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EFFECT OF SUPPLEMENTATION OF BROILER DIETS WITH GUAVA LEAVES AND/OR OLIVE OIL ON GROWTH, MEAT COMPOSITION, BLOOD METABOLITES AND IMMUNE RESPONSE

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ABSTRACT

This trail was conducted to study the effect of dietary supplementation of guava leaves (*Psidium guajava)* and/or olive oil on performance, meat composition, some blood metabolites, and immune response in broiler chicks. 240 (one-day) commercial broiler chicks (Cobb) were divided into four groups. Chicks were fed four experimental diets for 42 days; as a control diet without supplementation (group 1), control diets supplemented with 1% dried guava leaves (group 2), 1% olive oil (group 3) and mixture of 0.5% olive oil + 0.5% dried guava leaves (group 4). The results revealed that dietary supplementation of dried guava leaves and/or olive oil had a significant improved effect on BW, weight gain and FCR. Dietary supplementation of dried guava leaves and/or olive oil significantly increased DM and decrease ether extract of both breast and thigh meat. On other hand, supplementation of dried guava leaves and/or olive oil significantly ($P > 0.05$) decreased the level of lipids metabolites except for LDL-C. In addition, feeding of dried guava leaves and/or olive oil reflect higher bird immunity guardad by higher concentrations of total protein, globulin and higher values of total leukocytic count when compared to the control. Moreover, activity of antioxidant and liver enzymes were significantly improved by addition of guava leaves and/or olive oil. It could be concluded that supplementation of broiler diets with dried guava leaves and/or olive oil significantly improved performance and health status of the birds.

Key words: Guava leaves, Olive oil, Growth, meat composition, Immunity, Broiler.

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1. INTRODUCTION

The poultry production especially broilers is one of the largest and fastest growing agro-based industries in the World and provides the opportunity of meeting animal protein needs for humans. However, the adverse effect of animal protein is hypercholesterolemia causing heart and arteries diseases. It is generally accepted that dietary saturated fatty acids directly are related to plasma cholesterol levels. Fat supplementation in diets has been proven a valuable method for fulfilling the high energy requirements of rapidly growing broiler chickens. It has been well documented that the growth performance and feed conversion ratio of the broilers are influenced by dietary supplementation with fat (1). Olive oil with an abundant quantity of MUFAs (oleic acid with palmatic and lenoleic acids in smaller proportions) thought to not only contribute nutrients to the diets, but also to influence the fatty acids profiles in muscles and fat in monogastric animals (2). The beneficial effects of olive oil can be attributed not only

to the close relationship between unsaturated and saturated fatty acids, but also to the antioxidant property of its phenolic compounds. The main antioxidants of olive oil are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. In experimental studies, the antioxidant capacity of olive oil has been widely studied and it is known that after ingestion, the phenolic compounds of olive oil as oleuropein or hydroxytyrosol may display local antioxidant capacity in the gastrointestinal tract. Moreover, after absorption and metabolism of olive oil, the antioxidant actives may display their activity at the cellular level (3). Olive oil also increase birds immunity as dietary supplementation of either canola or olive oils (2%) to broiler chick increased immune response to Newcastle disease, final live BW and daily gain compared to control without affecting triglycerides, low density lipoprotein, very low density liopprotein and saturated fatty acids (SFA) when compared with the control (4). The leaves of guava are rich in flavonoids in particular quercetin, saponins, tannins, alkaloids anthraquinones, phlobatannins and cardiac glycosides. Moreover, theses flavonoids had antibacterial activity (5). Quercetin acts as calcium antagonist, affecting intestinal smooth muscle fibers and is responsible for the antispasmodic and anti-motility effect of guava leaves (6). 3,4-dihydroxybenzoic acid produced by peroxidase-dependent oxidation of quercetin enhanced their antibacterial and antioxidant effectiveness. High amounts of phenolic compounds in the leaves of white (*Psidium guajava* var. pyrifera L.) showed antioxidant activity (7). All parts of *Psidium guajava* including leaves have been used for treating stomachache and diarrhea in many countries. Leaves, pulp and seeds are used to treat respiratory and gastrointestinal disorders and as an antispasmodic, anti-inflammatory, as a cough sedative, anti-diarrheic, in the management of hypertension, obesity and in the control of diabetes mellitus. In addition, it possesses anticancer properties (8). The objective of the present study was to investigate the effect of dried guava leaves and/or olive oil supplementation on productive performance and general health status of growing chicks

