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USAGE OF OLIVE OIL TO ALLEVIATE THE ADVERSE EFFECT OF HIGH DIETARY CHOLESTEROL ON SOME BIOCHEMICAL AND HAEMATOLOGICAL TRAITS IN BROILER CHICKENS

(With 5 Tables)

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استخدام زيت الزيتون لتقليل التأثيرات غير المرغوب فيها للكوليسترول على بعض الخواص الكيميائية الحيوية والدموية فى دجاج التسمين

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تم إجراء هذه الدراسة لتعيين تأثير المستوى العالي من الكوليسترول (١%) والزيت الغنى بحمض الأوليك (زيت الزيتون ٥ %) على بعض الخواص الكيميائية الحيوية والدموية فى دجاج التسمين. وقد أوضحت النتائج أن التغذية على زيت الزيتون أدى إلى زيادة معنوية في قيم الجلوبيولين الكلى والجاما جلوبيولين بينما لم تتأثر بقية قيم البروتينات معنوياً. بالنسبة لقيم الكالسيوم والفوسفور غير العضوي فقد انخفضت جوهرياً في الدجاج الذي تناول غذاء يحتوى على مستوى عالي من الكوليسترول فقط بينما زادت قيم الماغنسيوم زيادة معنوية في نفس المجموعة. بخصوص أنماط الدهون والليبوبروتين فقد لوحظت زيادة معنوية عالية في قيم الدهون الثلاثية والكوليسترول وكذلك كوليسترول الليبوبروتين المنخفض والمنخفض جداً الكثافة (LDLc, VLDLc) بينما نقصت قيم كوليسترول الليبوبروتين عالي الكثافة (HDLc) وذلك في المجموعة التي تناولت مستوى عالي من الكوليسترول وقد أدت التغذية على زيت الزيتون إلى عكس هذه النتائج السالفة الذكر كلياً. من ناحية أخرى لوحظ أن مستوى تأكسد الدهون وشق الهيدروكسيل الحر قد زادت زيادة غير معنوية في المجموعة التي تناولت مستوى عالي من الكوليسترول بينما نقصت تلك القيم جوهرياً في المجموعة المغذاة على عليقة عالية المستوى من زيت الزيتون. ولكن لوحظ زيادة جوهرية في نشاط الجلوتاثيون ترانسفيراز وكذلك قيم الجلوتاثيون المختزل في نفس المجموعة . بالنسبة لصورة الدم لوحظ نقصاً معنوياً في قيم كرات الدم الحمراء وكرات الدم المضغوطة ومحتوى الهيموجلوبين عند التغذية على عليقة عالية المحتوى من الكوليسترول بينما زادت تلك القيم

جوهرياً بالإضافة إلى كرات الدم البيضاء ونسبة الخلايا الليمفاوية في الدجاج المغذى على زيت الزيتون. وأخيراً فقد أدى إضافة زيت الزيتون مع الكوليسترول في عليقة واحدة إلى التغلب والتخلص تقريباً من معظم النتائج غير مرغوب فيها والتي نتجت من الكوليسترول على الخواص الكيميائية الحيوية ومكونات الدم، فقد تحسنت كلها نحو مثيلاتها من المجموعة الضابطة ولذا ننصح باستخدام زيت الزيتون وخصوصاً مع الأغذية عالية المحتوى من الكوليسترول.

SUMMARY

The present study aimed to determine the effects of high dietary cholesterol (1%) and oleic acid rich oil (olive oil 5%) and a mixture of both on biochemical and haematological traits in chickens. The data obtained revealed that the feeding of olive oil alone resulted in significant increase in total serum globulins and gamma – globulins. The concentration of serum calcium or phosphorous was significantly decreased while that of magnesium was significantly increased in cholesterol - fed birds. On the other hand, serum triacylglycerol (TAG); total serum cholesterol, low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were significantly increased in birds kept on high dietary cholesterol, meanwhile, the values of high density lipoprotein cholesterol (HDLc) was significantly decreased. These data were reversed completely in olive oil - fed birds. Results also showed a non significant increase in values of lipid peroxidation and hydroxyl radical production in cholesterol – fed group, whereas the feeding of olive oil resulted in their significant decrease. The values of glutathione -S- transferase (GST) and reduced glutathione (GSH) showed a significant increase in hemolysate of olive oil fed birds, but they showed a non significant decrease in cholesterol -fed group. Concerning the blood picture, the cholesterol administration significantly decreased red blood cells (RBCs) count, packed cell volume (PCV) and haemoglobin (Hb) concentration, while the olive oil feeding led to significant increase in RBCs, white blood cells (WBCs) counts, PCV, haemoglobin concentration and lymphocyte percentage. In conclusion, the addition of olive oil to cholesterol containing feed, nearly counteract most of the undesirable effects resulted from cholesterol feeding.

