International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:3, 2015

Impact of Two Herbal Seeds Supplementation on Growth Performance and Some Biochemical Blood and Tissue Parameters of Broiler Chickens

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***Abstract*—**The effects of basil and/or chamomile seed supplementation on the growth of Hubbard broiler chicks were evaluated. The antioxidant effects of these supplements were also assessed. 120 1-day-old broiler chicks were randomly divided into four equal groups. The control group (group 1) was fed a basal diet (BD) without supplementation. Groups 2, 3, and 4 were fed the BD supplemented with 10g basil, 10g chamomile, and 5g basil plus 5g chamomile per kg of food, respectively. Basil supplementation alone or in combination with chamomile non-significantly ($P \geq 0.05$) increased final body weight (3.2% and 0.3%, respectively) and weight gain (3.5% and 3.6%, respectively) over the experimental period. Chamomile supplementation alone non-significantly ($P \geq 0.05$) reduced final body weight and weight gain over the experimental period by 1.7% and 1.7%, respectively. In comparison to the control group, herbal seed supplementation reduced feed intake and improved the feed conversion and protein efficiency ratios. In general, basil seed supplementation stimulated chicken growth and improved the feed efficiency more effectively than chamomile seed supplementation. The antioxidant activities of basil and/or chamomile supplementation were examined in the thymus, bursa, and spleen. In chickens that received supplements, the level of malondialdehyde was significantly decreased, whereas the activities of glutathione, superoxide dismutase, and catalase were significantly increased ($P<0.05$). Supplementation of basil and/or chamomile did not affect blood protein levels, but had lipid-lowering effects as evidenced by reduced serum levels of total lipids, triglycerides, and cholesterol. In conclusion, supplementation of basil and/or chamomile improved growth parameters in broiler chicks and had antioxidant and blood lipid-lowering effects. These beneficial effects of basil and/or chamomile supplementation resulted in economically viable production of high-quality white meat containing no harmful residues.

***Keywords*—**Herbal additives, basil, chamomile, broiler, growth performance, antioxidant.

I. Introduction

FOR several decades, antibiotics and hormones used as a source of feed additive, although great phobia of public concern about their side effects and residues in animal products. Recently, herbal supplements became a primary source of feed additives and antioxidants to improve general health conditions of human and animals. This is due to their potent action and relatively harmless effects.

Antioxidant effects of plants attributed to the presence of phenolic compounds, flavonoids and phenyl propanoids [1]. All parts of the plants, including seeds and flowers contain antioxidants [2].

Our demand of good-quality white meat is of great interest; so many researchers directed their efforts to evaluate herbal feed additives on broiler production as a good, fast and cheap source of white meat. They found that, herbal feed additives' formulations improved broiler weight gain, feed efficiency, lowered mortality and increased livability [3], [4].

Basil called Rehan in Arabic (*Ocimum basilicum* L.) belonging to plant family *Lamiaceae*, which contains essential oils rich in phenolic compounds, polyphenols such as flavonoids and anthocyanins [5]. Basil seeds improved productive performance of broiler chicks and decreased serum cholesterol but have no effect on carcass parameters [6]. Chamomile flowers have antimicrobial and anti-inflammatory effects on broiler chicks [7]; furthermore, it increased egg numbers of layers while decreased serum total cholesterol and GOT levels [8]. Moreover, chamomile flowers improved growth performance and feed conversion ratio of broilers [9], [10].

The objective of this study was to evaluate the effects of basil and/or chamomile supplementation on growth performance and their antioxidant activities on broiler chicks as well as their effects on protein pattern and lipid profiles.

II. Materials and Methods

A. Birds Used and Management

One hundred and twenty Hubbard broiler chicks of one day-old were obtained from a commercial hatchery then individually weighed and divided randomly into equal four groups with three replicates (each replicate contain 10 birds/cage). Chicks were reared in brooder batteries with wire mesh floors in an open house. The initial temperature was 32^{o}C then reduced sequentially according to chick age until reaching 26^{o}C at day 21. Chicks were kept on a 23 h. light program. Birds were vaccinated against the most common viral diseases which infect broiler chicks.

