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Testing of the Efficacy of Dietary Natural and Synthetic Antioxidants on Broiler Performance: A Comparative Study

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Abstract A comparative study was conducted to test of the efficacy of dietary natural and synthetic antioxidants on productive performance, blood biochemical composition and liver oxidation status of broiler chickens. One-day old Ross broiler chickens(n=210)were fed together on basal diet during the first week of age, then divided randomly into seven treated groups:T0(control group) without any addition,T1=0.75g *Withania somnifera* leaves(WSL)/kg diet,T2=1.5gWSL/kg diet,T3=0.75g rosemary leaves(RL)/kg diet,T4=1.5gRL/kg diet,T5=0.75g vitamin E/kg diet and T6=1.5g vitamin E/kg diet. Results showed that supplementing broilers with 1.5g WSL significantly increased final live body weight, body weight gain, dressing percentage and improved feed conversion ratio. Results of serum biochemical tests indicated that dietary supplementation with natural and synthetic antioxidants significantly ($p \leq 0.05$) reduced serum cholesterol as compared to control group. High levels of RL and vitamin E significantly decreased LDL as compared to control. Dietary supplementation with 1.5g RL significantly decreased serum albumin and glucose and increased globulin as compared to vitamin E supplemented groups. No significant differences were found between all experimental groups in serum HDL, VLDL, triglycerides, total protein, AST and ALT. In liver,1.5g WSL significantly reduced peroxide value and malondialdehyde levels as compared to RL. It can be concluded from this study that adding herbs like *Withania somnifera* as natural antioxidant to broiler diet had positive effect in reducing lipid oxidation and minimize harmful effect of oxidation damage.

Key words: Broiler, *Withania somnifera*, rosemary, vitamin E, blood and liver parameters, productive performance.

Introduction

Oxidative damage is one of the main problems facing poultry industry and lead to significant dropping in production. Oxidation increases as a result of a high intake of oxidized lipids, oxidation of sensitive polyunsaturated fatty acids (PUFA) or a low intake of nutrients involved in the antioxidant defense system (Meyorrissey *et al.*, 1998). Nutrition, environment and toxic substances are another factors involved in the occurrence of oxidative damage (Shehata and Yosef, 2010). Oxidation is inherent to metabolism, but an excessive

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formation of reactive oxygen species in oxidation processes can cause damage to vital components in biological systems(Halliwell *et al.*,1995; Hutadilok-Towatana *et al.*, 2006). Free radicals or reactive oxygen species capable of causing damage to deoxyribonucleic acid (DNA) have associated with various health problems (Cadens and Davies, 2000). Minimizing oxidative damage through scavenging free radicals mediated oxidative reactions, may be one of the most important approaches to prevent its hazard's effect. The main mode of action of different antioxidants are consists of removal of free radicals (FR) by transfer an H atom which stabilizes FR and becoming themselves low reactivity, thereby stopping the lipoperoxidative chain (Fellenberg and Speisky, 2006).

Recently many studies were conducted to evaluate the antioxidant activities of dietary natural and synthetic antioxidants. Natural antioxidants such as herbs are used as a substitute for synthetic antioxidants (Radwan *et al*., 2008) and are generally believed to be safer, healthier and less subject to hazards for human and animals which make them useful as natural animal feed additives (Faixova and Faix, 2008). Phytochemicals; flavonoids and polyphenolic compounds are potent free radical scavengers and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules (Devipriya and Shyamaladevi, 1999), this effect will rise their antioxidant activities like increasing the levels of glutathione and catalase (Sudheesh *et al.,* 1999), also, it has protective effect on liver by stimulating superoxide dismutase (SOD) in this organ (Huang *et al*., 1992).

Withania somnifera commonly known as Ashwagandha or Indian Ginseng, family Solanaceae, has multifaceted medicinal properties including antioxidant (Sundaram *et al*., 2011). The leaves contain flavonoids, withanolides and alkaloids (Khare, 2007). Studies shows that administration of active principles of *Withania somnifera* consisting of equimolar concentrations of sitoindosides VII-X and withaferin A, was found to increase superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase(GPX) activity in rat brain (Uddin *et al*., 2012).

