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Effect of Various Concentrations of an Anise Seed Powder (*Pimpinella Anisum* L.) Supplement on Selected Hematological and Biochemical Parameters of Broiler Chickens

ABSTRACT

The objective of the study was to investigate the influence of anise seed (*Pimpinella anisum* L.) powder supplemented to the drinking water on selected blood parameters of broilers. In total, 360 one-day-old Hubbard Classic chicks were randomly divided into four groups with three replicates of 30 birds each. Anise seed powder was added at 0, 500, 750, and 1000mg/L to the drinking water offered to the C (control), T1, T2, and T3 groups respectively. The supplement was supplied for 56 days. When broilers were 28 and 56 days old, blood samples were collected (30 birds per group) by brachial vein puncture to evaluate the cellular components of blood (RBC, WBC, Hgb, HCT, H/L). The following serum biochemical parameters were evaluated: CHOL, GLU, TP, albumin, globulins, Ca, P, TG, total lipids, UA, and creatinine, as well as AST and ALT enzyme activities. The statistical analysis indicated that the anise supplement significantly improved blood RBC, WBC, Hgb, HCT, TP, albumin, globulin, GLU, P, and Ca levels of broilers in groups T4, T3, and T2 compared with the control group on days 28 and 56, and on average. Also, T3, T2 and T1 presented lower H/L ratios and CHOL, TG, total lipids, creatinine, UA, AST and ALT serum levels compared with the control group. The highest inclusion levels of anise seed powder, i.e., 1000 and 750mg/L, had a stimulating effect on the physiological traits of the birds.

INTRODUCTION

The use of antibiotics as growth promoters in poultry production was banned in the European Union (Windisch *et al.*, 2008) due to the presence of their residues in poultry products and antibiotic resistance of human pathogens. Medicinal plants, used as growth promoters and flavor agents, may be used as an alternative to antibiotics, and improve the live performance of poultry, such as essential oils extracted from oregano, cinnamon, pepper, sage, thyme, rosemary (Hernandez *et al.*, 2004) or anise powder (Ciftci *et al.*, 2005; Al-Kassie 2008; Al-Shammari 2011) in broiler feeds.

Anise (*Pimpinella anisum* L.) has been widely used to maintain or improve animal and human health. It is an annual aromatic herb belonging to the *Apiaceae* family. It is cultivated mainly in southern Europe and Southeast Asia. Anise fruits, or the so-called seeds, are usable parts of the plant (Al-Beitawi *et al.*, 2009). They contain 2-6% of essential oils, phenolic acids, eugenol, estragole, and trans-anethole, which is a powerful phytoestrogen and the main component of the oil (80-95%) (Christaki *et al.*, 2012). Anise has been used over the years for its antioxidant (Gulcin *et al.*, 2003), antimicrobial (Al-Kassie 2008), antibacterial (Tabanca *et al.*, 2003), antipyretic (Afifi *et al.*, 1994), and antifungal (Soliman & Badea 2002) properties. Also, essential oil from



anise seeds can stimulate immunity (Yazdi *et al.*, 2014), as well as a digestion and milk secretion (galactagogue) (Frankič *et al.*, 2009). Anise has also been shown to have anti-cancer (Kadan *et al.*, 2012), antioxidative, antihemolytic, anti-inflammatory (Iyer *et al.*, 2013), antihyperglycemic, hypolipidemic (Rajeshwari *et al.*, 2011), cytoprotective, as well as anti-ulcer (Al Mofleh *et al.*, 2007) and anti-osteoporosis characteristics (Hassan & Saed 2011).

The number of studies on the physiological responses of poultry to the supplementation of anise seeds in the drinking water is limited (Al-Shammari, 2011). Some experiments evaluated the performance of broilers fed anise (Al-Beitawi *et al.*, 2009; Al-Kassie 2008; Ciftci *et al.*, 2005; El-Deek *et al.*, 2003; Soltan *et al.*, 2008). These studies tested different levels of anise seeds powder added to the diet in order to determine its on the live performance of broilers. Therefore, the aim of this study was to evaluate the potential of anise seed (*Pimpinella anisum* L.) powder supplemented to the drinking water as a physiological stimulator on the blood profile of broiler chickens.