2. MATERIALS AND METHODS

240 (one-day-old) unsexed broiler chicks of a commercial meat type (Cobb) were used in this study. The chicks were weighed and randomly divided into 4 experimental groups with three replicates per group (60 chicks/group; 20 chicks/replicate). First group served as control diet without additives, while groups 2, 3, and 4 fed control diet supplemented with 1% dried guava leaves, 1% olive oil and mixture of 0.5% olive oil + 0.5% dried guava leaves. Birds were reared in a naturally ventilated house with continuous lighting. There were three feeding phases: starter phase (0-21 days; CP 22.72% and ME 3204.83 kcal/kg diet); and grower phase (22-35 days; CP 20.51% and ME 3202.33 kcal/kg diet) and finisher phase (36-42 days; CP 18.04 % and ME 3204.43 kcal/kg diet). The diets were offered in mash form. Feed and water were provided *ad-libitum*.

All diets were formulated to cover the nutrient requirements of chicken (9). Ingredients and the composition of the experimental diets are shown in Table 1. Chicks were vaccinated against Newcastle and Gumboro diseases. Body weight was determined through the period of starter and finisher and feed intake was recorded for the corresponding periods and feed conversion ratio was calculated.

2.1.Additives used:

1-Olive oil: consists of saturated fatty acids: polyunsaturated fatty acids (9.24 g/100 g) and monounsaturated fatty acids (61.259.24 g/100 g). Energy: 899.87 Kcal.
2-Ground guava leaves: contain 94.34% dry matter, 13.2% crude protein, 4% ether extract and 24% ash.

2.2.Growth performance parameters:

Body weight was determined through the period of starter and finisher and feed intake was recorded for the corresponding periods. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

2.3.Meat composition:

At day 42, 3birds/group were randomly selected and slaughtered. Samples from breast and thigh muscle were collected for determination of dry matter, crude protein, fat and ash (10).

2.4.Clinicopathological and immunological examination:

At 42 days, 3 birds were randomly selected from each group and slaughtered for collection of blood samples. Blood was divided in to two parts, the first part was collected in clean centrifuge tube containing anticoagulant (EDTA), used for counting total leukocyte and differential leukocyte (16). The second part was collected into clean centrifuge tube without anticoagulant for separation of serum to determine total protein (11), albumin (12), while globulin was calculated.

High-density lipoprotein (HDL) (13) Total cholesterol and triglyceride (15); and low density lipoprotein (LDL) were calculated according to (18). Aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) were determined as described by (14, 17). Superoxide dismutase (SOD) activity and glutathione peroxidise (GSH-PX) were measured according to (19).

2.5.Statistical analyses:

Data were analyzed by using the General Linear Model procedure (20). Difference between treatment means were compared using Duncan's multiple range test Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

3. RESULTS

3.1. Growth performance:

Growth performance of broiler chicks fed the experimental diets is shown in Table 2. The result revealed significant ($P<0.05$) increased in average final BW and body weight gain of the groups fed diet supplemented with dried guava leaves and/or olive oil compared to control group.

3.2. Meat composition:

Dietary supplementation with dried guava leaves and/or olive oil significantly ($P < 0.05$) increased dry matter and decrease ether extract of both breast and thigh meat as shown in Table 4.