Rosemary (*Rosmarinus officinalis*), belonging to Lamiaceae family is used for flavoring foods and beverages and also for several pharmaceutical applications (Polat *et al.*, 2011). Rosemary has high amount of a rosmaric acid (Nielsen *et al*., 1999), flavonoids and phenolic acid (Ho *et al*., 2000) that have antioxidant properties. Some previous experiments showed that phenolic compound of rosemary oil, such as carnosic acid acts as antioxidants (McCarthy *et al.*, 2001). It was also found that low levels of dietary rosemary leaves meal could be safely used in broiler diet to promote growth and to impart healthful constituents to the consumer (Ghazalah and Ali, 2008). Dietary synthetic antioxidant like vitamin E (α-tocopherol) is a biological antioxidant soluble in fat (Halliwell and Gutteridge, 1991) and inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane (McDowel, 1989). It has been reported that the supplementation of high levels of vitamin E does not change the metabolism of broilers significantly, and as it protects the biologic membranes and effects the cell multiplication, the utilization of vitamin E can be beneficial for those very rapid growth animals such as broilers (Arslan *et al*., 2001). The objective of this study was to investigate the effect of supplementing broiler chickens diet with different levels of natural (Ashwagandha and rosemary leaves) or synthetic (vitamin E) antioxidants on blood parameters, liver tissue oxidative status, productive performance and carcass quality.

Materials and methods

This experiment was carried out at the Poultry Farm of Animal Resources Dept., College of Agriculture/University of Baghdad during the period 20/11/2012 until 26/12/2012.Two hundred and ten 1-day old Ross strain unsexed broiler chicks at average weight of (41.8g/bird) were reared together on floor pen as one group during the 1st week of age and they reach 135.5g/chick. At 8th day old, the chicks were randomly distributed into seven groups. Each group was replicated three times with 10 chicks per one. All birds were fed basal diet formulated according to NRC (1994) (Table 1).

Table1. Ingredients and calculated analysis of the basal diet.

Ingredient	**%**
Yellow corn	40
Wheat	28.5
Soybean meal(44%)	25
Premix*****	5
Vegetable oil	0.5
Dicalcium phosphate	0.1
Limestone	0.7
Salt	0.2
Total	100
Calculated analysis**	
ME(kcal/kg)	2950
Crude protein%	21.5
C/P ratio	173.2
Methionin+cysteine%	0.73
Lysine%	0.82
Available phosphorus%	0.50
Crude fiber	3.84
Crude fat	3.10

*Provides per kg of diet: 40% crude protein,2500kcal/kg ME, 9%fat,4.5%crude fiber, methionine and lysine;** NRC(1994).

The experimental treatments were as follows: T0 (control) = basal diet without any addition, T1= 0.75g *Withania somnifera* dried leaves (WSL)/kg basal diet T2=1.5g WSL/kg basal diet, T3=0.75g rosemary dried leaves (RL)/kg basal diet, T4= 1.5g RL /kg basal diet, T5= 0.75g vitamin E/kg basal diet, T6=1.5g vitamin E/kg basal diet.

Artificial lighting regime was provided constantly during experimental period, the water and mash feed were provided *ad libitum* through 35 days experimental period. All chickens were vaccinated against avian disease. Live body weight, body weight gain, feed consumption and feed conversion ratio were recorded for each replicate during the experimental weeks (2,3,4 and 5). At the end of 5 weeks, two broiler chickens from each replicate were taken randomly and slaughtered. Dressed carcass were weighted and calculated as percentage of live body weight. The dressed carcass was partitioned into main cuts; breast, wings, back, neck and thighs cuts. The cuts weights were estimated as percentage of dressed carcass. At the time of slaughter, 6 birds per treatment were taken randomly and slaughtered; blood was collected immediately in no heparinized tubes and centrifuged to obtain serum. Liver from each slaughtered chicken was derivation. Serum and liver samples were stored at -20◦C until they were analyzed. Serum glucose, total protein, albumin, cholesterol, triglycerides, high density lipoprotein(HDL),low density lipoprotein(LDL), alanine amino transferase(ALT), aspartate amino transferase(AST) and alkaline phosphatase(ALP) were measured with the help of commercial kits. The albumin content was deducted from the total protein to obtain globulin level. Very low density lipoprotein (VLDL) and non HDL was calculated by following formulas:

VLDL=TG/5

Non HDL= Total cholesterol - HDL

Liver malondialdehyde(MDA) was measured according to Witte *et al*.,(1970), liver free fatty acids(FFA) and peroxide value(P.V) were measured according to Egan *et al*.,(1981).

All data obtained were analyzed using ANOVA (SAS, 2010). Significant differences among treatment was determined by Duncan's multiple rang tests (Duncan, 1955). P value less than 0.05 was considered as statistically significant.

Results and Discussion

The live body weight, body weight gain, feed consumption and feed conversion ratio of broiler chickens in different groups during the experimental periods are illustrated in Table 2. The data showed that body weight at the end of 3 weeks of age was significantly ($p \leq 0.05$) higher in broiler chickens which received basal diet with 0.75g of RL or 1.5g vitamin E as compared to 0.75g vitamin E group, while body weight of broilers received 0.75g WSL was significantly (p≤0.05) the highest as compared with control and all other treated groups at the end of 4 weeks of age. Final body weight at the end of 5 weeks of age revealed that, broilers received 1.5g WSL showed significant (p≤0.05) increasing as compared to control whereas; all treated groups didn't differ significantly among each other.

The present data indicated that the body weight at the last two weeks of experimental periods (4 and 5 weeks) increased significantly by adding *Withania somnifera* leaves to diet comparable to control group.

Table 2. Live body weight (g), body weight gain (g/week), feed consumption (g/week) and feed conversion ratio of broiler during experimental weeks.

Treatment / Parameter	T0	T1	T2	T3	T4	T5	T6
Live body weight(wk)							
2	423.00	454.66	442.66	458.34	453.67	435.00	485.66
3	761.13ab	785.33ab	775.53ab	830.07a	804.0ab	727.53b	884.73a
4	1161.11b	1408.89a	1156.11b	1198.89b	1213.33b	1118.89b	1195.55b
5	1983.2b	2179.4ab	2285.0a	2115.0ab	2175.6ab	2036.7ab	2076.7ab
Body weight gain(wk)							
1-2	287.50	319.16	307.16	322.84	318.17	299.50	350.16
2-3	338.13	330.67	332.87	371.73	350.33	292.53	399.07
3-4	399.97b	623.55a	380.57b	368.82b	409.33b	391.35b	350.82b
4-5	822.1b	770.6b	1128.9a	916.11ab	962.0ab	917.8ab	881.1ab
Feed consumption(wk)							
2-3	627.26ab	572.79c	598.34bc	635.27a	600.77bc	625.78ab	602.64bc
3-4	659.24b	673.88b	713.47a	710.86a	700.43a	627.60c	705.26a
4-5	1236.74f	1572.49c	1577.79bc	1300.67e	1607.73ab	1511.47d	1614.50a
Feed conversion ratio							
2-3	1.86	1.86	1.80	1.73	1.72	2.26	1.52
3-4	1.66ab	1.11b	1.93ab	2.23a	1.71ab	1.69ab	2.02ab
4-5	1.52b	2.06a	1.42b	1.51b	1.68ab	1.64ab	1.85ab

Means having different letters in the same raw are significantly different (p≤0.05)

Average body weight gain of experimental groups showed that at the first and second (1-2 and 2-3) interval weeks of age, there were no significant differences between all experimental groups (T0,T1,T2,T3,T4,T5 and T6). Broiler chickens received 0.75g WSL obtained significantly (p≤0.05) higher body weight gain as compared to control and other treated groups at the third interval (3-4) weeks. At the last interval weeks(4- 5) of age, dietary supplementing with 1.5g WSL recorded significantly(p≤0.05) higher body weight gain as compared to control and to

0.75g WSL, while, the control group didn't differ from the values of T3, T4, T5, and T6.