B. Experimental Design and Feeding Program

First group (G1) of chicks served as control one, the other three groups were fed on basal diet (BD) plus different levels

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of herbal plants (basil and/or Chamomile seeds) as shown in Table I. Basil and Chamomile were purchased from a local market.

TABLE I
THE APPLIED EXPERIMENTAL DESIGN

Group.	Diet	Basil seed g/ kg diet	Chamomile seed g / kg diet
1 (Control)	Basal Diet (BD)	-	-
2	BD	10g/kg	-
3	BD	-	10g/kg
4	BD	5g/kg	5g /kg

Ingredient composition and chemical analysis of basal diet were calculated according to [11] and described in Table II. Starter diet was given from 0-3 weeks of age. After that, chicks were subjected to finisher diet. Diets were formulated according to Hubbard® recommendation book.

TABLE II
INGREDIENT COMPOSITION AND CHEMICAL ANALYSIS (%) OF THE BASAL DIET

Ingredients	Diet	
	Starter (0-3 weeks)	Finisher (4-5 weeks)
Yellow corn, ground	54.2	60.6
Soybean meal (44%CP)	31.9	25.33
Corn gluten meal (60%CP)	7.1	4.81
Corn oil	2.98	5.44
Ground limestone	1.5	1.5
Monocalcium phosphate	1.4	1.4
Common salt	0.3	0.3
Vitamin and Trace mineral mixture *	0.3	0.3
DL-Methionine	0.1	0.1
L-Lysine	0.1	0.1
Coccidiostate †	0.02	0.02
Antimold	0.1	0.1
Chemical analysis		
Crude protein	23.1	19.39
ME Kcal / kg diet	3053	3252.6
Calorie / protein ratio	132.2	167.7
Calcium	0.9	0.9
Phosphorus, available	0.45	0.45
Lysine	1.3	1
Methionine	0.57	0.48

*Protoba Mix produced by EL-TOBA Co. For Premixes & Feed ELSADAT CITY EGYPT. Each 3 Kilograms contain: Vitamin A 12000000 iu, Vitamin D3 3000000 iu, Vitamin E 40000 mg, Vitamin K3 3000 mg, Vitamin B1 2000 mg, Vitamin B2 6000 mg, Vitamin B6 5000 mg, Vitamin B12 20 mg, Niacin 45000 mg, Biotin 75 mg, Folic acid 2000 mg and Pantothenic acid 12000 mg. Manganese 100000 mg, Zinc 60000 mg, Iron 30000 mg, Copper 10000 mg, Iodine 1000 mg, Selenium 200 mg and Cobalt 100 mg.

† Curacoxin (Diclazuril 0.5%) by ARABCOMED.

C. Studied Parameters

1. Evaluation of Growth Performance

Body weights (BW) of chicks were recorded individually at hatch day and then weekly recorded up to five weeks of age; furthermore, body weight gain (BG) was weekly calculated as the differences between two successive weights. Moreover, the relative growth rate (RGR), feed intake and feed conversion ratio of birds among different groups were weekly recorded.

2. Carcass Traits

Five birds from each treatment were randomly selected at the end of the experiment, fastened for 12 h., weighed and slaughtered to complete bleeding. After that, dressing percentage was recorded. Moreover, liver, heart, gizzard, spleen, thymus and bursa were calculated as a percentage from the hot weight of the birds.

3. Biochemical Tissue Parameters

Thymus, bursa and spleen were collected and washed with ice-cold Tris-HCl buffer (0.05 M, pH 7.4) containing 0.25 M sucrose. The tissues were blotted, dried, weighed and then homogenized in the ice-cold buffer with twelve strokes in a tight-fitting Potter-Elvehjem homogenizer and then used for determination of reduced glutathione (GSH), tissue lipid peroxides as malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) activity. GSH was assayed by spectrophotometric technique; the method is based on reductive cleavage of 5.5. dithiobis 2-nitrobezoic acid (DTNB) by sulfhydryl group resulted in yellow color with maximum absorbance at 412 nm [12], while MDA was measured spectrophotometrically after the reaction with thiobarbituric acid [13]. Moreover, SOD was determined according to the method of [14] and finally,CAT was determined according to the method of [15].

