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Effect of Dietary Anise Seeds Supplementation on Growth Performance, Immune Response, Carcass Traits and Some Blood Parameters of Broiler Chickens

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Abstract: The objective of the present study was to investigate the effect of dietary anise seeds supplementation on growth performance, immune response, some blood parameters and carcass traits of broiler chickens. Two hundred and seventeen Arbor Acre one day old broiler chicks were randomly allotted into 7 groups (31 per each) of mixed sex. Anise seed was supplemented to the basal diet at 0.0 (control), 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 g/kg diet (groups 2-7), respectively and the trail was lasted for 6 weeks. The analysis of variance of the data indicated that anise supplementation at 0.5 and 0.75 g/kg of diet (groups 3 and 4) significantly ($p \leq 0.05$) improved body weight gain, performance index and relative growth rate of broiler chicken, while had no significant effect on feed intake and feed conversion ratio when compared with the control. Moreover the highest inclusion level of anise (1.5 g/kg diet) of broiler chicken diet (group 7) reduced growth performance. On the other hand anise supplementation at 0.5 g/kg (group 3) of broiler diet improve blood picture (RBCs counts, WBCs count, HB and PCV%) clearly than other anise supplementation levels and significantly ($p \leq 0.05$) increased lymphocytes when compared with the control, while anise supplementation in broiler chickens diets increased serum albumin, decreased globulin concentration, increased albumin/globulin ratio, non significantly reduced serum GOT and the lower levels of anise (groups 2 and 3) reduced serum concentration of GPT, glucose, cholesterol while had no effect of serum phospholipids and uric acids concentrations when compared with the control. Anise supplementation had non significant effect on HI antibody titer to Newcastle disease vaccine, dressing percent and the anise level at 0.5 g/kg (group 3) non significantly increased thymus gland weight relative to the body weight and had no effect on both bursa and spleen index while the higher level (group, 7) had negative effect on spleen, bursa and thymus gland weight percent, that indicate anise at 0.5 g/kg supplementation had stimulatory immune effect, my provide hepatoprotective effect and improve the economical efficiency of production while, the higher level may be had negative effect.

Key words: Anise, broiler chickens, growth performance, immune response, carcass traits, economical efficiency

INTRODUCTION

Antibiotics Growth Promoters (AGP) for poultry diets have been banned for use in the European Union and pressure from consumer groups and major poultry buyers has threatened their removal from diets in the US. Therefore, searches for alternate products can aid in growth promotion, improved feed utilization and maintenance of gut health are taking place. Herbs species and various plant extracts have received increasing attention as possible AGP replacements (Hertrampt, 2001; Revington, 2002; Huyghebaert, 2003). The world Health Organization estimated that, 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts or their active components (Ciftci *et al*., 2005). Those plants and their components are perceived as natural and safe by consumers. Such components are already established as flavorings in human and animal feeds.

Aromatic plants have been used traditionally in the therapy of some diseases for a long time in the world. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpeniods, lignans, sulfides, polyphenolics, caroteniods, coumarins, saponins, plant sterols and phthalides have been identified (Craig, 1999).

Research interest has focused on various herbs that posses hypolipidemic, antiplatelet, antitumor, or immune stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. In addition to their antimicrobial activity (Elgayyar *et al*., 2001; Singh *et al*., 2002; Valero and Salmeron, 2003), they posses biological activities such as that of antioxidants (Lopez-Bote *et al*., 1998; Botsoglou *et al*., 2002; Miura *et al*., 2002) and as hypocholesterolemics (Craig, 1999) and stimulate effect on animal digestive systems (Jamroz and Kamel, 2002; Ramakrishna *et al*., 2003; Ciftci *et al*., 2005), to increase

production of digestive enzymes and improve utilization of digestive products through enhanced liver functions (Langhout, 2000; Williams and Losa, 2001; Hermandez *et al*., 2004). In limited research, some aromatic plants and their components on the performance, the addition of these substances to the feeds and water improved feed intake, feed conversion ratio and carcass yield (Bassett, 2000; Hertrampt, 2001; Turker, 2002; Alcicek *et al*., 2003).

As an aromatic plant, anise (*Piminella anisum* L.) is an annual herb in digeneous to Iran, India, Turkey and Egypt and many other warm regions in the world. As a medicinal plant, anise has been used as stimulating effect of digestion and antiparasitic (Cabuk *et al*., 2003), antibacterial (Singh *et al*., 2002), antifungal (Soliman and Badea, 2002) and antipyretic (Afifi *et al*., 1994) and could have some direct antiviral effects.

Very few studies in poultry performance have been conducted using anise or other essential oils (Lee *et al*., 2004; Florou-Paneri *et al*., 2005; Ertas *et al*., 2005; Ciftci *et al*., 2005), while only one trial showed some effect of the anise seeds on broiler performance (Al-Kassie, 2008) but no review explained the effect of that seeds on the carcass quality, immune response and blood parameters so that we needs to be tested.

In this study, we aimed the use of anise seed in broiler nutrition as a natural growth promoting, digestive and immune stimulant substance. For this purpose, the different level of anise seeds were added in basal diet and studied to determine the effect on performance, carcass traits, some blood parameters, immune response and economical efficiency of production compared to the control.

MATERIALS AND METHODS

This research was carried out at the Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Alexandria University, to investigate the effect of different dietary additional levels of anise seed on growth performance, nutrient digestibility, carcass traits, some blood constituents and immune response of broiler chickens.

Birds, Accommodation and management: A total of 217 Hubbard one-day-old broiler chicks were used in this study. The broiler chicks were randomly allotted into 7 equal groups (31 per each) of mixed sex. The chicks were housed in a clean well ventilated room, previously disinfected with formalin. The room was provided with electric heaters to adjust the environmental temperature according to the age of the birds. Feeds and water were supplied *ad-libitum*. Prophylactic measures against the most common infectious diseases were carried out. The chicks were vaccinated against Newcastle disease with different types of Newcastle disease vaccine as presented in Table 1.