Key Words: *Olive oil–Biochemical & Haematological Traits-Broiler-Chickens*

INTRODUCTION

Hypercholesterolemia has been widely accepted as a high risk factor for development of atherosclerosis and ischemic heart disease (Dawber, 1980).

The serum cholesterol, triacylglycerol, LDLc and VLDLc showed a positive correlation to dietary cholesterol in human (Liburn and Bacon, 1990; Washburn and Marks, 1990). Also, Korshom et al. (1992) found that the total serum lipids, triacylglycerols and total serum cholesterol were significantly increased in birds kept on 1% cholesterol enriched diet.

On the other hand, Jain and Konard (1978) observed that, when rabbit given cholesterol alone, it reduced the total serum protein and albumin. In contrary, Altura et al. (1990) concluded that the high dietary cholesterol has non – significant effects on serum protein and liver enzymes activities.

The use of nutritional supplements either alone (Silagy and Neil, 1994) or in combination with a drug (Contacos et al., 1993) has been shown to be effective in lowering total serum cholesterol and triacylglycerols concentrations in hyperlipidemic subjects. Recently some authors study the effects of plant oils on lipids metabolism (Korshom et al., 1992 and Roch et al., 1998).

It is well known that, the prevalence of coronary artery disease, morbidity and mortality is low in Mediterranean regions, where the traditional diet which is rich in olive oil provides high amounts of monounsaturated fatty acids (MUFA) (Roche et al., 1998). Olive oil may have beneficial effects not only on blood lipid profile, cardiovascular health but also on incidence of cancer (Trichopoulou and Lagiou, 1997).

Olive oil has a fatty acid composition: its oleic acid content ranges from 56% to 84%, whereas linoleic acid constitutes 3-21% (Tiscornia et al., 1982). Although the major effects of high MUFA fat intakes on serum cholesterol are attributed to replacement of saturated fatty acids in the diet (Hegsted et al., 1993), some studies have attributed a cholesterol lowering effect to increase of HDL more than polyunsaturated fat does (Mensink and Katan, 1992). Therefore, this study was planned to throw light on the effects of high dietary cholesterol on both biochemical and haematological traits in chickens

and a trial for using olive oil to counteract the adverse effects of high dietary cholesterol.

MATERIAL and METHODS

One day old (unsexed) Arbor Acres broiler chicks were used in this experiment. Birds were fed on a commercial starter broiler diet (Table 1) for two weeks before experiment. The chicks were vaccinated with a prophylactic dose against Newcastle disease and supplied with furaltidone, erythromycine and amprole as a prophylactic dose against bacterial and protozoal diseases.

Experimental design:

Eighty chicks were randomly divided into four equal groups (20 for each) as follows:

Group I : Kept on basal diet only (control).
Group II: Fed basal diet plus 1% cholesterol.
Group III: Given basal diet and 5% olive oil.
Group IV: Kept on basal diet plus 1% cholesterol and 5% olive oil.

Cholesterol and olive oil were mixed well with the diet and prepared freshly. The diet and water were provided ad libitum. Samples were taken from the feeds and chemically analyzed according to the methods of AOAC (1990).

Table 1: Composition and calculated analysis of the basal diet.

Ingredient	%
Yellow corn	68.30
Soybean meal (48%)	25.50
Fish meal (72%)	4.00
Limestone	1.20
Salt	0.30
DL-methionine	0.25
L-Lysine	0.25
Vitamin mixture*	0.10
Mineral mixture**	0.10
Total	100.00
Calculated analysis:	
Metabolizable energy Kcal/kg	3082
Crude protein, %	21.7
Available phosphorous, %	0.41
Methionine, %	0.58
Soluble amino acids, %	0.89
Lysine, %	1.20
Choline, mg/kg	1300
Determined values:	
Crude protein, %	22.00
Ether extract, %	2.50
Calcium, %	0.96

* Supplies per kg diet: Vit. A, 5000 IU; Vit. D, 1100 ICU; Vit. E, 11 IU; riboflavin, 4.4 mg; Ca-pantothenate, 12mg; nicotinic acid, 44mg;Vit.B12, 66μg ; Vit. B6, 2.2 mg; menadione, 1.1mg; folic acid, 0.55 mg; biotin, 0.11mg; thiamine, 2.2mg and ethoxyquin, 125mg.