4. Biochemical Blood Parameters

By the end of the experimental period, five blood samples were collected from each group and centrifuged at 3000 rpm for 10 minutes in order to separate the serum for determination of serum total protein, albumin, globulin, triglycerides, total lipids and total cholesterol level. They were measured spectrophotometrically using commercial biochemical diagnostic kits supplied by Bio-Merieux Laboratory Reagents and Products, France according to the manufacturers' instructions.

D. Statistical Analysis

The obtained data firstly, subjected for testing normality between groups and also between replicates within groups using procunivariate procedures, then the analysis of variance between groups was performed using SAS [16]. Finally significant differences among studied groups were assessed using Duncan.

III. RESULTS AND DISCUSSION

A. Body Weight Development

Statistical analysis of the obtained data revealed that no difference between one day broiler chicks body weight of different experimental groups (Table III). Basil seeds supplementation (group 2) improved ($P \geq 0.05$) broiler chicken body weight during 1st, 2nd and 3rd weeks by about 2.3%, 2.5% and 3.9%; respectively when compared to control, while basil seeds supplementation significantly increased body weight during 4th week of broiler age by about 8.3% when

compared to control, but the final body weight of broiler chicks fed on the basal diet supplemented with 10g basil seeds/kg diet non-significantly improved weight by about 3.2% when compared with the control. The improvement in body weight may be due to the presence of fat soluble, unidentified factors and essential fatty acids in medicinal and aromatic plants [17], or due to stimulating effect on the digestive system of broilers [18]. Such improvement may be due to the antipyretic, antispasmodic, stomachic antioxidant and antimicrobial activities of basil [19], [20]. These finding were disagree with those of [6] who noted that adding basil seeds (3g/kg) to broiler diet resulted in significantly increased body weight by about 8.4% compared to the control.

On the other hand, chamomile seeds supplementation non-significantly decreased broiler weights at different stages of the experimental period when compared with the control. The obtained data are disagree with those obtained by [7] who stated that 0.25% of chamomile flower powder supplementation improved growth rate of broiler chicks compared with control one. Moreover, addition of 5g/kg diet of both basil and chamomile seeds (group 4) non-significantly improved final broiler chick weight by about 3.5% when compared with the control. However, it was observed that basil seeds' supplementation (10g/kg) had more growth stimulant effects when compared with chamomile seeds' supplementation and generally, the lower response in the present study may be related to the higher inclusion rate of basil and chamomile seeds compared with mentioned literatures.

TABLE III
EFFECT OF DIETARY SUPPLEMENTATION OF (BASIL AND/OR CHAMOMILE) ON BODY WEIGHT OF BROILER CHICKS

Age/week	Groups(Mean ± SE)			
	G1	G2	G3	G4
Day one	46.40 ±0.22	46.42 ±0.18	46.63 ±0.20	46.93 ±0.18
Week1	143.00 ±4.14	146.33 ±3.47	140.50 ±3.00	147.17 ±1.97
Week2	313.28 ±8.29^{b}	321.17 ±7.72^{b}	314.00 ±8.38^{b}	349.67 ±11.28^{a}
Week3	764.46 ±13.97^{ab}	794.00 ±12.30^{a}	753.83 ±11.35^{b}	788.67 ±14.54^{ab}
Week4	1196.96 ±7.74^{b}	1295.86 ±20.35^{a}	1192.00 ±21.12^{b}	1303.67 ±15.91^{a}
Week 5	1791.92 ±9.43^{ab}	1848.79 ±21.83^{a}	1760.67 ±29.00^{b}	1854.33 ±25.13^{a}
Final body weight relative to control	100	103.2	98.3	103.5

Means within the same row bearing different superscripts are significant at (P<0.05).

B. Growth Performance Parameters

Effect of dietary basil and/or chamomile seeds' supplementation on some growth performance parameters of broiler chicken are presented in Table IV. It was noticed that basil seeds or combination of both basil and chamomile seeds' supplementation (groups 2 and 4) respectively increased total body gain and relative growth rate (RGR) of broiler chicks throughout the whole experimental period by about (3.3% and 0.2%) and (3.6% and 0.1%) relative to control group respectively, while chamomile seeds alone supplementation decreased (P≥0.05) total gain and RGR by about 1.7% and 0.1% when compared with the control.