Table 1: Vaccination program of broiler chicks during the experimental period

Age (days)	Vaccine	Route of vaccination
7	Hitchner[1]	Eye drop
12	Gumbora[2]	“”“”“”
14	Killed ND[3]	I/M injection
17	Lasota[4]	Eye drop
20	Gumbora	“”“”“”
26	Lasota	“”“”“”
30	Gumbro	“”“”“”
35	Lasota	“”“”“”

[1]B1 Hitchner (Izovac), [2]Izovac Gumbro Batch No. 7125, [3]Killed N.D. (Intervet), [4]ND vaccine Lasota (Vet. Ser. And Vacc., Res. Insti. Cairo, Egypt)

Table 2: Ingredient composition and chemical analysis of the basal diet

	Starter diet	Grower diet	Finisher diet
Ingredients:			
Yellow Corn	61.08	65.08	66.88
Soy meal (44 (%))	24.00	18.50	16.00
Corn G. meal	10.00	10.00	10.00
Dry fat[1]	-	2.00	3.000
Limestone	1.30	0.80	0.500
Di-calcium phosphate	2.50	2.50	2.500
Salt (NaCl)	0.37	0.37	0.370
Vitamin-trace m. Mixture[2]	0.35	0.35	0.350
DL-ethionine	0.10	0.10	0.100
L-Lysine	0.20	0.20	0.200
Coccidiostate[3]	0.10	0.10	0.100
C. Analysis:			
Moisture (%)	11.22	11.34	11.31
Crude protein (%)	22.20	20.17	19.20
Ether extract (%)	2.76	4.86	5.910
Ash (%)	6.76	6.37	6.320
ME Kcal/kg[4]	2957.40	3088.00	31720
C/P ratio	133.22	153.10	165.21

[1]Dry fat (Magnapacl) produced by Marel Misr Co. (Egypt) and contain 84% palm oil and 9% Ca. [2]Protoba Mix produced by El-Toba cO. For Premixes and Feed Elsadat City Egypt. Each 3 Kilograms contain: Manganese 100000 mg, Zinc 600000 mg, Iron 30000 mg, Copper 10000 mg, Iodine 1000 mg, Selenium 200 mg, Cobalt 100 mg, 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; 3: Kill cox, Produced by Arabian company for pharmaceutical industries; [4]Calculated according the NRC (1994)

Experimental design and feeding program: The broiler chicks were randomly allotted into 7 groups; each group of (31 per group) received one out of the different experimental diets during the experimental period (6 weeks experiment). Diets were formulated based on corn and soybean meal to meet the requirements according to NRC (1994). The ingredient composition and chemical analysis (AOAC, 1985) of the Basal Diet (BD) are presented in Table 2. The applied experimental design is presented in Table 3. All the experimental diets as well as fresh water were constantly available throughout the experimental period.

Table 3: The experimental design during experimental period

Group No.	Diet	Anise seed supplementation level (g/kg)
1	Basal diet	---
2	“”	0.25
3	“”	0.5
4	“”	0.75
5	“”	1.0
6	“”	1.25
7	""	1.5

Measurements: Body weight development, body weight gain and feed intake of broiler chicks in different groups were weekly recorded. Feed Conversion Ratio (FCR) and Relative Growth Rate (RGR) were calculated according to Lambert *et al*. (1936) and Brody (1968), respectively.

Immune response measurements: Haemagglutination Inhibition test: 4 sets of blood samples were collected from the experimental birds of each group at 14, 24, 34 and 42 days of age. Blood samples was collected without anticoagulant for separation of sera to detect the titer of antibodies against Newcastle disease vaccine using Haemaglutination Inhibition test (HI) as an indicative of the bird’s immune response in the different experimental groups. Micro technique of HI test was done according to Takatasy (1955). Geometric Mean Titre (GMT) was calculated according to Brugh (1978).

Phagocytic activity and index: phagocytic activity was determined according to Kawahara *et al*. (1991). Fifty micrograms of Candida albicans culture were added to 1 mL of citrated blood, collected at the end of experiment by slaughtering 5 birds from each group. Treated blood samples were put in shaker water bath at 23-25°C for 3-5 h. Smears of blood were made and then stained with Geimsa stain. Phagocytosis was estimated by determining the proportion of macrophages which contain intracellular yeast cells in a random sample of 300 macrophages and expressed as percentage of Phagocytic Activity (PA). The number of phagocytized Candida cells was counted in the phagocytic cells to calculate the phagocytic index according to the following equations:

$$\text{Phagocytic activity} = \frac{\text{Macrophages containing yeast}}{\text{Total number of macrophages}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{Number of cell phagocytized}}{\text{Number of phagocytic cell}} \times 100$$

Differential leucocytic count: This test was done at the end of experimental period as blood film was prepared according to the method described by Lucky (1975). Ten drops from May-Grunwald stain stock solution were added to equal amount of distilled water on a dry unfixed smear then mixed and left for 1 min for staining. The dye was decanted without rinsing. Diluted Geimsa stain was poured over the film as counter stain and left for 20 min then rinsed in water current and examined by oil emersion lens. The percentage and absolute value for each type of cells were calculated according to Maxine and Benijamin (1985).

Estimated blood parameters: At the end of the experimental period, blood samples were taken from 5 birds of different groups. The blood samples were left to drop on the side of the tube to prevent destruction of RBCs. Each blood sample was left to coagulate at room temp. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 min. The clear serum was transferred carefully to clean and dry vials and kept in deep freezer until analysis for determination of total serum protein, albumin, SGPT and SGOT, alkaline phosphatase, total cholesterol (LDL and HDL were determined also), triglycerides, phospholipids, uric acid and creatinine according to Doumas *et al*. (1981), Reinhold (1953), Reitman and Frankel (1957), Kind and King (1954); Zak *et al*. (1954), Sidney and Barnard (1973), Giorgio (1974), Henry (1978), Patton and Crouch (1977) and Fossatti and Prencipe (1980), respectively.