The present data showed that final body weight and weight gain at the last interval weeks(4-5) of age was increased due to adding 1.5g WSL to diet comparable to control group, this improvement could be related to the effect of *Withania somnifera* in stimulating the thyroid gland directly and/or through the pituitary gland to secret the thyroid hormones. Thyroid hormones increase metabolic rate, which lead to increase amino acid metabolism (More *et al*., 1980), farther more, thyroid hormones accelerate cellular reactions in most organs and tissues of the body, including the liver in which amino acids are synthesized and formed, this means that *Withania somnifera* has anabolic effects and enhancing the synthesis of proteins in liver and increasing the body weight in human and animals (Anabalagan and Sadique, 1981).

Average feed consumption of broiler chickens during (2-3) weeks of age was significantly($p \leq 0.05$) the less in group received 0.75g WSL as compared to control group, while, the higher values was found in 0.75g RL group comparable to 1.5g RL, 1.5g vitamin E, 0.75 or 1.5g WSL. At the last interval(4-5) weeks of age, unsupplemented group(T0) was significantly ($p \leq 0.05$) the less in feed consumption as compared to all supplemented groups, while, the highest values was found in group dietary fed with 1.5g vitamin E.

Feed conversion ratio(FCR) for different experimental treatments revealed that during the interval(3-4)weeks of age, group received 0.75g RL was worse than control and all other experimental groups, while, FCR of group received 0.75g WSL was the better as compared to control and all other supplemented groups. It can be seen also from Table 2 that during (4-5) weeks of age), treated group with 1.5g WSL recorded mathematically the best FCR as compared to all other groups, on other hand, no significant differences were found among T0,T2,T3,T4,T5 and T6.

Results of resent study showed significant increasing in final body weight, body weight gain and an improvement in feed conversion ratio by increasing the level of WSL in diet, this result may suggested that *Withania somnifera* have anabolic effects and enhancing the synthesis of proteins in liver and increasing the body weight in human and animals(Anabalagan and sedique, 1981).

Statistical analyses of carcass yield in different groups are illustrated in Table 3. The present data showed significant ($p \leq 0.05$) increasing in dressing percentage in

Treatment Parameter	T0	T1	T2	T3	T4	T5	T6
Triglycerides mg/dl	130.50	110.50	177.0	100.0	115.0	111.5	M134.0
Cholesterol mg/dl	185.0a	141.50b	153.0b	141.50b	133.0b	145.0b	142.50b
HDL mg/dl	105.25	104.55	99.35	95.10	78.15	88.05	99.60
LDL mg/dl	53.80a	29.60ab	35.25ab	31.85ab	24.40b	34.65ab	16.10b
VLDL mg/dl	26.10	22.10	27.40	22.0	23.0	22.30	26.80
Non HDL mg/dl	79.75a	46.95ab	53.65ab	46.40ab	54.85ab	56.95ab	42.90b

Means having different letters in the same raw are significantly different ($p \leq 0.05$).

Group recived 1.5g WSL as compared to broilers received 1.5g RL in diet while there were no significant differences between all other groups. No significant differences among the different broiler chickens groups in the weight of dressed carcass, breast% and in the two thighs cut%. Wings cut% decreased significantly in groups supplemented with 0.75 or 1.5g WSL, 1.5g RL, 0.75 or 1.5g vitamin E as compared to control. The percentage of back cut increased significantly($p \leq 0.05$) in broiler chickens supplemented with 1.5g vitamin E as compared to control. Data also showed that all supplemented groups significantly ($p \leq 0.05$) obtained higher neck cut% as compared to control. Results illustrated in Table 3 indicated that dietary supplementation with WSL or RL as natural antioxidants or with vitamin E as synthetic antioxidant leads to significant increasing in the percentage of neck cut and significant reduction in the percentage of wings cut as compared to unsupplemented group.

Serum lipid profiles of broiler chickens in different groups are illustrated in Table 4. Data showed that cholesterol was significantly ($p \leq 0.05$) lower in all treated groups which received dietary natural and synthetic antioxidants as compared to control.