Basil and/or chamomile seeds' supplementation (groups 2 - 4) reduced feed intake of broiler chicks throughout the whole experimental period by about 2.1%, 2.6% and 2.8% respectively when compared with the control. Reduction of feed intake with basil and/or chamomile seeds' supplementation may be related to stimulating digestive process of the birds with herbal seeds supplementation [21]. The present data are in harmony with those obtained by [6], [7].

Moreover, basil and/or chamomile seeds' supplementation showed better feed conversion ratio (FCR) when compared with the control; while, basil and/or chamomile seeds supplementation improved protein efficiency ratio (PER) by about 8%, 4% and 8%; respectively when compared with the control. Basil seeds' supplementation at 10g/kg diet more effective on growth performance and feed efficiency parameters of broiler chicks than chamomile seeds addition which required further investigation to proof the results with different levels of the studied herbal seeds.

Improvement of FCR and PER with basil or basil plus chamomile seeds supplementation may be related to lower feed intake and higher gain of broiler chicks, and also this could be attributed to the presence of essential oils in basil. These volatile essential oils have been shown to contain biologically active components [22]. Juvocimere I and II contained in basil have been reported as potent juvenile analogs [23], while, chamomile flowers has endocrine stimulating effect via enhancement of thyroxin hormone activity with consequence acceleration of nutrients metabolites resulted in increasing body weight [24]. Anti-inflammatory, antimicrobial, antioxidant and antifungal effects of chamomile flowers that attributed to presence of some essential compounds resulted in improvement of body weight gain and feed conversion ratio [25]. The present data are in harmony with those obtained by [6], [10]. Also [7] observed that the addition of chamomile at a level of 0.25% in the broiler diet gave better feed utilization.

TABLE IV
EFFECT OF DIETARY SUPPLEMENTATION OF (BASIL AND/OR CHAMOMILE) ON GROWTH PERFORMANCE PARAMETERS OF BROILER CHICKS

Parameter	Groups (Mean ± SE)			
	G1	G2	G3	G4
Overall body gain (g/bird)	1744.6 ±31.3^{ab}	1802.4 ±26.8^{ab}	1714.0 ±30.7^{b}	1807.4 ±31.2^{a}
Daily body gain (g/bird/bird)	49.8	51.5	49.0	51.6
Overall RGR (%)	189.9 ±0.1^{ab}	190.2 ±0.1^{a}	189.6 ±0.2^{b}	190.0 ±0.1^{ab}
Total feed intake (g/bird)	3255	3180	3170	3165
Daily feed intake (g/bird)	93	90.9	90.6	90.4
Daily feed intake relative to control	100	97.9	97.4	97.2
Total FCR value	1.86	1.76	1.84	1.75
FCR relative to control	100	94.6	98.9	94.1
Protein efficiency ratio (PER)	2.5	2.7	2.6	2.7
PER relative to control	100	108	104	108

Means within the same row bearing different superscripts are significant at (P<0.05).

C. Carcass Traits of Broiler Chicks

Basil and/or chamomile (groups 2–4) supplementation improved (P≥0.05) dressing percentage (Table V) of broiler chicken by about 2.7%, 0.8% and 2.6% respectively when compared with the control which fed on the basal diet without supplementation. Moreover, herbal seed supplementation had no significant effect on liver and gizzard relative weights, however, basil or chamomile supplementation alone (groups 2 and 3) respectively had no effect on heart relative weight while, combination of both herbal seeds significantly increased relative heart weight by about 19.2% when compared with the control. Similar results were obtained by [26] who recorded non-significant differences for carcass parameters between basil fed broilers and control ones and with those obtained by [9] who concluded that chamomile had no effects on relative weights of carcass parameters in broiler chicks. On the other hand, our results disagreed with [27] who reported that use of essential oil of chamomile flowers extract enhanced hepatic metabolize that resulted in increased liver weight percentage in rats. Regarding immune organs' relative weights (Table V), it was observed that basil and/or chamomile seeds' supplementation (groups 2–4) non-significantly improved spleen and bursa relative weights by about (11.1% and 9.1%) for all groups when compared with the control. Moreover, basil alone or combined with chamomile seeds (groups 2 and 4) respectively improved (P≥0.05) thymus gland relative weight while chamomile addition alone (group 3) significantly improved thymus gland relative weight by about 13.3% when compared with the control. Immune organs relative weights improvement with herbal seeds supplementation indicated that herbal seed had immune stimulant effects on broiler chicks and chamomile seeds more immune stimulant than basil seed supplementation.

