton feed. While broilers fed diet supplemented with 1000 g/ton had the highest relative economic efficiency followed by those fed diet supplemented with 500 g/ton and then those received 250 g/ton by 12.5, 11.00 and 4.90% respectively, compared to control.

CONCLUSION

Based in the data presented above, it could be concluded that added NSP liquid enzyme in drinking water by 8 ml/L water might be involved in growth performance, lipid peroxidation and modified plasma and muscle lipids profile in broiler chickens.

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تأثير اضافة مستويات من زيت بذرة البرسيم علي الاداء الانتاجي وخصائص الدم البيوكيميائية ومحتوي العضلات من الاحماض الدهنية لبداري التسمين.

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تهدف هذه الدراسة الي تقييم تأثير اضافة زيت بذرة البرسيم علي الأداء الانتاجي وخصائص الدم البيوكيميائية ومحتوي العضلات من الاحماض الدهنية لبداري التسمين. استخدم في هذه الدراسة 600 كتكوت غير مجنس عمر يوم واحد من سلالة الكب ، وقسمت عشوائيا الي اربع مجموعات تجريبية بكل منها 150 كتكوت. تتغذي المجموعة الاولى وهي مجموعة المقارنة على عليفة تغطي الاحتياجات الغذائية لسلالة الكب بدون اي اضافات، أما المجاميع التجريبية الثلاثة الأخرى فتتغذي على العليفة الكنترول مضاف اليها مستويات مختلفة من زيت بذرة البرسيم (250، 500، 1000 جم/طن علف). تشير نتائج هذه التجربة الي عدم وجود اي اثار معنوية لاضافة زيت بذرة البرسيم على كلا من وزن الجسم، معدل الزيادة في وزن الجسم، ونسبة النفق، بينما كان هناك انخفاض معنوي في كمية العلف المأكولة وتحسن في الكفاءة التحويلية. كذلك فان اضافة زيت بذرة البرسيم الي علائق بداري التسمين لم يؤثر معنويا على الوزن النسب لكلا من الذبيحة، القلب، الكبد، القانصة وعضلات الفخذ او الصدر، في حين كان هناك انخفاض معنوي في الوزن النسبي لدهون البطن وخاصة مع مستوي 1 كجم /كجم علف. لم يحدث تغير معنوي في قياسات الدم البيوكيميائية لكلا من البروتين الكلي، الألبومين، الجلوبيولين، الجلوكوز ونشاط انزيمات الكبد (جلوتاميك اكسالواسيتك ترانزأمينز، جلوتاميك بيروفيك ترانزأمينز). حدث انخفاض معنوي في مستوي الكولسترول الكلي ببلازما الدم نتيجة اضافة زيت بذرة البرسيم. لم يتأثر علاوة على ذلك مستوي بعض الاحماض الدهنية بالعضلات مثل (البالمك، الأوليك) وكذا مستوي المالوندالدهيد في الكبد بينما حدث زيادة معنوية في مستوي العضلات من حامض اللينولينك وكذا فيتامين هـ بزيادة مستوي الاضافة من زيت بذرة البرسيم من 250: 1000 جم /كجم علف. تشير نتيجة الدراسة الاقتصادية الي حدوث تحسن ملحوظ في الكفاءة الاقتصادية لقطعان بداري التسمين باستخدام زيت بذرة البرسيم حتى مستوي 1كجم/كجم علف.

نخلص من هذه الدراسة انه يمكن استخدام زيت بذرة البرسيم في علائق بداري التسمين لتحسين الاداء الانتاجي والحالة الفسيولوجية وحالة مضادات الاكسدة وجودة الذبيحة وكذا الكفاءة الاقتصادية حتى مستوي 1كجم/كجم عليفة.

Table (1): The composition and calculated analysis of basal diets

Ingredient	Experimental diet		
	Starter 0-14 days	Grower 15-30 days	Finisher 31-42 days
Yellow corn	575	615	650
Soybean meal (44%)	320	278	240
Corn gluten meal (60%)	58	50	40
Premix ¹	3	3	3
Vegetable oil	7.5	17.5	30.5
Dicalcium phosphate	15	15	15
Limestone	13.9	13.2	12.5
Salt	3	3	3
DL-Methionine	1.85	1.8	1.65
L. Lysine	2.75	3.5	4.35
Total	1000	1000	1000
Calculated analysis			
Crude protein (Analyzed) (%)	23	21	19
Digestible Lys	1.19	1.14	1.11
Digestible Meth + Cysteine	0.84	0.78	0.71
Digestible Thr	0.77	0.70	0.63
Calcium	1.0	0.94	0.91
Available phosphorus	0.46	0.45	0.44
ME, MJ/Kg	12.43	12.85	13.15

^{**} Each 3kg of premix contained: Vit. A 12000IU, Vit. D 2200IU, Vit. E 10mg, Vit. K₃ 2000mg, Vit. B₁ 1000mg, Vit. B₂ 3000mg, Vit. B₆ 1300mg, Vit. B₁₂ 10mg, Pantothenic acid 10mg, Niacin 30mg, Folic acid 1000mg, Biotin 50mg, Choline chloride 300mg, Manganese 60mg, Zinc 50mg, Copper 10mg, Iron 30mg, Iodine 1000mg, Selenium 100mg, Cobalt 100mg and CaCo₃ to 3g.