Carcass characteristic: At the end of the experimental period, 5 chicks from each group were randomly selected and scarified to calculated the carcass and dressing percentages, also organ weight and its relative to the live weight were calculated.

Economical efficiency of production: Total production cost was calculated including prices of 1 day old chicks, feeding, heating, veterinary care, management and housing. Selling price was calculated by multiplying total live weight of the birds produced by the price per unit weight commonly offered in the market. Economical efficiency was estimated as:

$$\text{Economical efficiency} = \frac{\text{Net revenue}}{\text{Total production cost}} \times 100$$

Statistical analysis: The analysis of variance for the obtained data was performed using (SAS, 1987) to assess significant differences.

RESULTS AND DISCUSSION

Body weight development: The effect of dietary anise seeds supplementation on body weight development of broiler chicks is presented in Table 4. The analysis of variance of the data at the start of the experiment showed that there was no significant difference in body weight between different experimental groups, while there were differences between the broiler fed different levels of anise seeds supplementation began in the 3rd week and more appeared at the end of the experiment.

Table 4: Effect of dietary anise seed supplementation on body weight development (g/bird) of broiler chickens in different experimental groups

Age (week)	Groups No. 1	2	3	4	5	6	7
0	42.7±0.58^{a}	42.74±0.61^{a}	42.58±0.64^{a}	42.71±0.58^{a}	42.81±0.62^{a}	42.68±0.57^{a}	42.68±0.57^{a}
1	165.75±3.55^{a}	164.32±3.83^{a}	170.29±3.36^{a}	175.23±3.26^{a}	162.87±3.90^{a}	170.39±3.52^{a}	164.48±4.59^{a}
2	416.82±6.66^{a}	416.39±8.05^{a}	423.55±7.53^{a}	436.13±5.83^{a}	413.87±8.5^{a}	428.87±7.54^{a}	409.13±9.16^{a}
3	773.58±11.81^{b}	762.61±13.94^{b}	805.23±15.04^{a}	812.91±12.95^{a}	789.87±15.12^{ab}	801.87±14.09^{ab}	760.23±15.46^{b}
4	1211.80±18.28^{a}	1196.19±24.91^{a}	1277.97±23.88^{a}	1216.87±20.09^{a}	1223.68±20.90^{a}	1246.77±24.75^{a}	1176.07±21.60^{a}
5	1615.55±27.98^{b}	1678.23±34.61^{ab}	1747.55±33.76^{a}	1714.84±30.04^{a}	1725.23±29.59^{a}	1683.36±37.98^{b}	1673.45±27.59^{b}
6	2137.73±35.14^{b}	2145.74±49.50^{b}	2311.42±47.31^{a}	2290.97±46.57^{a}	2212.45±44.77^{ab}	2141.26±40.24^{b}	2078.00±38.19^{b}

Values are means±standard error; Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 5: Effect of dietary anise seed supplementation on average growth performance parameters of broiler chickens

Parameters	Groups No. 1	2	3	4	5	6	7
Total B. gain (TBG/g)	2094.73±39.52^{b}	2103.0±48.92^{b}	2268.84±46.69^{a}	2183.10±79.86^{ab}	2169.65±44.18^{ab}	2098.58±39.69^{b}	2035.26±37.64^{b}
TBG (RTC)*	100	+0.39	+8.31	+4.22	+3.58	+0.18	-2.84
Daily B. gain (g)	49.88±0.82^{b}	50.07±1.16^{b}	54.02±1.11^{a}	53.53±1.09^{a}	51.66±1.05^{ab}	49.97±0.95^{b}	48.46±0.90^{b}
Average daily feed intake (g)	96.86±3.72^{a}	99.32±3.86^{a}	101.12±7.40^{a}	100.31±5.25^{a}	97.31±5.25^{a}	91.97±3.04^{a}	93.34±2.94^{a}
FI (RTC)*	100	+2.54	+4.4	+3.56	+0.46	-5.05	-3.63
FCR	1.96±0.04^{a}	2.02±0.05^{a}	1.90±0.04^{a}	1.90±0.04^{a}	1.91±0.04^{a}	1.86±0.04^{a}	1.95±0.04^{a}
FCR (RTC)*	100	103.06	96.94	96.94	97.45	94.90	99.49
PI	111.04±3.54^{b}	109.94±5.05^{b}	125.03±5.02^{a}	123.71±4.97^{a}	118.85±4.77^{ab}	117.59±4.38^{ab}	108.96±3.97^{b}
RGR**	192.15±0.04^{c}	192.14±0.09^{c}	192.74±0.05^{a}	192.65±0.05^{a}	192.38±0.05^{b}	192.16±0.05^{b}	191.92±0.06^{d}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$) *FI= Feed Intake; FCR = Feed Conversion Ratio; RTC = Relative to The Control; ** Relative growth rate

It was observed that broiler chick groups which fed on the basal diet supplemented by 0.50 and 0.75 g of anise seeds/kg diet (groups 3 and 4) showed significantly ($p \leq 0.05$) higher body weight by about 8.1 and 7.2%, respectively, when compared with the control, while chicks groups which fed on the basal diet supplemented by 0.25, 1.0 and 1.25 g/kg diet (groups 2, 5 and 6) showed non significantly ($p \leq 0.05$) higher by about 0.4, 3.5 and 0.2%, respectively when compared with the control one, on the other hand the higher supplementation level of anise seed 1.5 g/kg diet (group 7) recorded non significant ($p \leq 0.05$) reduction in the body weight by about 2.8% when compared with the control.