D. Biochemical Tissue Parameters

The results are shown in Table VI. Supplemented groups with both basil and chamomile either alone or in combination have antioxidant activity revealed in a significant decrease in MDA level and a significant increase in GSH level as well as SOD and CAT activity in all examined tissues in comparison with those of the control group. These findings closed to [28] who reported that basil possessed good antioxidant properties attributed to free volatile aglycones in two different methods as the 2,2/-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH) and ferric reducing/antioxidant power assay (FRAP) when compared with that of the essential oil and well-known antioxidant butylatedhydroxytoluene (BHT). Basil has shown antioxidant activity due to its aromatic compounds and phenolic acids [20]; the main phenolics reported in basil are phenolic acids and flavonol-glycosides [29]. In addition, antioxidant effect of phenolics leads to absorbing and neutralizing free radicals [30]. On the other hand, antioxidant activities of water and alcohol extracts of chamomile were evaluated in linoleic acid and liposome model systems [31]. Furthermore, methanolic extract of chamomile significantly increased all of antioxidant enzymes as well as significantly decreased (P< 0.05) lipid peroxidation in CCL4 intoxicated rats [32].

TABLE V
EFFECT OF DIETARY BASIL AND/OR CHAMOMILE SEEDS SUPPLEMENTATION ON CARCASS OF BROILER CHICKS

Parameters	Groups (Mean ± SE)			
	G1	G2	G3	G4
Dressing (%)	69.9± 0.9	71.8±0.6	70.5±0.7	71.7±0.3
Liver (%)	2.6±0.2	2.5±0.2	2.4±0.1	2.4±0.2
Heart (%)	0.5±0.0^{b}	0.6±0.0^{b}	0.5±0.0^{b}	0.6±0.01^{a}
Gizzard (%)	1.7±0.2	1.9±0.1	1.7±0.1	1.7±0.1
Spleen (%)	0.09±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Thymus (%)	0.15±0.0^{b}	0.16±0.0^{ab}	0.17±0.0^{a}	0.16±0.0^{ab}
Bursa (%)	0.11±0.0	0.12±0.0	0.12±0.0	0.12±0.0

Means within the same row bearing different superscripts are significant at (P<0.05).

TABLE VI
EFFECT OF BASIL AND/OR CHAMOMILE ON MALONDIALDEHYDE, GLUTATHIONE, SUPEROXIDE DISMUTASE AND CATALASE ACTIVITY OF BROILER CHICKS

Parameter	Organ	Group (Mean ± SE)			
		G1	G2	G3	G4
MDA (nmol /g wet tissue)	Thymus	136.8 ± 2.5^{a}	100.1 ± 2.6^{c}	124.9 ± 2.5^{b}	80.7 ± 2.4^{d}
	Bursa	165.4 ± 2.9^{a}	133.5 ± 1.7^{b}	112.1 ± 2.3^{c}	99.3 ± 1.2^{d}
	Spleen	186.7 ± 1.4^{a}	139.9 ± 1.8^{c}	137.3 ± 1.8^{c}	148.9 ± 1.4^{b}
GSH (µmol/g wet tissue)	Thymus	13.8 ± 0.6^{c}	29.5 ± 0.6^{a}	31.5 ± 0.3^{a}	22.9 ± 0.8^{b}
	Bursa	36.4 ± 1.3^{d}	75.6 ± 1.3^{b}	93.2 ± 1.4^{a}	57.0 ± 1.3^{c}
	Spleen	37.0 ± 1.4^{c}	64.9 ± 1.1^{b}	81.4 ± 1.4^{a}	62.1 ± 1.6^{b}
SOD (u/mg protein)	Thymus	68.7 ± 5.2^{b}	89.6 ± 3.2^{a}	90.7 ± 1.5^{a}	76.8 ± 2.3^{ab}
	Bursa	187.8 ±3.8^{a}	191.7 ±2.6^{a}	185.6 ±2.7^{a}	189.0 ±2.4^{a}
	Spleen	79.4 ±1.6^{c}	94.5± 1.5^{b}	111.5 ±1.8^{a}	91.9 ±1.5^{b}
CAT (k/sec/mg protein)	Thymus	41.5 ±3.5^{c}	71.9 ±5.4^{ab}	76.9 ±5.7^{a}	68.8 ±6.4^{b}
	Bursa	81.5 ±1.8^{c}	114.8 ±1.4^{a}	117.8 ±1.3^{a}	95.7 ±1.5^{b}
	Spleen	63.5 ±4.3^{c}	97.2 ±2.3^{b}	123.8 ±2.3^{a}	98.7 ±4.6^{b}