Table 4. Serum lipid profile.

Treatment Parameter	T0	T1	T2	T3	T4	T5	T6
Dressing carcass%	75.230ab	74.78ab	76.41a	74.47ab	71.99b	75.68ab	73.76ab
Carcass weight(g)	1822.50	1829.33	1815.0	1833.33	1850.0	1836.67	1841.67
Thigh%	27.601	27.07	25.90	28.13	26.11	27.55	27.62
Breast%	37.04	39.58	40.18	34.71	36.52	37.02	34.02
Wings%	15.45a	9.47b	8.64b	10.25ab	9.11b	9.22b	9.60b
Back%	13.77b	17.91ab	18.88ab	18.10ab	19.55ab	18.47ab	20.57a
Neck%	3.99b	6.03a	6.47a	6.22a	5.94a	6.31a	5.69a

Means having different letters in the same raw are significantly different ($p \leq 0.05$)

In recent study, we found that dietary natural and synthetic antioxidants leads to reduced LDL levels in broiler serum as compared to control group, this reduction was statistically significant ($p \leq 0.05$) in groups received the high levels of both RL or vitamin E(T4 and T6), while it was insignificant in groups received WSL (T1 and T2) or low levels of RL or vitamin E (T3 and T5). No significant differences were found between all experimental groups(T0,T1,T2,T3,T4,T5 and T6) in serum triglycerides, HDL and VLDL while non HDL was significantly($p \leq 0.05$) the lower in group received 1.5g vitamin E(T6) as compared to control.

Reducing the level of cholesterol by dietary natural antioxidant may reflect the hypocholesterolemic properties attributed to the defatted part of plant leaves which are rich in fibrous content and may block intestinal cholesterol absorption, leading to reduced its level in blood(Lansky *et al.*,1993).In this field, Ebihara and Schneeman (1989) reported that the cholesterol lowering effect of *Withania somnifera* could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion.

The HDL cholesterol is reported to provide protection against heart disease in contrast to non HDL cholesterol fraction (Anderson and Tietyen-Clark, 1986). Reducing levels of LDL cholesterol may be attributed to an enhanced plasma clearance of LDL.

Data in Table 5 showed the statistical analysis of serum glucose, total protein, albumin, and globulin, ALT, AST and ALP. Concerning glucose and albumin, it can be seen that the lowest levels was significantly($p \leq 0.05$) recorded in group fed with 1.5g (RL) while 0.75g vitamin E group(T5) had the highest value, from other hand, all treated groups(T1,T2,T3,T4,T5 and T6) didn't differ significantly to control.

Supplementing broilers diet with natural or synthetic antioxidants didn't lead to significant differences with control group in total protein and globulin, while, broiler chickens which received 1.5g RL(T4) was significantly($p \leq 0.05$) higher in globulin as compared to those treated with 0.75 or 1.5g vitamin E(T5 and T6).

Reducing the level of glucose in group recived1.5g rosemary leaves in diet as natural antioxidant compared to those received dietary synthetic antioxidant (vitamin E) could be attributed to the high crude fiber content (cellulose 15.59, hemicellulose 6.79 and lignin 5.94%) (Ghazalah and Ali, 2008) in particular cellulose from the cell walls of the leaves which may prevent enzymes to reach glucose by enfolding them, this action lead to delayed gut glucose absorption(Mohammed, 2013). The recent result agreed with Tekeli *et al.* (2006) who found that rosemary leaves could be used to decrease blood glucose. Also, in this study, the significant increase in serum globulin in group supplemented with rosemary leaves indicates its effective role as natural antioxidant in increasing immunity due to its role in developing and protecting cells and inhibiting non-enzymatic oxidation (Houghton *et al.*, 1995).

Table 5. Serum biochemical tests.