Table (2): Effect of alfalfa seed oil supplementation levels on performance traits

Treatments	Initial body weight, gram	Final body weight, gram/42 day	Body weight gain, gram/42 day	Feed intake, gram/42 day	FCR	Mortality, %
Control (C) Basal diet	43.66±0.33	2340 ±32	2296.3±32.7	3966±16.77 ^a	1.65±0.15 ^a	0.66±0.33
250 gram/ton feed	44.0±0.57	2375±24	2332±24.8	3853±16.3 ^{ab}	1.62±0.026 ^{ab}	0.33±0.33
500 gram/ton feed	44.66±1.19	2444±33	2400.7±33.3	3822±24.7 ^{ab}	1.56±0.013 ^{bc}	0.33±0.33
1000 gram/ton feed	44.98±0.17	2460±58	3415±58.6	3794±8.1 ^b	1.54±0.03 ^c	0±0.00
significance	0.56	0.17	0.18	0.05	0.03	0.48

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan ,s Multiple Range Test.

Table (3): Effect of alfalfa seed oil supplementation levels on carcass traits.

Treatments	Carcass, g/100g bw	Heart weight, g/100g bw	Liver weight, g/100g bw	Gizzard weight, g/100g bw	Abd. fat weight, g/100g bw	Breast muscle weight, g/100g bw	Thigh muscle weight, g/100g bw
Control (C) Basel diet	70.32±2.7	0.511±0.02	3.06±0.15	2.46±0.09	1.44±0.112a	21.995±0.92	19.816±1.41
250 gram/ton feed	70.53±2.6	0.514±0.02	3.15±0.20	2.45±0.16	1.238±0.07ab	22.088±1.45	19.715±0.94
500 gram/ton feed	70.63±1.5	0.514±0.04	3.125±0.09	2.44±0.14	1.193±0.06ab	22.101±0.66	19.753±0.76
1000 gram/ton feed	70.41±2.8	0.513±0.024	3.158±0.07	2.48±0.10	1.083±0.06b	22.311±0.79	19.928±0.60
Significance	0.99	0.90	0.96	0.91	0.03	0.12	0.23

-Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

-Values are expressed as means ± standard error. Gram/100 gram body weight (g/100g BW)

Table (4): Effect of alfalfa seed oil supplementation levels on some blood constituents of broilers.

Treatments	Total protein mg/dl	Albumin mg/dl	Globulin mg/dl	GPT u/l	GOT u/l	Cholesterol mg/dl	Glucose mg/dl
Control (C) Basel diet	4.28±0.14	2.4±0.05	1.85±0.15	54.33±4.6	242±15	159±6a	183±18
250 gram/ton feed	4.2±0.09	2.35±0.03	1.81±0.07	50.83±3.2	230±6	140±3b	182±10
500 gram/ton feed	4.26±0.08	2.41±0.09	1.81±0.13	51.5±2.2	228±7	134±6b	183±8
1000 gram/ton feed	4.16±0.14	2.38±0.10	1.76±0.17	50.33±2.8	230±8	127±2b	186±19
Significance	0.89	0.92	0.88	0.83	0.71	0.002	0.91

^{a,b} Mean values with different letters in the same column differ significantly at $p < 0.05$. Values are expressed as means ± standard error.

Glutamic oxaloacetic transaminase (GOT); glutamate pyruvate transaminase (GPT)

Table (5): Effect of alfalfa seed oil supplementation levels on muscle fatty acids and liver MDA content in broilers.

Treatments	Palmatic, mg/100 g fat	Oleic Acid, mg/100 g fat	Linolenic Acid, mg /100 g fat	Liver MDA, nanomole/gram liver	Vitamin E, mg/gram muscle
Control (C) Basel diet	0729±0.019	0.309±0.002	0.326±0.007 ^c	5.32±0.44	0.049±0.005 ^b
250 gram/ton feed	0.641±0.040	0.313±0.006	0.336±0.003 ^{bc}	5.15±0.48	0.055±0.001 ^{ab}
500 gram/ton feed	0.641±0.040	0.323±0.008	0.439±0.031 ^{ab}	5.14±0.31	0.059±0.001 ^a
1000 gram/ton feed	0.616±0.076	0.321±0.008	0.479±0.061 ^a	4.91±0.26	0.061±0.001 ^a
Significance	0.22	0.45	0.02	0.90	0.047

^{a,b}Mean values with different letters in the same column differ significantly at $p < 0.05$.

Values are expressed as means ± standard error.

Table (6): Effect of alfalfa seed oil supplementation levels on economic efficiency of broiler chick.

Item	Treatment			
	T1 control	T2 (250g ASO/ton)	T3 (500g ASO/ton)	T4 (1000g ASO/ton)
Total feed intake (g)	3966	3853	3822	3794
Price/kg diet (L.E.)	6.80	6.82	6.83	6.86
Total feed cost (L.E.)	26.97	26.27	26.10	26.02
Average body weight (g)	2340	2375	2444	2460
Selling price* (L.E.)	58.50	59.37	61.10	61.50
Net revenue** (L.E.)	31.53	33.10	35.00	35.48
Relative E.EF.***	100	104.9	111.0	112.5

* Selling price = 25.0 L.E. × Average body weight (kg)

** Net revenue = Selling price of meat yield-feed cost.

*** Assuming that the relative economic efficiency of control diet equal to 100.

- The price of 1 kg alfalfa seed oil= 60 L.E.