The highest body weight was recorded in broiler group No. 3 (2311.42 g) which fed on the basal diet supplemented by 0.5 g anise seed/kg diet, followed by group No. 4 (2290.97 g) which fed on 0.75 g anise seed/kg diet, followed by group No. 5 (2212.45 g) fed on 1.0 g anise seed/kg diet and the lowest body weight was recorded in broiler chicks in group No. 7 (2078.0 g) which fed on the highest level (1.5 g/kg diet) of anise seeds. As shown anise seeds supplementation at 0.5 g and 0.75 g/kg diet significantly improved body weight when compared with the control and with both lower (0.25 g/kg) and higher (1.25 and 1.5 g/kg) levels of anise seeds. These differences among the groups may be due to active ingredient such as anothole of the anise which have digestive stimulating effect (Cabuk *et al*., 2003). Besides, essential oils of the anise have been reported to posses antimicrobial (Dorman and Deans, 2000; Burt and Reinders, 2003; Valero and Salmeron, 2003), anticoccidial (Giannenas *et al*., 2003), antifungal (Pina-Vaz *et al*., 2004) and antioxidant effects (Lee and Shibamoto, 2002; Gulcin *et al*., 2004). The reason of reducing body weight with the higher level of anise seed supplementation (1.5 g/kg diet) could be affected negatively digestive system.

The data are in agreement with (Alcicek *et al*., 2003; Ertas *et al*., 2005), who stated that addition of 200 ppm essential oil mix derived from oregano, clove and anise improved body weight, while addition of 100 or 400 ppm reduced live body weight when compared with the negative control of broiler chickens. On the other hand that data are disagreement with that obtained by (Ciftci *et al*., 2005), they reported that higher addition of anise oil (400 mg/kg) was more effective on broiler performance than the lower supplementation levels (100 or 200 mg/kg) when compared with the control. Also Al-Kassie (2008), they indicated that anise seed supplementation up to 1% improved live body weight and considered as a potential growth promoter in poultry. That difference may be due to using different feed ingredient of broiler feeding and also the different environmental conditions.

Growth performance: Table 5 shows the effect of dietary anise seeds supplementation at different levels on body weight gain, feed intake, Feed Conversion Ratio (FCR), Performance Index (PI) and Relative Growth Rate (RGR) of broiler chicks. From the data, it was observed that the highest body gain was recorded in broiler chicks group

Table 6: Effect of dietary anise seed supplementation on blood picture (erythrocyte count (RBCs), leucocytes counts (WBCs), hemoglobin (Hb) and packed cell volume (PCV)%) of broiler chickens

	Groups No.						
Parameters	1	2	3	4	5	6	7
RBCs count (10^6)	2.0±0.09^{a}	2.18±0.26^{a}	2.02±0.13^{a}	1.98±0.22^{a}	2.3±0.12^{a}	2.33±0.11^{a}	2.2±0.07^{a}
WBCs count (10^3)	21.75±0.85^{b}	21.50±0.50^{b}	22.25±0.85^{ab}	22.75±0.75^{ab}	24.25±0.48^{a}	21.75±0.48^{b}	22.0±0.91^{b}
HB%	10.0±0.58^{b}	10.0±0.41^{b}	12.25±0.75^{a}	10.50±0.50^{ab}	11.25±0.75^{ab}	8.25±0.75^{b}	9.50±0.65^{b}
PCV%	30.25±1.10^{b}	32.00±1.22^{bc}	37.0±1.08^{a}	31.75±0.48^{abc}	34.75±1.44^{abc}	27.75±1.65^{b}	27.25±2.39^{b}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at (p= 0.05)

Table 7: Effect of dietary anise supplementation on hemagglutination inhibition (HI), Geometric mean antibody titer (log_2) against ND virus vaccine of broiler chickens

Age	Groups No.						
(days)	1	2	3	4	5	6	7
14	1.67±0.33^{a}	1.67±0.33^{a}	1.33±0.33^{a}	2.33±0.33^{a}	1.67±0.67^{a}	2.00±0.58^{a}	2.00±0.00^{a}
24	2.00±0.00^{a}	2.33±0.88^{a}	1.00±0.00^{a}	1.33±0.33^{a}	1.67±0.33^{a}	1.67±0.33^{a}	1.33±0.33^{a}
34	2.67±0.33^{a}	3.33±0.33^{a}	3.33±0.33^{a}	2.67±0.67^{a}	3.33±0.67^{a}	1.33±0.33^{a}	3.33±0.67^{a}
42	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}

No. 3 (2268.84 g/bird) which fed on the basal diet supplemented by 0.5 g of anise seed/kg diet, while anise addition at 0.25, 0.75, 1.0 and 1.25 g/kg diet (groups 2, 4, 5 and 6) non significantly ($p \leq 0.05$) improved body gain by about 0.4, 4.2, 3.6 and 0.2%, respectively when compared with the control, but the higher inclusion rate (1.5 g/kg diet) of anise seeds (group 7) non significantly ($p \leq 0.05$) reduced body gain by about 2.9% when compared with the control one.

The effect of different amounts of anise on the feed intake is presented in Table 5. The average daily feed intake not differed significantly ($p \leq 0.05$) between groups. The improvement was observed by about 2.5, 4.4, 3.6 and 0.5% in groups 2, 3, 4 and 5 which fed on the basal diet supplemented with 0.25, 0.5, 0.75 and 1.0 g of anise/kg, when compared with control group, while the higher level (1.25 and 1.5 g/kg diet) of anise (groups 6 and 7) reduced feed intake by about 5.1 and 3.6%, respectively.