Treatment / Parameter	T0	T1	T2	T3	T4	T5	T6
Glucose mg/dl	155.50ab	167.50ab	170.0ab	162.0ab	135.50b	196.50a	156.50ab
Total protein g/dl	4.09	4.11	4.12	4.01	4.11	4.11	3.76
Albumin mg/dl	2.92ab	2.68ab	2.77ab	3.0ab	2.53b	3.29a	2.9ab
Globulin g/L	1.17ab	1.43ab	1.35a	1.0ab	1.58a	0.81b	0.84b
ALT IU/L	4.50	4.50	3.0	2.0	2.5	3.0	4.5
AST IU/L	19.50	26.0	27.0	28.0	26.0	25.5	36.5
ALP IU/L	42.90b	112.55a	109.95a	107.60a	84.15ab	110.0a	53.45ab

Means having different letters in the same raw are significantly different ($p \leq 0.05$)

The combinations of the results illustrated in Tables 4 and 5 leads to associate the relationship between decreasing the levels of cholesterol, albumin/globulin and LDL in broilers received rosemary leaves and the possible explanation could be as follows; most of the circulating cholesterol is carried in birds by HDL (α-2 globulin fraction) and LDL (β-globulin fraction) (Zantop,1997), these lipoproteins became the principle cholesterol transport, this finding is consistent with a study performed by Polat *et al.* (2011).

Liver function enzymes (ALT and AST) didn't differ significantly between treated and control groups, this result agreed with Radwan *et al.*,(2008). Supplementing broilers with WSL (T1 and T2), 0.75g RL(T3) or 0.75g vitamin E (T5) lead to significant($p \leq 0.05$) increasing in ALP as compared to control group, while all groups that were recived natural and synthetic antioxidants didn't differ significantly among each other, this increasing in ALP could be related to increasing osteoblastic activity due to dietary supplementing with natural and synthetic antioxidants (Arsalan *et al.*,2001).This result agreed with Mohammed (2013) who found significant increasing in ALP in layer hens supplemented with RL and vitamin E.

Liver MDA, FFA% and P.V are illustrated in Table 6. The present results showed that MDA decreased significantly ($p \leq 0.05$) in groups received 1.5g WSL(T2), also with 1.5g vitamin E(T6) compared to 0.75g RL(T3), however, all other treated groups didn't differ significantly with control. The ability of vitamin E in protecting liver from lipid peroxidation and keep cells membranes from damage is resulted from the methyl groups of tocopherol that interact with the Cis double bounds of the fatty acids to form a stable complex in membrane phospholipids(Parker and Landvik, 1990), this action leads to reduced MDA synthesis, thus, decreasing its levels in this organ(Shahin *et al.*, 2001). Regarding FFA%, we didn't found significant differences between all experimental groups while dietary supplementation with 1.5g WSL (T2) lead to significant($p \leq 0.05$) reduction in P.V compared to 1.5g RL, 0.75g and 1.5g of vitamin E.

Table 6. Liver antioxidant status.

Treatment / Parameter	T0	T1	T2	T3	T4	T5	T6
MDA(mg/kg tissue)	0.62ab	0.71ab	0.57b	0.73a	0.70ab	0.64ab	0.57b
FFA%	0.3	0.2	0.2	0.2	0.20	0.2	0.5
P.V(meq/kg tissue)	1.0c	1.2b	1.0c	1.4abc	2.0a	1.8ab	1.9ab

Means having different letters in the same raw are significantly different ($p \leq 0.05$).

Reducing the levels of MDA and P.V in liver tissues due to supplementing broilers with 1.5g *Withania somnifera* leaves/kg diet means that this plant offered hepat sitoindosider VIII-X oprotective effect which could be due to(i) presence of natural antioxidant like withaferin A and sitoindosider VIII-X which fairly potent anti-inflammatory, antioxidant and immune modulator activities (Bhattacharya *et al*., 1997; Dhuley, 2000).

Results of present study indicated that adding 1.5g *Withania somnifera* leaves/kg diet as natural antioxidant, had hepatoprotective effect on broilers liver tissues, furthermore, increasing live body weight, body weight gain, dressing percentage and improved feed conversion ratio which means together, reducing the hazard's effect of oxidation damage as compared to rosemary leaves or synthetic vitamin E.

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