** Mineral mixture: provides (milligrams per kg diet), Mn, 60; Zn, 50; Fe, 30; Cu,5; I, 1.1 and Se, 2.

Collection of blood samples:

After 6 weeks from starting the experiment the birds were fasted over night then sacrificed. Ten ml of blood were taken from each bird. Each blood sample was divided into 3 parts. The first part was received in EDTA containing tubes for determination of blood picture. The 2nd part of the blood sample was taken without any anticoagulant for determination of serum constituents.

While the 3rd part of the blood was collected in tubes containing acid citrate dextrose as anticoagulant and stored at 4°C for 2 hr., then

centrifugated at 3000 r.p.m. for 5 min. to remove blood plasma and washed with phosphate buffer (pH 7.4) and recentrifuged. The supernatant was aspirated and discarded. The RBCs were washed twice times and then diluted ten times with phosphate buffer and the mixture was vortexed for 5 min. to obtain hemolyzate of red blood cells.

Analytical Methods:

The collected blood samples for haematological examinations were used within 6 hr. for red blood cells (RBCs) count, white blood cells (WBCs) count, haemoglobin (Hb) estimation, packed cell volume (PCV) and differential leukocytic count (Sood, 1985).

Sera samples were used for determination of total protein (Lowry et al., 1951), albumin (Doumas et al., 1971), gamma globulin (Varely, 1979), total cholesterol (Richmond, 1972), triacylglycerol (Sidney and Bernard, 1973), high density lipoprotein, HDLc (Lopes – Virella et al., 1977), low density lipoprotein, LDLc & very low density lipoprotein, VLDLc (Bauer, 1982). Mineral concentrations (Ca, P and Mg) were determined using atomic absorption spectrophotometer, AAS (Perkin Elmer Model 2380, USA).

Glutathione S–transferase (GST), reduced glutathione (GSH), Hydroxyl radical (OH°) and lipid peroxidation (LP) were determined in red blood cells hemolyzate according to Vessey & Boyer (1984), Sedlak and Lindsay (1968), Olah et al., (1990) and Placer et al. (1966), respectively.

Data obtained were statistically analysed by the analysis of variance (ANOVA) according to Snedecor and Cochran (1982) and the differences between the means were tested for significance according to Duncan (1955).

RESULTS

The obtained results were listed in four tables. Table (2) shows that, the feeding of cholesterol resulted in a non – significant decrease in the values of serum proteins fractions, while it significantly ($P<0.05$) decreased the calcium and phosphorous concentrations. The values of magnesium showed a significant increase in this group. The supplementation of olive oil led to a significant increase in values of total serum globulins and gamma globulin fraction, when compared with the other groups.

On the other hand, Table (3) reveals that, cholesterol feeding significantly ncreased the triacylglycerols, total serum cholesterol, LDL cholesterol and VLDL cholesterol values, meanwhile it significantly ($P<0.05$) decreased the HDL cholesterol. The opposite was observed in the case of the olive oil treated chickens.

Results presented in Table (4) demonstrate that cholesterol containing feed insignificantly decreased the levels of GST and GSH. A non-significant alteration in the values of the lipid peroxidation and hydroxyl radical was noticed in the same group. The olive oil fed- group showed the highest values of GST and GSH and lowest values of lipid peroxidation and hydroxyl radical when compared with the other groups.

Concerning the blood picture, Table (5) shows that, the cholesterol feeding significantly ($P<0.05$) decreased RBCs count, PCV value and Hb concentration, while, the olive oil feeding resulted in a significant increase in RBCs count, WBCs count, PCV, Hb content and lymphocyte percentage.

Furthermore, the data in Tables (3, 4 and 5) also reveal that, mixing of olive oil with cholesterol in the diet nearly counteract the most deleterious effects exerted by chocholesterol feeding on the biochemical and haematological parameters investigated.

DISCUSSION

The obtained results (Table 2) revealed that feeding cholesterol resulted in a non–significant decrease in all protein fractions. These data were in accordance with the findings of Altura et al. (1990), but not in agreement with those of Jain and Konard (1978) who reported that, feeding cholesterol to rabbit, reduced the values of total serum protein and albumin.

The olive oil treatment led to a significant increase in total serum globulins, gamma globulins and lymphocyte %, which gave an evidence that olive oil may have immuno-stimulant effects.

This study reveals that feeding cholesterol significantly decreased the values of serum calcium & phosphorous, but significantly increased the values of serum magnesium. It is well known that the divalent cations can increase the excretion of lipids in animals and human subjects, since these cations form insoluble salt complex with lipids (Renaud et al., 1983). Cholesterol feeding (Table 1) significantly increased magnesium concentrations which nearly agrees with those

obtained by Altura et al. (1990) who observed that the magnesium values were increased in hyperchloesterolemic animals. They believed that hypercholesterolemic state results in loss of Mg from soft tissues, thereby generating an underlying Mg deficiency.