MATERIAL AND METHODS

The present experiment was conducted on a private poultry farm in Babylon, Iraq. A total of 360 one-day-old Hubbard Classic unsexed broiler chicks were allotted to four treatment groups (n=90), with three replicates (pens) of 30 birds each. Birds were reared in floor pens (4 m²) with wood-shaving deep litter and equipped with hanging drinkers and feeders. All chicks were vaccinated against infectious bronchitis, Newcastle disease, and infectious bursal disease, according to the vaccination program implemented on the farm. The birds were fed *ad libitum*. The diet was formulated according to the NRC (National Research Council, 1994), and was isocaloric and isonitrogenous (Table 1).

Anise seed powder was obtained commercially (Center of Herbal Medicine/Hatra Herbs, Iraq). The control group of birds (C) drank regular drinking water without any additives. Three different quantities of anise seeds powder were added to the drinking water: 500 mg/L, 750 mg/L and 1000 mg/L, and were offered to groups T1, T2 and T3 respectively.

When broilers were 28 and 56 days old, blood samples were collected by brachial vein puncture using sterile lancets, and placed in centrifuge tubes containing the anti-coagulant K3-EDTA. The following blood count parameters were determined: erythrocyte

(RBC) and leucocyte counts (WBC), hemoglobin concentration (Hgb), hematocrit (HCT), and heterophil to lymphocyte ratio (H/L) (Archer 1965; Gross & Siegel 1983; Natt & Herick 1952). Part of the collected blood was centrifuged at 1000 rpm for 10 min, and the following biochemical parameters were evaluated in the blood plasma: levels of total cholesterol (CHOL), glucose (GLU), total protein (TP), albumin and globulin, calcium (Ca), phosphorus (P), triglycerides (TG), total lipids, uric acid (UA), creatinine, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. All blood serum biochemical parameters were determined photometrically using commercially available testing kits (Biodiagnostic Co).

Table 1 – Ingredients and chemical composition of the basal diet fed to Hubbard Classic broilers for 56 days

| Ingredients | Inclusion (%) | |
|--|---------------|--------|
| Corn, yellow | 43.5 | 44.3 |
| Corn (grain) | 18.0 | 18.0 |
| Soybean meal (45% CP) | 25.8 | 23.6 |
| Animal protein concentrate ¹ | 10.0 | 10.0 |
| Common salt | 0.30 | 0.30 |
| CaCO ₃ | 0.40 | 0.40 |
| Sunflower salt | 2.00 | 3.40 |
| Total | 100 | 100 |
| Calculated chemical composition | | |
| Crude protein (%) | 22.11 | 21.20 |
| Metabolizable energy (kcal/kg) | 3005.5 | 3105.6 |
| Metabolizable energy/crude protein ratio | 136.0 | 146.5 |
| Digestible lysine (%) | 1.14 | 1.08 |
| Digestible methionine+cysteine (%) | 0.78 | 0.75 |
| Calcium (%) | 1.03 | 1.03 |
| Available phosphorus (%) | 0.47 | 0.47 |
| Crude fiber (%) | 3.603 | 3.466 |
| Ether extract (%) | 5.23 | 6.64 |

¹ Protein concentrate was imported from Provimi Co., Jordan, and provided per kg: 40% crude protein; ME 2100 kcal/kg; 5% crude fat; 20% ash; 5.6% calcium; 5% available phosphorus; 3.85% lysine; 3.7% methionine and 4.2% methionine + cystine.

The data were statistically analyzed by the General Linear Models procedure of the SAS software (SAS, 1996). Statistically significant differences observed among treatment were separated using the Duncan's multiple range test with 5% probability level.