The analysis of variance of the data revealed that anise seed supplementation had no significant ($p \leq 0.05$) effect on FCR of broiler chicks. The best FCR was recorded in chick group No. 6 which fed on basal diet supplemented by 1.25 g anise/kg diet and that improvement related to the lower feed intake and comparable body weight gain compared with the control, while the lower level (0.25 g anise/kg) slightly increased FCR by about 3.1% when compared with the control and the other experimental groups (3, 4, 5 and 7) non significantly ($p \leq 0.05$) improved FCR by about 3.1,3.1%, 2.6 and 0.5%, respectively when compared with control. Moreover, anise supplementation at 0.5 and 0.75 g/kg diet (groups 3 and 4) significantly ($p \leq 0.05$) improved PI and RGR of broiler chicks while anise addition at 1.0 and 1.5 g/kg (groups 5 and 6) non significantly ($p \leq 0.05$) improved both PI and RGR but the lowest and highest addition amount of anise 0.25 and 1.5 g/kg (groups 2 and 7) non significantly ($p \leq 0.05$) reduced the mentioned parameters when compared with the control. The results are in agreement with results of studies in which different essential oils were added to poultry diets. In these studies reported that essential oils derived from different aromatic plants have improved weight gain and FCR (Ather, 2000; Bassett, 2000; Williams and Losa, 2001; Tucker, 2002; Kamel, 2001; Alcicek *et al*., 2003; Giannenas *et al*., 2003), the inclusion levels of essential oils varied from 20-200 ppm and the authors noticed that improvement in weight gain and feed intake when compared with the control. Also the data are in agreement with that obtained by Jamroz *et al*. (2003), they supplemented a wheat-barley diet for broilers with avilamycin or 150 or 300 ppm of a plant extract containing capsaicin, carvacrol and cinnamic aldehyde and stated that the antibiotic and the 2 levels of the plant extract improved FCR by 5.8, 3.1 and 7.1%, respectively, also, Ciftci *et al*. (2005), who reported that anise oil supplementation in broiler feed had no significant effect on feed intake and improved both daily weight gain and FCR compared with the negative control. At the same time, the data are also harmony with those obtained by Botsoglou *et al*. (2002), who reported that when dietary oregano essential oils, at the concentrations of 50 and 100, were fed to broiler chickens for a period 38 days had no effects on body gain and FCR. Ertas *et al*. (2005) reported that addition of 200 ppm essential oil mix derived from oregano, clove and anise improved body gain and FCR compared to the control in broiler.

Blood picture: The effects of dietary anise supplementation on some blood picture of broiler chickens in different groups are presented in Table 6. The results regarding RBCs count showed that there was non significant ($p \leq 0.05$) increase with anise supplementation groups by about 9, 1, 15, 16.5 and 10%

Table 8: Effect of dietary anise seeds supplementation on phagocytic activity, phagocytic index and differential leucocytes count percent of broiler chick groups

	Groups No.						
Parameters	1	2	3	4	5	6	7
Phagocytic activity	19.75±0.48^{b}	20.50±0.50^{b}	21.50±1.04^{ab}	20.00±0.4^{b}	20.75±0.63^{b}	21.00±0.71^{ab}	22.75±0.75^{a}
Phagocytic index	1.55±0.24^{bc}	1.48±0.13^{c}	1.73±0.11^{bc}	1.35±0.10^{c}	1.50±0.16^{c}	1.85±0.10^{b}	2.18±0.05^{a}
Lymphocytes	42.25±0.75^{c}	43.50±0.50^{ab}	45.50±0.87^{a}	45.00±0.82^{ab}	44.75±0.48^{ab}	42.50±0.65^{c}	41.25±0.63^{c}
Monocytes	1.25±0.25^{a}	1.75±0.25^{a}	1.75±0.48^{a}	1.00±0.00^{a}	1.25±0.25^{a}	1.75±0.25^{a}	2.25±0.48^{a}
Basophile	7.00±0.41^{a}	7.25±0.25^{a}	7.25±0.86^{a}	7.00±0.41^{a}	8.25±0.48^{a}	7.50±0.65^{a}	7.50±0.50^{a}
Eosinophil	9.75±0.63^{a}	10.00±0.41^{a}	9.00±0.41^{a}	8.50±0.96^{a}	9.00±1.00^{a}	8.25±0.95^{a}	9.25±0.75^{a}
Neutrophil	39.75±0.85^{a}	37.75±0.75^{ab}	36.50±1.66^{b}	38.75±0.48^{ab}	36.75±1.11^{ab}	40.25±1.49^{ab}	39.75±0.85^{a}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 9: Effect of dietary anise seeds supplementation on serum total protein, albumin, globulin and albumin/globulin (A/G) ratio of broiler chickens

	Groups No.						
Parameters	1	2	3	4	5	6	7
Total protein (g/dL)	4.18±0.10^{a}	4.33±0.19^{a}	3.88±0.10^{a}	4.10±0.21^{a}	3.65±0.18^{a}	3.95±0.21^{a}	3.50±0.16^{a}
Albumin (g/dL)	2.08±0.13^{b}	2.15±0.10^{b}	2.58±0.06^{a}	2.28±0.08^{ab}	2.38±0.10^{ab}	2.03±0.14^{b}	2.40±0.18^{ab}
Globulin (g/dL)	2.10±0.15^{a}	2.180±0.20^{a}	1.30±0.17^{b}	1.83±0.12^{ab}	1.28±0.18^{b}	1.93±0.15^{ab}	1.10±0.13^{b}
A/G ratio	1.02	1.03	1.98	1.34	2.16	1.07	2.06

Values are means±standard error. Mean values with different letters at the same row differ significantly at (p= 0.05)

in group 2, 3, 5, 6 and 7, respectively when compared with the control (No. 1) while group No. 4, which fed on the basal diet with 0.75 g anise/kg diet exhibited non significant decrease in RBCs count. Regarding HB and PCV%, the results revealed that anise supplementation at 0.5 g/kg diet (group, 3) significantly ($p \leq 0.05$) increased HB and PCV% by about 22.5 and 22.3%, respectively, when compared with the control while the other addition levels had non significant effect. On the other hand the anise supplementation increased WBCs count by about 2.3, 4.6, 11.5, 0.0 and 1.1% for groups 3-7, respectively when compared with the control. Generally, we notice that anise addition at 0.5, 0.75 and 1.0 g/kg diet improve the blood picture of the broiler chickens while the lowest and higher levels had no significant effect of those parameters.