Results in Table (3) reveal a high significant increase in values of triacylglycerol, total cholesterol, LDLc & VLDLc and a significant decrease in level of HDLc in birds kept on cholesterol enriched diet, which agree with the results obtained by Bordia et al. (1979). It is well known that feeding cholesterol alters lipid and lipoprotein metabolism. Furthermore, some investigators found a positive correlation between dietary cholesterol and serum values of tricylglycerol and total cholesterol in human and animals (Liburn and Bacon, 1990, Washburn and Marks, 1990 and Korshom et al., 1992) .

Olive oil feeding significantly decreased serum triacylglycerol, total cholesterol, LDLc & VLDLc concentrations while, it significantly increased HDL concentration These results are in accordance with those reported by Korshom et al. (1992) and Trichopoulou and Lagiou (1997) in birds and human, respectively. The high content of plant sterols in olive oil may be responsible for the hypocholesterolemic effect, since the plant sterols are generally assumed to exert this effect by interfering with cholesterol absorption and synthesis (Korshom et al., 1992). Change in liporprotein patterns resulted from cholesterol and olive oil feeding may illustrate alterations in values of cholesterol and triacylglycerols. Higher values of LDL& VLDL in feeding cholesterol birds may promote the production of triglycerides – rich lipoprotein which in turn stimulates the formation of LDL and promoting the catabolism of HDL (Patsch et al., 1987) .

From the present study, it is evident that high level of cholesterol with diet, insignificantly increased the values of lipid peroxidation and hydroxyl radicals, while the olive oil feeding significantly decreased them and in addition it significantly increased the values of GST and GSh. It is well known that the most important factor in protection against reactive oxygen radicals in the red blood cells, is the glutathion redox system (Novak et al., 1991). The antioxidant effects of olive oil was suggested by Manna et al. (1997) who reported that the dietary intake of olive oil polyphenols may lower the risk of reactive oxygen metabolite – mediated diseases. Furthermore, Visioli and Galli (1998) concluded that, the hydroxytyrosol of olive oil exerts antioxidant activity

both by chelating free metal ions and by scavenging newly formed free radicals.

Table (5) reveal that, the cholesterol feeding significantly decreased the RBCs count, Hb content and PCV value while, the feeding of olive oil increased them beside its ability in producing leukocytosis and lymphocytosis. The antioxidant effect (Manna et al., 1997 & Visioli and Galli, 1998) as well as the immunostimulant effect may reflect the effects of olive oil in improving the blood picture.

The alleviation of deleterious effects resulting from high dietary cholseterol was tried by using plants and fish oils (Alder and Holub, 1997). However, the use of monounsaturated fatty acids in the form of olive oil is preferred than the polyunsaturated ones, since the latter decreases the antioxidant defenses of the body (Leibovitz et al., 1990). Olive oil has been shown that it contains high amount of plant sterols (Fabris and Vitagliano, 1956), antioxidant effects (Manna et al., 1997), increasing insulin secretion (Roche et al., 1998), increasing HDL & decreasing LDL (Pastsch et al., 1987 and Roch et al., 1998), in addition to it immuno stimulant effects (Tables 2, 5). All these beneficial effects may illustrate the mode of action of olive oil in relieving the undesirable action of high dietary cholesterol.

Conclusion: Adding of olive oil to the diet of broiler chicken, improved the blood picture, increased the antioxidant agents, stimulated the immunity, decreased total cholesterol level, increased the beneficial lipoprotein cholesterol (HDLc), decreased the harmful lipoprotein cholesterol (LDLc). In other words, it positively counteracts the most deleterious effects exerted by cholesterol feeding on the biochemical and haematological criteria.

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Table (2) : Serum protein patterns and mineral concentrations in chickens affected by cholesterol and / or olive oil (mean ± S.E.) .

	Control Group	Cholesterol fed group	Olive oil fed group	Olive oil + Cholesterol fed group
Total protein (g/dl)	3.39±0.08[a]	3.16±0.07[a]	3.58±0.08[a]	3.40±0.09[a]
Albumin (g/dl)	1.81±0.04[a]	1.65±0.03[a]	1.69±0.05[a]	1.73±0.04[a]
Globulin (g/dl)	1.56±0.036[b]	1.51±0.028[b]	1.89±0.04[a]	1.67±0.03[ab]
γ- globulin (g/dl)	0.53±0.021[b]	0.48±0.03[b]	0.69±0.02[a]	0.58±0.04[ab]
Calcium (g/dl)	10.20±0.13[a]	8.07±0.19[b]	9.00±0.61[a]	9.30±0.73[a]
Inorg. Phosph. (mg/dl)	5.39±0.095[a]	4.10±0.07[b]	4.63±0.34[a]	4.54±0.48[a]
Magnesium (mg/dl)	12.03±0.99[b]	14.16±1.81[a]	11.38±0.68[b]	13.34±1.23[a]

a,b means within the same row with the same superscripts are not significantly different ($P < 0.05$).