RESULTS

The effects of *Pimpinella anisum* L. supplementation on blood count parameters are presented in table 2. The RBC, WBC, Ht, and Hgb obtained when broilers were 28 and 56 days old, as well as the overall mean of the analyzed parameters indicated a significant influence of the additive (p≤0.05). Broilers receiving anise seed powder in the drinking water presented higher RBC



Table 2 – Effect of the supplementation of *Pimpinella anisum* L. in the drinking water on blood cell counts of Hubbard Classic broilers

| Parameter | Age (day) | Treatments | | | | SEM |
|--|-----------|------------|-----------|-----------|-----------|-------|
| | | C | T1 | T2 | T3 | |
| | | \bar{x} | \bar{x} | \bar{x} | \bar{x} | |
| RBC ($10^6 \times \text{mm}^{-3}$) | 28 | 3.11b | 3.15b | 3.62a | 3.65a | 0.031 |
| | 56 | 3.14b | 3.62ab | 3.73a | 3.75a | |
| | Total | 3.12b | 3.36ab | 3.67a | 3.70a | |
| HCT (%) | 28 | 27.00b | 30.00ab | 32.00a | 33.00a | 0.179 |
| | 56 | 28.00c | 32.00b | 32.00b | 34.00a | |
| | Total | 27.00b | 31.00ab | 32.00a | 33.00a | |
| Hgb concentration ($\text{g} \times \text{dL}^{-1}$) | 28 | 9.45c | 9.13b | 10.10a | 10.20a | 0.141 |
| | 56 | 9.59d | 10.10c | 10.35b | 10.72a | |
| | Total | 9.52c | 9.61b | 10.22a | 10.46a | |
| WBC ($10^3 \times \text{mm}^{-3}$) | 28 | 21.61c | 23.25b | 24.11ab | 25.74a | 0.611 |
| | 56 | 22.73c | 23.85b | 24.42b | 26.70a | |
| | Total | 22.17c | 23.55bc | 24.26ab | 26.22a | |
| H/L ratio | 28 | 0.290a | 0.245b | 0.220c | 0.221c | 0.689 |
| | 56 | 0.300a | 0.231b | 0.220c | 0.210c | |
| | Total | 0.295a | 0.239b | 0.228b | 0.215bc | |

RBC - erythrocytes, HCT - hematocrit, Hgb - hemoglobin, WBC - leucocytes, H/L - heterophils to lymphocytes ratio.

a, b - means within rows with different letters differ significantly at $p \leq 0.05$.

and WBC, which resulted in higher hematocrit and hemoglobin levels. In addition, the HCT difference between T3 and the control group was about 18%, on average. The results also showed significant decrease ($p \leq 0.05$) of the H/L ratio of T1, T2 and T3 relative to the control group at both evaluated ages and of overall average (about 30%).

Table 3 shows the results of selected biochemical blood traits of broilers drinking water supplemented with *Pimpinella anisum*. Glucose, total protein, albumin, globulin, calcium, and phosphorus levels were significantly different ($p \leq 0.05$) across treatment groups as measured at 28 and 56 days of age and as overall average, with higher values obtained in the anise-fed groups (T1, T2 and T3) compared with the control group. The addition of anise seed powder to the drinking water also resulted in significant reduced ($p \leq 0.05$) levels of total cholesterol, total lipids, triglycerides, as well as of AST and ALT activities when compared with the control group. Moreover, significant decreases in UA and creatinine levels were recorded, independently of period, as a result of anise seed supplementation.

DISCUSSION

The H/L ratio is considered a stress indicator. According to Gross & Siegel (1983), the H/L ratio seems to be a more valuable tool to explain the different stress factors to which birds are exposed than corticosterone

serum levels. Environmental conditions, especially in intensive rearing systems, can generate stress. Natural substances like probiotics, prebiotics, organic acids or plant-derived products are recommended for stress reduction (Cetin *et al.*, 2011; Ghareeb *et al.*, 2008; Windisch *et al.*, 2008). In the present study, broilers were reared under intensive, conventional management conditions. The anise additive evaluated in the current study may play an important role in alleviating stress, as shown by the reduction in H/L ratio in the anise-supplemented group relative to the control group.