3.3. Lipid profiles and antioxidant enzymes:

Effect of dietary dried guava leaves and/or olive oil supplementation on total cholesterol, triglycerides or lipoproteins showed significant changes ($P<0.05$) as shown in Table 4. Except LDL was not significantly changed.

3.4. Immune response:

Feeding broiler on diets supplemented with dried guava leaves and/or olive oil had significant ($P<0.05$) increase in serum total protein, globulin concentration, total leukocytes, lymphocyte and monocyte count when compared to control as shown in Table 5.

Table 1. Physical and chemical composition (%) of the experimental diets.

	Experimental diets		
Ingredients	Starter	Grower	Finisher
Yellow corn	57.35	62.40	69.1
Soybean meal, 48%	25.4	23.25	18.7
Corn gluten, 60%	6.5	3.8	3.5
Fish meal, 65%	3.5	3.5	2.5
Soybean oil	3.5	3.3	2.5
Calcium carbonate	1.2	1.2	1.2
Calcium dibasic phosphate	1.5	1.5	1.5
Common salt	0.3	0.3	0.3
Premix[1]	0.3	0.3	0.3
DL- Methionine, 98%	0.2	0.2	0.18
Lysine, Hcl, 78%	0.15	0.15	0.12
Toxenil	0.1	0.1	0.1
Calculated composition			
ME, Kcal/Kg	3204.83	3202.33	3204.436
CP, %	22.72	20.513	18.043
EE, %	6.138	6.0326	5.376
CF, %	2.411	2.3988	2.3689
Ca, %	1.202	1.198	1.1378
Available phosphorus, %	0.464	0.458	0.4307
Lysine, %	1.293	1.0949	1.0119
Methionine, %	0.651	0.5487	0.5352

[1] Muvco premix: Each 2.5kg contain vit. A (10, 000000 IU), vit. D3 (2, 000000 IU), vit. E (10 g), vit.k3 (1000 mg), vit. B1 (1000 mg), vit. B2 (5g), vit. B6 (1.5 g), pantothenic acid (10 g), vit. B12 (10 mg), niacin (30 g), folic acid (1000 mg), biotin (50 g), fe (30 g), Mn (60 g), Cu (4 g), I (300 mg), Co (100 mg), Se (100 mg) and Zn (50 g).

Table 2. Effect of dietary dried guava leaves and/or olive oil on the overall (0-6 wks) performance of broiler chickens (mean ± SE).

	Experimental diet			
Parameters	Control	1% Guava leaves	1% Olive oil	0. 5% Guava leaves + 0. 5% Olive oil
Initial body weight (g)	37.52 ± 0.02	37.52 ±0.01	37.51 ±0.01	37.51 ±0.02
Final body weight (g)	2006.42 ±8.28^{d}	2090.81±14.81^{c}	2239.76 ±12.62^{a}	2166.07 ±10.22^{b}
Absolute weight gain (g)	1968.90 ±8.27^{d}	2053.29 ±14.80^{c}	2202.25 ±15.62^{a}	2128.56 ±10.24^{b}
Total feed consumption	3891.77 ±5.44^{a}	3696.03 ±6.74^{ab}	3663.98 ±3.97^{b}	3787.18 ±5.03^{ab}
Feed conversion ratio	1.98 ±0.01^{a}	1.80 ±0.02^{b}	1.67 ±0.05^{c}	1.78 ±0.03^{bc}

^{abcd} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 3. Effect of dietary dried guava leaves and/or olive oil on chemical composition (fresh basis) of breast and thigh muscles of broiler chicks (mean ± SE).