Table (3): lipids and lipoproteins cholesterol patterns in chickens affected by cholesterol and / or olive oil (mean ± S.E.).

	Control Group	Cholesterol fed group	Olive oil fed group	Olive oil + Cholesterol fed group
Triacylglycerol (mg /dl)	104.60±4.12 [b]	136.07± 5.46 [a]	92.46±3.16 [c]	109.61±3.33 [b]
Total cholesterol (mg/dl)	124.37±3.66 [b]	158.38±4.71 [a]	101.80±3.80 [c]	125.77±5.39 [b]
HDLc (mg/dl)	38.71±1.69 [b]	31.46±1.89 [c]	46.93±3.90 [a]	40.39±3.07 [ab]
LDLc (mg/dl)	64.71±2.49 [b]	99.71±3.34 [a]	36.00±2.71 [c]	63.34±2.99 [b]
VLDLc (mg/dl)	20.92±1.23 [bc]	27.20±1.66 [a]	17.90±1.23 [c]	21.92±2.06 [bc]

a,b,c means within the same row with the same superscripts are not significantly different ($P < 0.05$).

Table (4) : Glutathione S-transferase, glutathione, lipid peroxidation and hydroxyl radicals in chickens affected by cholesterol and / or olive oil (mean ± S.E.).

	Control Group	Cholesterol fed group	Olive oil fed group	Olive oil + cholesterol fed group
Glutathione – s-tranferarse (μg/mg prot.)	6.34±0.39 [b]	6.00±0.41 [b]	8.34±0.34 [a]	6.76±0.46 [b]
Reduced glutathione (μg/mg prot.)	0.94±0.07 [bc]	0.86±0.065 [c]	1.30±0.09 [a]	0.98±0.06 [b]
Lipid peroxidation (nm MDA* / mg prot.)	0.36±0.016 [a]	0.40±0.03 [a]	0.21±0.012 [b]	0.37±0.02 [a]
Hydroxyl radicals (nm MDA*/mg prot.)	0.21±0.015 [a]	0.22±0.016 [a]	0.15±0.009 [b]	0.23±0.013 [a]

* MDA = Malonyl – dialdehyde.

a,b,c means within the same row with the same superscripts are not significantly different ($P < 0.05$).

Table (5) :Blood picture in chickens affected by cholesterol and / or olive oil (mean ± S.E.).

	Control Group	Cholesterol fed group	Olive oil fed group	Olive oil + cholesterol fed group
R.B.Cs count ($x10^6$)	2.79 ± 0.22^{b}	2.1 ± 0.19^{c}	3.41 ± 0.15^{a}	2.44 ± 0.21^{cb}
W.B.Cs count ($X10^3$)	25.4 ± 0.58^{b}	25.5 ± 1.43^{b}	29.34 ± 1.17^{a}	26.2 ± 1.25^{b}
P.C.V (%)	31.3 ± 0.46^{b}	27.7 ± 1.28^{c}	34.92 ± 1.16^{a}	30.0 ± 1.56^{b}
Hb(gm %)	7.21 ± 0.18^{b}	6.51 ± 0.17^{c}	8.96 ± 0.51^{a}	6.84 ± 0.78^{bc}
Heterophils(%)	24.9 ± 0.54^{a}	25.40 ± 1.71^{a}	22.2 ± 1.34^{a}	24.6 ± 1.39^{a}
Basophils(%)	1.80 ± 0.21^{a}	1.1 ± 0.7^{a}	1.1 ± 0.23^{a}	1.00 ± 0.21^{a}
Eosinophils(%)	4.9 ± 0.15^{a}	6.1 ± 0.28^{a}	4.2 ± 0.41^{a}	6.0 ± 0.34^{a}
Monocytes (%)	3.4 ± 0.27^{a}	2.9 ± 0.28^{a}	2.7 ± 0.19^{a}	2.7 ± 0.27^{a}
Lymphocytes(%)	64.9 ± 0.43^{b}	64.5 ± 1.56^{b}	69.8 ± 1.34^{a}	65.7 ± 2.53^{b}

a,b,c means within the same row with the same superscripts are not significantly different ($P< 0.05$).