Anise supplementation at 0.25 and 0.5 g/kg of broiler diet reduced serum levels of ALT, glucose, and cholesterol, but had no effect on serum uric acid level when compared with the control group (Tabanca *et al.*, 2003). On the other hand, Kucukkurt *et al.* (2009) did not find any differences in plasma cholesterol, glucose and total protein concentrations in laying quails fed with anise seeds at 0, 10, 20, 30, 40, and 50 g/kg; however, serum triglyceride level was lower only in 10 g/kg diet when compared with the control group. Similar results were observed by Christaki *et al.* (2011), who found that total cholesterol and TG serum levels were considerably lower in Japanese quails supplemented with ground anise seed at 10 g/kg and 20 g/kg. Other medicinal herbs or plants have the same effect on biochemical profile of poultry blood. The additive of rosemary plant was incorporated into the diets at 5.7 g/kg, 8.6 g/kg and 11.5 g/kg (Polat *et al.*, 2011), and reduced total



Table 3 – Effect of the supplementation of *Pimpinella anisum* L. in the drinking water on blood biochemical parameters of Hubbard Classic broilers

| Parameter | Time (day) | Treatments | | | | SEM |
|--------------------------------------|------------|------------|-----------|-----------|-----------|-------|
| | | C | T1 | T2 | T3 | |
| | | \bar{x} | \bar{x} | \bar{x} | \bar{x} | |
| AST ($U \times L^{-1}$) | 28 | 123.3a | 120.8a | 107.4b | 104.5b | 4.340 |
| | 56 | 139.7a | 126.3b | 113.3c | 110.4c | |
| | Total | 131.5a | 123.6b | 110.4c | 107.5c | |
| ALT ($U \times L^{-1}$) | 28 | 38.21a | 25.28b | 26.18b | 24.10b | 5.025 |
| | 56 | 40.42a | 29.43b | 27.65b | 25.65b | |
| | Total | 39.31a | 27.35b | 26.91b | 24.87b | |
| GLU ($mg \times dL^{-1}$) | 28 | 183.0b | 187.9b | 197.8a | 200.0a | 6.508 |
| | 56 | 184.3b | 188.9b | 201.0a | 208.3a | |
| | Total | 183.6b | 188.4b | 199.4a | 204.2a | |
| TP ($g \times dL^{-1}$) | 28 | 2.30c | 3.10b | 3.95a | 4.15a | 6.830 |
| | 56 | 2.40c | 3.30b | 4.10a | 4.35a | |
| | Total | 2.35c | 3.20b | 4.20a | 4.25a | |
| Albumin ($g \times dL^{-1}$) | 28 | 1.03b | 1.00b | 1.35a | 1.27a | 6.950 |
| | 56 | 1.09c | 1.10b | 1.40a | 1.44a | |
| | Total | 1.06b | 1.05b | 1.37a | 1.35a | |
| Globulin ($g \times dL^{-1}$) | 28 | 1.27c | 2.10b | 2.60a | 2.88a | 0.539 |
| | 56 | 1.31c | 2.20b | 2.70a | 2.91a | |
| | Total | 1.29c | 2.15b | 2.65a | 2.89a | |
| CHOL ($mg \times dL^{-1}$) | 28 | 177.10a | 155.38b | 134.18b | 125.74b | 5.836 |
| | 56 | 185.10a | 177.38ab | 172.81b | 154.74bc | |
| | Total | 181.10a | 166.38b | 153.49b | 140.24bc | |
| Total lipids ($mg \times dL^{-1}$) | 28 | 633.10a | 586.23b | 463.31c | 451.00c | 35.32 |
| | 56 | 696.12a | 598.12a | 497.23b | 488.43b | |
| | Total | 664.61a | 592.17ab | 480.27b | 469.71b | |
| TG ($mg \times dL^{-1}$) | 28 | 194.4a | 183.1b | 185.1b | 177.3c | 4.032 |
| | 56 | 199.3a | 188.1b | 188.1b | 182.0b | |
| | Total | 196.8a | 185.6b | 186.6b | 179.7c | |
| UA ($mg \times dL^{-1}$) | 28 | 4.88a | 4.73a | 4.72a | 3.99b | 0.700 |
| | 56 | 5.00a | 4.88a | 4.84a | 4.15b | |
| | Total | 4.94a | 4.81a | 4.78a | 4.07b | |
| Creatinine ($mg \times dL^{-1}$) | 28 | 0.96a | 0.95a | 0.91b | 0.79c | 0.630 |
| | 56 | 0.98a | 0.96a | 0.93b | 0.81c | |
| | Total | 0.97a | 0.96a | 0.92a | 0.76b | |
| Ca ($mg \times dL^{-1}$) | 28 | 8.91 | 11.80a | 11.43a | 11.68a | 0.910 |
| | 56 | 9.40c | 11.26b | 12.66a | 13.11a | |
| | Total | 9.16b | 11.53ab | 12.045a | 12.39a | |
| P ($mg \times dL^{-1}$) | 28 | 3.71c | 4.81b | 5.59a | 5.58a | 0.440 |
| | 56 | 3.90c | 4.85b | 5.61a | 5.59a | |
| | Total | 3.80c | 4.83b | 5.60a | 5.58a | |