Hemagglutination Inhibition test (HI) to newcastle disease vaccine: Table 7, illustrates the effects of dietary anise supplementation on the results of HI antibody titer to Newcastle disease vaccine of broiler chickens. The analysis of variance of the obtained data showed non significant ($p \leq 0.05$) variations in HI titer at 14th, 24th, 34rd and 42nd days of broiler chickens fed on the basal diet or supplemented with anise seeds at different levels and these results are similar to that concluded by Al-Ankari *et al*. (2004), who reported that the mean antibody titer against NDV live vaccine response showed no evidence that Habek Mint had stimulated or suppressed the immune system of the broiler chickens.

Phagocytosis and differential leukocytic count: The effect of dietary anise supplementation on phagocytosis and differential leukocytic count are presented in Table 8. The analysis of variance of the data showed that there was an increase in phagocytic activity in all broiler groups fed on the anise supplemented diet when compared with control. The highest phagocytic activity was observed in group 7 which fed basal diet supplemented with highest level of anise (1.5 g/kg diet), followed by group 3, which fed on the basal diet supplemented by 0.5 g anise/kg and then followed by group 6 which fed on the basal diet supplemented by 1.25 g anise/kg. From this result, it was observed that the groups fed on 0.5, 1.25 and 1.5 g of anise/kg diet (groups 3, 6 and 7), respectively showed higher phagocytic index when compared with the control, while group 2, 4 and 5, which fed on the basal diet supplemented by 0.25, 0.75 and 1.0 g of anise/kg diet recorded non significant ($p \leq 0.05$) reduction of phagocytic index compared with the control one. The obtained data indicated that anise supplementation at 0.5 g/kg diet (group 3) increased lymphocytes significantly ($p \leq 0.05$) by about 7.7% when compared with the control, while anise addition at 0.25, 0.75, 1.0 and 1.25 g/kg diet (group 2, 4, 5 and 6), respectively non significantly ($p \leq 0.05$) increased lymphocytes by about 2.96, 6.5, 5.9 and 0.6%, respectively when compared with the control. On the other hand anise addition at 1.5 g/kg negatively effect on those type of immune cell and non significantly ($p \leq 0.05$) reduced by about 2.4%. Moreover, the analysis of variance of the obtained data revealed that there are non significant variations between different experimental broiler groups in the concentration of monocytes, basophile, eosinophile and neutrophile. From the data can be concluded that dietary anise addition in broiler chicken diets has an non specific immunostimulatory effects on broiler chicken

Table 10: Effect of dietary anise seeds supplementation on some blood parameters of broiler chickens

	Groups No.						
Parameters	1	2	3	4	5	6	7
GOT (U/100 mL)	68.25±1.11^{a}	64.50±1.32^{a}	67.75±1.65^{a}	67.50±1.23^{a}	67.00±1.08^{a}	65.50±0.65^{a}	65.75±1.03^{a}
GPT (U/100 mL)	73.75±1.44^{a}	72.25±1.11^{ab}	73.50±0.87^{a}	74.00±1.78^{a}	74.00±1.58^{a}	69.25±1.03^{b}	75.25±1.03^{a}
Glucose (mg/dL)	82.75±1.38^{b}	83.75±1.25^{ab}	78.00±1.68^{c}	80.75±1.44^{b}	79.50±1.26^{b}	80.25±2.49^{b}	87.75±0.48^{a}
Alkaline phosphatase (mg/dL)	11.50±1.04^{a}	11.50±0.65^{a}	11.00±1.58^{a}	11.00±0.41^{a}	11.25±0.63^{a}	11.25±0.85^{a}	11.25±0.86^{a}
Cholesterol (mg/dL)	204.75±2.95^{a}	196.5±5.69^{a}	193.5±4.11^{a}	194.75±2.02^{a}	194.75±2.17^{a}	201.0±2.68^{a}	198.5±2.63^{a}
Cholest LDL (mg/dL)	106.5±3.80^{a}	105.0±2.12^{a}	105.5±2.33^{a}	106.0±1.58^{a}	107.25±3.12^{a}	105.25±2.53^{a}	107.0±4.26^{a}
Cholest HDL (mg/dL)	41.50±1.04^{b}	41.75±0.85^{b}	41.25±1.03^{b}	40.50±0.87^{b}	45.75±0.75^{a}	45.75±1.65^{a}	42.25±1.97^{b}
Triglyceride (mg/dL)	198.25±4.92^{a}	193.75±6.14^{a}	190.75±2.93^{a}	186.00±2.61^{a}	190.75±3.71^{a}	185.00±1.96^{a}	184.25±1.25^{a}
Phospholipids (mg/dL)	106.25±2.02^{a}	100.5±3.01^{a}	106.5±1.04^{a}	105.25±2.56^{a}	110.5±0.87^{a}	105.75±3.77^{a}	101.25±2.10^{a}
Uric acid (mg/dL)	2.40±0.27^{a}	2.33±0.11^{a}	2.40±0.11^{a}	2.20±0.06^{a}	2.38±0.10^{a}	2.63±0.21^{a}	2.23±0.05^{a}
Creatinine (mg/dL)	0.73±0.11^{b}	0.70±0.08^{b}	0.85±0.10^{ab}	1.20±0.21^{a}	1.13±0.05^{a}	0.83±0.15^{ab}	0.80±0.07^{ab}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

immune cell. These results are similar to that concluded by Walter and Bilkei (2004) who reported that the non-T/on-B cells in peripheral blood lymphocytes was significantly higher in Oregpig (dried leaf flower of Origanum vulgare, enriched with 500 g/kg cold pressed essential oils of the leaf and flower) received pigs than in the control animals, Moreover, the proportion of CD4+ CD8+ double positive T-lymphocytes in peripheral blood and mesenteric lymph node was higher in Oregpig receiving pigs than in the control animals so that dietary oregano has non specific immunostimulatory effects on porcine immune cells.