	Experimental diet			
Parameters	Control	1% Guava leaves	1% Olive oil	0. 5% Guava leaves + 0. 5% Olive oil
Breast, DM %	24.54 ± 0.09^{c}	27.20 ± 0.46^{a}	25.40 ± 0.12^{b}	25.83 ± 0.20^{b}
Breast , CP %	23.48 ± 0.25	22.49 ± 0.17	22.12 ± 0.45	23.48 ± 0.76
Breast , EE %	2.26 ± 0.10^{a}	1.79 ± 0.03^{b}	1.22 ± 0.04^{c}	1.60± 0.07^{b}
Breast , Ash %	0.22 ± 0.01^{a}	0.17 ± 0.01^{b}	0.22 ± 0.02^{a}	0.25 ± 0.01^{a}
Thigh, DM %	23.55 ± 0.22^{d}	26.13 ± 0.10^{a}	24.19 ± 0.17^{c}	25.50 ± 0.14^{b}
Thigh, CP %	22.44 ± 0.52	22.36 ± 0.23	22.24 ± 0.15	23.31 ± 0.27
Thigh, EE %	2.60 ± 0.35^{a}	2.30 ± 0.06^{ab}	1.39 ± 0.10^{c}	1.70 ± 0.06^{bc}
Thigh, Ash %	0.20 ± 0.03	0.18 ± 0.01	0.18 ± 0.01	0.23 ± 0.01

^{abc} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 4. Effect of dietary dried guava leaves and/or olive oil on some blood biochemical parameters and antioxidant effect of broiler chicks (mean ± SE).

Parameters	Experimental diet			
	Control	1% Guava leaves	1% Olive oil	0. 5% Guava leaves + 0. 5% Olive oil
Total protein, g/dl	4.02 ±0.02^{c}	4.76 ±0.15^{ab}	4.45 ±0.09^{b}	4.90 ±0.12^{a}
Albumin, g/dl	1.69 ±0.06	1.94 ±0.09	1.69±0.02	1.83 ±0.10
Globulin, g/dl	2.33 ±0.04^{b}	2.82 ±0.24^{a}	2.76±0.07^{a}	3.07 ±0.02^{a}
A / G ratio	0.73 ±0.04	0.70 ±0.09	0.61±0.01	0.59 ±0.03
ALT, U/L	9.42 ±0.31^{a}	8.25±0.43^{b}	7.58±0.36^{b}	6.13±0.07^{c}
AST, U/L	64.11 ±0.81^{a}	60.11±0.49^{b}	59.79±1.47^{bc}	56.50±1.13^{c}
Triglyceride, mg/dl	54.36 ±1.02^{a}	51.19 ±0.72^{b}	50.25 ±1.18^{b}	49.31 ±0.85^{b}
HDL-cholesterol, mg/dl	88.52 ±1.07^{a}	83.29 ±1.19^{b}	79.32 ±1.20^{c}	80.04 ±1.16^{bc}
LDL-cholesterol, mg/dl	42.19 ±1.17	40.07±1.16	38.83±1.59	40.13±1.16
Superoxide dismutase	0.33 ±0.01^{d}	0.54± 0.02^{c}	0.82 ±0.07^{b}	0.92 ±0.03^{a}
Glutathione Peroxidase	0.43 ±0.03^{c}	0.56 ±0.02^{b}	0.69 ±0.02^{a}	0.66 ±0.04^{a}

^{abcd} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 5. Effect of dietary dried guava leaves and/or olive oil on total and differential leukocytic count (cell $\times 10^3$ /µl) of broiler chicks (mean ± SE).

Parameters	Experimental diet			
	Control	1% Guava leaves	1% Olive oil	0. 5% Guava leaves + 0. 5% Olive oil
Leukocytic count	24.93 ±0.31^{b}	27.88±0.34^{a}	25.51 ±0.10^{b}	27.73 ± 0.60^{a}
Heterophil	8.36 ±0.17	8.60 ±0.18	8.80 ±0.15	8.77 ±0.15
Esinophil	0.74 ± 0.01	0.73 ±0.02	0.72 ±0.01	0.73 ±0.02
Basophil	0.23 ±0.01	0.21 ±0.01	0.21 ±0.01	0.22 ±0.01
Lymphocyte	14.16 ±0.09^{c}	16.53 ±0.29^{a}	14.15 ±0.09^{c}	15.66 ±0.36^{b}
Monocyte	1.44 ±0.04^{b}	1.81 ±0.11^{a}	1.63 ±0.04^{ab}	1.76±0.01^{a}