AST -aspartate aminotransferase, ALT -alanine aminotransferase, GLU -glucose, TP - total protein, CHOL - total cholesterol, TG - triglycerides, UA -uric acid, Ca - calcium, P - phosphorus.
a, b - means within rows with different letters significantly differ at $p \leq 0.05$.



cholesterol and creatinine levels. Creatinine is a chemical waste molecule generated from muscle metabolism. The kidneys maintain blood creatinine level within a normal range. It is possible that the feeding anise seeds stimulates the liver and the kidneys to function more efficiently. The supplementation of the drinking water of broilers with thyme (*Thymus vulgaris*), coneflower (*Echinacea purpurea*), and garlic (*Allium sativum*) improved not only the live performance, but also the immune parameters and the biochemical profile of birds (Rahimi *et al.*, 2011). Those authors found that garlic significantly reduced TC, LDL, and TG serum levels, and thyme increased the hematocrit and the hemoglobin levels.

Anise seeds positively affect the digestibility of nutrients, enhance the digestion of protein, cellulose and fat, improve ileal digestibility of nutrients, increase the activities of pancreatic lipase and amylase, have antioxidant activity, preventing fatty acids oxidation, and increase nutrient utilization (Al-Shammari 2011; Ertas *et al.*, 2005; Ertas *et al.*, 2005; Hernandez *et al.*, 2004; Jamroz & Kamel 2002; Ramakrisna *et al.*, 2003). These properties of the anise seeds may have stimulated digestion of the broilers in the present experiment, as shown by the low activities of liver enzymes (AST and ALT) of broilers supplemented with anise seeds. The significant increase in calcium and phosphorus serum levels in the experimental groups supplemented with various doses of anise seeds powder relative to the control group may be due to the antiosteoporotic properties of this plant (Putnam *et al.*, 2007).

The beneficial properties of anise were reflected in the cellular and biochemical traits of blood of all treatment groups (T3, T2 and T1), resulting from the improvement of biological and metabolic processes as well as optimized utilization of nutrients in the digestive system. Anise may enhance broiler resistance to various stress factors, reduce lipid synthesis, enhance growth and protein synthesis, change carbohydrate metabolism, increase blood levels calcium, phosphorus, protein, and glucose, stimulate erythrocyte synthesis and cell differentiation, and stimulate of blood circulation.

CONCLUSION

Results of the experiment showed that adding 500, 750, 1000 mg/L of anise seeds powder into the drinking water of Hubbard Classic broiler chickens significantly improved their blood profile. Anise seeds can be considered as an effective physiological promoter in growing broilers.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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