Effect of anise supplementation on serum protein, albumin and globulin levels: The effects of dietary anise supplementation on serum total protein, albumin, globulin and albumin/globulin (A/G) ratio of broiler chickens in different groups are illustrated in Table, 9. Analysis of variance of the obtained data revealed that anise supplementation at 0.5, 0.75, 1.0, 1.25 and 1.5 g/kg diet (Groups 3-7), respectively showed a non significant ($p \leq 0.05$) reduction in serum total protein level by about 7.2, 1.9, 12.7, 5.5 and 16.3%, respectively, when compared with the control, while the lowest level (0.25 g/kg) of anise supplementation recorded a non significant ($p \leq 0.05$) increase by about 3.6%. The data revealed that anise supplementation at 0.5 g/kg (group, 3) significantly ($p \leq 0.05$) increased serum albumin concentration by about 24% when compared with the control, while supplementation at 0.25, 0.75, 1.0 and 1.5 g/kg diet (groups, 2, 4, 5 and 7), respectively recorded non significant ($p \leq 0.05$) increase in serum albumin concentration by about 3.4, 9.6, 14.4 and 15.4%, respectively when compared with the control. On the other hand the higher anise levels (1.25 g/kg) supplementation (groups 6) non significantly ($p \leq 0.05$) decreased serum albumin concentration by about 2.4%. Serum globulin concentration recorded a contrast value to the serum albumin concentration and generally anise supplementation in broiler chickens diets increased serum albumin, decreased globulin concentration and increased albumin/globulin ratio. The control group and low level of anise supplementation (group, 2) recorded the highest values of globulin concentration and that may be attributed to enhance the resistance of chickens against different stress factors.

Some blood serum parameters: Table 10 shows the effects of dietary anise supplementation on SGPT, SGOT, glucose, alkaline phosphatase, cholesterol, cholest (Low density lipoprotein LDL and high density lipoprotein HDL) triglycerides, creatinine and uric acid of broiler chickens in different groups. It can be noticed that there was a non significant ($p \leq 0.05$) reduction in serum GOT in broiler chicken fed on the basal diet supplemented by anise at different levels by about 5.5%, 0.7, 1.1, 1.8, 4.0 and 3.7% (groups 2-7), respectively when compared with the control. While, Serum GPT showed some variation as anise supplementation at 0.25 and 0.5 g/kg (groups 2 and 3) recorded non significant ($p \leq 0.05$) reduction in SGPT when compared with the control and anise supplementation at 1.25g/kg significantly ($p \leq 0.05$) decreased SGPT, while other levels of anise supplementation (groups 4, 5 and 7) recorded non significant higher values of SGPT concentration. Generally GOT and GPT considered as liver enzyme which increased with liver damage (heptatocellular degeneration), so the decrease in SGOT and SGPT may provide evidence for the occurrence of hepatoprotective effect of anise and its essential oil (Langhout, 2000; Williams and Losa, 2001; Hermandez *et al*., 2004). Moreover, the analysis of variance of the obtained data revealed that anise supplementation non significantly ($p \leq 0.05$) decreased alkaline phosphatase values.

Regarding serum glucose data revealed that there was a significant ($p \leq 0.05$) reduction in the broiler chick group fed on the basal diet supplemented with 0.5 g anise/kg (group, 3) by about 5.7% when compared with the control, while anise supplementation at 0.75, 1.0 and 1.25 g/kg (groups 4-6) non significantly ($p \leq 0.05$)

Table 11: Effect of dietary anise seed supplementation on some carcass traits of broiler chickens

	Groups No.						
Items	1	2	3	4	5	6	7
Dressing (%)	71.43±1.14[a]	72.33±1.11[a]	72.69±1.04[a]	73.49±1.66[a]	69.88±1.45[a]	70.36±1.39[a]	71.46±0.85[a]
Heart Wt (%)	0.50±0.07[a]	0.49±0.02[a]	0.50±0.05[a]	0.52±0.04[a]	0.44±0.03[a]	0.48±0.03[a]	0.44±0.03[a]
Gizzard Wt (%)	1.85±0.12[a]	2.16±0.14[a]	2.09±0.22[a]	1.94±0.15[a]	1.80±0.11[a]	1.80±0.10[a]	1.89±0.19[a]
Liver Wt (%)	2.21±0.16[a]	1.97±0.21[a]	2.09±0.03[a]	2.05±0.15[a]	2.40±0.21[a]	2.06±0.20[a]	2.30±0.16[a]
Abdominal fat wt (%)	2.01±0.05[b]	1.84±0.05[b]	1.91±0.19[b]	2.45±0.44[ab]	2.33±0.39[ab]	2.24±0.08[b]	2.86±0.43[a]
Spleen index	0.16±0.02[a]	0.10±0.01[b]	0.13±0.004[ab]	0.16±0.02[a]	0.16±0.03[a]	0.13±0.02[ab]	0.10±0.00[b]
Bursa Wt (%)	0.21±0.01[a]	0.19±0.02[a]	0.21±0.02[a]	0.12±0.0[b]	0.16±0.03[ab]	0.21±0.01[a]	0.14±0.03[ab]
Thymus gland Wt (%)	0.36±0.07[a]	0.43±0.13[a]	0.48±0.09[a]	0.26±0.05[a]	0.27±0.03[a]	0.37±0.10[a]	0.37±0.04[a]

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 12: Economic efficiency of production as affected by dietary anise supplementation of broiler chickens

	Groups No.						
Items	1	2	3	4	5	6	7
No. of broiler chicks	31	31	31	31	31	31	31
Broiler chicks cost (USD)	24.80	24.80	24.80	24.80	24.80	24.80	24.80
Feed Cost (USD)	62.87	64.61	65.98	65.65	63.85	60.51	62.65
Other costs (USD/Group)*	15.25	15.25	15.25	15.25	15.25	15.25	15.25
Total Costs (USD)	102.92	104.66	106.03	105.70	103.90	100.56	102.70
Returns (USD)	141.93	142.60	153.93	152.60	147.26	142.60	137.94
Net Income (USD)	39.01	37.94	47.90	46.90	43.36	42.04	35.24
T. Return/T. Cost ratio (%)	137.90	136.25	145.18	144.37	141.73	141.81	134.31
Net income/T. Cost (%)	37.90	36.25	45.18	44.38	41.73	41.81	34.31

*Other costs, includes, Vet. Management and drugs, electric, water, farm rent and labor

decreased serum glucose levels by about 2.4, 3.9 and 3.0%, respectively, on the other hand the lowest and highest level of anise supplementation (groups 2 and 7) increased glucose levels when compared with the control. These results are supported by the data obtained by Lemhadri (2004) who reported that an aqueous oregano extract exhibits an anti-hyperglycemic activity in STZ rats without affecting basal plasma insulin concentrations.