^{abc} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

4. DISCUSSION

The obtained results herein revealed that the supplementation of dried guava leaves and/or olive oil enhanced the growth performance of broilers. These results are in accordance with previous results (22) which affirmed that dietary supplementation of 0.5% and 2% olive oil to broiler chicks significantly (P<0.05) increased final live body weight and daily gain broiler chicken and had no significant effect on the amount of feed intake compared to control. Beneficial effects of supplementing olive oil on broilers performance may be due to it facilitates overall digestion and absorption of nutrients, including crucial fat, soluble vitamins. Researchers estimate that 55- 66% of polyphenols from olive oil are absorbed after ingestion, primarily in the small intestine. Guava leaves extract and olive oil has anti-inflammatory antibacterial and antimicrobial activities that induce positive effects on broilers gut health (5, 8). On the other hand, some researchers (22) and (23) declared that use of 0.04% or 0.06% of guava leaves extract in poultry ration didn`t have significant effect on BW and weight gain. The changes in broilers body composition because of dried guava leaves and/or olive oil are supported by previous work (21) who mentioned that 0.04% or 0.06% of guava leaves extract have significant effect on meat composition. It is documented that dietary PUFA or olive oil addition promotes lean tissue deposition, inhibits lipid synthesis and increases fatty acid oxidation (23). These effects could explain the reason why the fat content of the carcass reduced by dietary plant oils inclusion. The effect of dried guava leaves and/or olive oil supplementation on lipid profile showed significant (P > 0.05) decreased level of lipids metabolites except for LDL-C. These results agree with the previous one (24) who showed that *psidium guava* extract administration to broiler diet could decrease triglycerides, cholesterol, VLDL and LDL cholesterol and also increase HDL cholesterol. On the other hand, other research (4) stated that colorimetric analysis of serum revealed that, triglycerides, total cholesterol and VLDL values weren't significantly differ among broiler groups fed diet supplemented with 2% olive oil alone or with canola oil. In addition, 3% olive oil showed decrease in LDL and triglyceride did not reduce the HDL level in broiler diet (25). Triglycerides are secreted from the liver into the blood by triglyceride-rich lipoproteins; therefore, impaired hepatic lipogenesis results in decreased triglyceride concentrations in plasma (26).

Super oxide dismutase enzyme and glutathione peroxidase enzyme had significantly (P < 0.05) increased by dried guava leaves and/or olive oil supplementation. These result are similar to previous results (7) which reported the presence of higher amounts of phenolic compounds with antioxidant activity in the leaves of white (*Psidium guajava* var. *pyrifera L.*). Furthermore, in vitro study, phenolic compounds in olive oil inhibit low-density lipoprotein (LDL) oxidation (27). This may also explained as enrichment muscle cell membranes by PUFAs led to a decrease in the oxidative stability of the muscle tissue and an increase in the antioxidant enzyme activity. Furthermore, in case of Olive oil, enrichment in PUFAs of muscle cell membranes led to a decrease in the oxidative stability of the muscle tissue and an increase in the antioxidant enzyme activity, similar results have been reported in rats (28).

Feeding broiler on diets supplemented with dried guava leaves and/or olive oil had significant (P<0.05) increase in serum total protein, globulin concentration, total leukocytes, lymphocyte and monocyte count. Positive effects of supplementing guava leaves on broilers immunity may be due to the presence of presence of flavonoids, which

have anti-microbial activity (5). Flavonoids derivatives have been found to inhibit the growth of *Staphylococcus aureus* at the dilution of 1: 10,000 (30). Terpenes and a certain degree of lipophilicity might determine toxicity by the interactions with the membrane constituents and their arrangement, (29). This is medically important in the treatment of inflamed tissues. Lectins in guava were shown to bind to *E. coli* preventing its adhesion to the intestinal wall and thus preventing infection (31).