Means within the same row bearing different superscripts are significant at (P<0.05)

E. Biochemical Blood Parameters

The results are listed in Table VII. Concerning the effect of basil and/or chamomile on blood protein, reported biochemical results displayed no adverse effects on blood components as a result of adding different natural feed additives reflected in normal blood protein and its fractions. Protein patterns concentration exhibited healthy, non-pathological and non-toxic effect of basil addition on liver. The same findings were obtained by [33] with biotonic as herbal feed additive. Total protein and albumin increased significantly due to supplementation with chamomile, the same findings were reported in sheep by [34]. This increase in plasma protein may be attributed to the improved nitrogen absorption [35] and reflects no pathological disorders in the liver [36].

Regarding the effect of basil and/or chamomile on lipid profiles, both additives had lipid lowering effects reflected in decreased serum total lipid, triglyceride and cholesterol. These results agreed with those obtained by [26] who reported that sweet basil (SB) significantly decreased serum cholesterol, triglycerides levels. In addition, Chamomile flowers' supplementation resulted in significantly decreased serum total cholesterol and triglyceride levels in broilers [9], in sheep [34] and in goat [37]. The reduction of total blood cholesterol may be due to an active substrate of SB supplementation as discussed by [38] where the most of herbal feed additives inhibits metabolism of lipid via interfering of cholesterol micelles solubilization in the gastrointestinal tract with consequence decrease in cholesterol absorption and increase in fecal bile acid cholesterol excretion. Furthermore, herbal feed additives (as green tea) reduced pancreatic and gastric lipase activity leading to interfering with fat digestion [39].

TABLE VII
EFFECT OF BASIL AND/OR CHAMOMILE ON SERUM PROTEIN AND ITS FRACTIONS AS WELL AS ON SERUM LIPID PROFILES OF BROILER CHICKS

Parameter	Group (Mean ± SE)			
	G1	G2	G3	G4
Total protein (g/dl)	7.4 ±0.1^{b}	7.0 ±0.2^{c}	7.7 ±0.1^{a}	6.9 ±0.3^{c}
Albumin (g/dl)	4.2 ±0.1^{ab}	4.1 ±0.2^{b}	4.6 ±0.1^{a}	3.8 ±0.1^{c}
Globulin (g/dl)	3.2 ±0.1^{a}	2.9 ±0.1^{b}	3.2 ±0.1^{a}	3.2 ±0.1^{a}
Total lipids (mg/dl)	492.7 ±35.2^{a}	471.6 ±41.3^{b}	488.7 ±35.2^{a}	436.6 ±41.3^{c}
Total cholesterol (mg/dl)	133.4 ±1.9^{a}	129.6 ±4.6^{a}	128.0 ±4.2^{a}	119.9 ±.2.8^{b}
Triglycerides (mg/dl)	128.3 ±2.4^{a}	118.0 ±2.3^{b}	127.6 ±3.3^{a}	116.4 ±1.9^{b}

Means within the same row bearing different superscripts are significant at ($P<0.05$)

IV. CONCLUSION

The results of this study confirmed that using basil and/or chamomile supplementation resulted in economically viable production of high-quality healthy white meat containing no harmful residues.

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