Statistical analysis revealed that anise supplementation non significantly ($p \leq 0.05$) decreased cholesterol concentrations by about 4.0, 5.5, 4.9, 4.9, 1.8 and 3.1% (groups 2-7) when compared with the control. While cholesterol LDL (Low Density Lipoprotein) showed some non significant variation between groups and HDL (High Density Lipoprotein) increased with anise supplementation at 1.0, 1.25 and 1.5 g/kg (groups 5-7) when compared with the control. The data are in agreement with those obtained by Craig (1999) who reviewed the role of herbs and their essential oils as to their cholesterol lowering properties and Hood *et al*. (1978) who tested a capsule containing an essential oil which forced fed to the laying hens individually at 10, 50, 100 and 200 mg/kg/day and reported that no significance differences among treatments in plasma cholesterol levels were observed. The authors ascribed the non significant effect of the selected essential oil components to either ineffective inhibition of HMC-CoA reductase or to their fast degradation in liver. Analysis of variance of the data revealed that anise seed supplementation had no significant effect on serum phospholipids and uric acid of broiler chickens when compared with the control, but generally the triglycerides non significant ($p \leq 0.05$) decreased by about 2.3, 3.8, 6.2, 3.8, 6.7 and 7.1% in groups 2-7, respectively when compared with the control. Moreover, anise supplementation at 0.75 and 1.0 g/kg diet (groups 4 and 5) significantly ($p \leq 0.05$) increased creatinine concentration values when compared with the control, while anise supplementation at 0.5, 1.25 and 1.5 g/kg (groups 3, 6 and 7) non significantly ($p \leq 0.05$) increased creatinine level and only 0.25 g/kg of anise supplementation (group 2) slightly decreased the creatinine when compared with the control.

Carcass characteristics: The effects of dietary anise supplementation on dressing percent and some organs (gizzard, heart, liver, visible abdominal fat, spleen, bursa and thymus gland) weights relative to the live body weight of broiler chicks in different groups at the end of experimental period are summarized in Table 11.

The analysis of variance of the data indicated that chicken which fed on the basal diet supplemented with 0.25, 0.5, 0.75 and 1.5 g anise/kg diet (groups 2, 3, 4 and 7) showed non significantly ($p \leq 0.05$) improvement in dressing percent value by about 1.3, 1.8, 2.9 and 0.04%, respectively when compared with control group. Moreover, anise supplementation at 1.0 and 1.25 g/kg diet (groups 5 and 6) recorded non significantly ($p \leq 0.05$) reduction in dressing percent when compared with

control. The data are in agreement with those obtained by Zhang *et al*. (2005) reported that essential oil (150 g/ton) supplementation in broiler diet improve dressing percent compared with untreated group. On the other hand, anise supplementation non significantly ($p \leq 0.05$) effect on heart percent, gizzard weight percent and liver weight percent relative to the body weight when compared with control. Analysis of variance of the data revealed that 0.25 and 0.5 g anise/kg diet (groups 2 and 3) non significantly ($p \leq 0.05$) reduced abdominal fat weight percent relative to the body weight by about 8.46 and 4.98%, respectively when compared with the control while the higher amount of anise supplementation increased abdominal fat percent. Moreover, anise supplementation at 0.5 g/kg diet (group 3) non significantly increased thymus gland weight percent and had no effect on both bursa and spleen index when compared with the control, while the higher level of anise addition (group 7) had negative effect on spleen, bursa and thymus gland weight percent, that indicate anise at 0.5 g/kg supplementation had immune stimulatory effect and the higher level may be had a negative effect.

Economical efficiency of production: Feeding costs, total production costs, net income (USD/group) and economic efficiency of production in different broiler groups shown in Table 12. It was clear from the data that the total costs of production 102.92, 104.66, 106.33, 105.7, 103.9, 100.56 and 102.7 for groups 1-7, respectively. These results revealed that the possibility of increasing economic efficiency by anise supplementation of broiler chicken diet. The greatest economical efficiency was obtained by group 3 (which fed on the basal diet supplemented by 0.5 g anise/kg) by about 19.21% when compared with the control group, followed by group 4, 5 and 6 which fed on basal diet supplemented by 0.75, 1.0 and 1.25 g anise/kg, respectively, by about 17.1, 10.11 and 10.32, respectively while the lowest and highest levels of anise supplementation (0.25 and 1.5 g/kg) for groups 2 and 7 decreased economical efficiency of production by about 4.4 and 9.5%, respectively when compared with the control.

Conclusion: It can be concluding that anise seed (0.5 g/kg diet) supplementation improve body weight gain, FCR, performance index and relative growth rate of broiler chicken. Moreover, anise supplementation (0.5 g/kg) non significantly increased thymus gland weight relative to the body weight and had no effect on both bursa and spleen index, while the higher level (group, 7) had negative effect on spleen, bursa and thymus gland weight percent, that indicate anise at 0.5 g/kg supplementation had stimulatory immune effect, my provide hepatoprotective effect and improve the economical efficiency of production while, the higher level may be had negative effect.

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