It could be concluded that supplementation of broiler chicken diets with dietary dried guava leaves and/or olive oil had significantly improved growth performance, serum lipid profiles, antioxidant activity, liver enzymes, total leukocytic count, increased dry matter and decrease ether extract of both breast and thigh meat.

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تأثير إضافة أوراق الجوافة وزيت الزيتون على معدلات النمو ومكونات اللحم وبعض مكونات الدم والمناعة في علائق بداري التسمين

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قسم التغذية والتغذية الإكلينيكية – كلية الطب البيطري – جامعة الزقازيق – مصر

الملخص العربي

تم إجراء هذا البحث لدراسة أثر إضافة أوراق الجوافة وزيت الزيتون على معدلات النمو ومكونات اللحم وبعض مكونات الدم والمناعة في علائق بداري التسمين تحت الظروف المحلية. استخدم عدد 240 كتكوت تسمين (سلالة الكب) قسمت عشوائيا إلى أربعة مجموعات متساوية بكل منها ثلاثة مكررات كل مكرر به 20 كتكوت. تم تغذية الطيور على علائق متساوية في محتوى الطاقة والبروتين لكل مرحلة عمرية. كانت المعاملات كالتالي: 1–عليقة ضابطة، 2–عليقة ضابطة أضيف إليها ا أوراق الجوافة 1% ، 3–عليقة ضابطة أضيف إليها ا زيت الزيتون 1% و4–عليقة ضابطة أضيف إليها أوراق الجوافة (1%) وزيت الزيتون (0.5%). تم وزن الكتاكيت فى بداية ونهاية التجربة وحساب متوسط وزن الجسم، معدل الزيادة في وزن الجسم، معدل استهلاك العلف ومعامل التحويل الغذائي. في نهاية التجربة تم اخذ 3 عينات دم عشوائيا من كل مجموعة لقياس تركيز كلا من، الكولسترول الكلى، الدهون الثلاثية، البروتين الكلى، الألبومين، الجلوبيولين وبعض الانزيمات المضادة للأكسدة وإنزيمات الكبد وعدد كرات الدم البيضاء وايضا تم تقييم مكونات جسم الطيور. وأظهرت النتائج أن إضافة أوراق الجوافة وزيت الزيتون على حدة او كخليط ادت الى تحسين معنوي على وزن الطيور، معدل الزيادة في الوزن ومعامل التحويل الغذائي زاد معنويا ومعدل استهلاك العلف خاصة في المجاميع التي أضيف إلى غذائها زيت الزيتون بنسبة1 %. إضافة أوراق الجوافة وزيت الزيتون أدت إلي تحسين معنوي في مستوي الكوليسترول الكلي والدهون الثلاثية وايضا الانزيمات المضادة للأكسدة وانزيمات الكبد. أيضا وجد تحسن معنوي في مستوي البروتين الكلي والجلوبيولين في مصل الدم. إضافة أوراق الجوافة وزيت الزيتون أيضا أدت إلي زيادة معنوية في عدد كرات الدم البيضاء خاصة عند إضافتهم كخليط او اضافة 1% أوراق الجوافة فقط. أيضا وجد تأثير معنوي على التركيب الكيميائي لجسم للطيور. من هذه النتائج يتضح أن إضافة أوراق الجوافة وزيت الزيتون إلى علائق بداري التسمين له تأثير معنوي على معدلات النمو حيث أظهرت النتائج تحسين معنوي في مستوي الكوليسترول الكلي والدهون الثلاثية والانزيمات المضادة للأكسدة وانزيمات الكبد ومستوي البروتين الكلي والجلوبيولين في مصل الدم وأيضا عدد كرات الدم البيضاء.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):23–32, ديسمبر 2013)