

Effect of Natural Spices on Plasma Proteins in Broiler Chicks

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Abstract

This study was designed to compare four different medicinal plants, cinnamon (*Cinnamomum verum*), cummin (*Cuminum cyminum*), fenugreek (*Trigonella foenum-graecum*) and ginger (*Zingiber officinale*), as natural feed additives with the Doxystin: "Doxycycline HCl 50 mg and Colistin sulfate" (known antimicrobial growth promoter) on plasma biochemical values of broiler chicks includes total protein, albumin, globulin, albumin/ globulin (A/G) ratio and the Alkaline phosphatase activity. Two hundred and forty, (one day-old) broiler chicks were randomly assigned to six groups of similar mean weight each included four replicates of ten chicks. The control group received broilers basal diet. For the treated groups, the basal diet was supplemented with one of the following: the antimicrobial (Doxystin) as 0.5% or one of the spices *C. verum*, *C. cyminum*, *T. foenum-graecum* and *Z. officinale* as 2%. The results showed significant ($P < 0.05$) increase in the serum total protein and the globulins concentrations in *T. foenum-graecum* group compared to the control group and other spice treated groups, also the globulins levels were significantly ($P < 0.05$) higher in Doxystin and *C. cyminum* groups compared to the control group. The serum albumin/globulin ratio was significantly ($P < 0.05$) decreased in all experimental groups compared to the control group.

Treatment with different spices in the present work increased the total proteins and the serum globulins fraction of the blood proteins, albumin remains unchanged and this effect lowered A/G ratio. These findings suggest improved rate of growth and immunity in the broiler chicks. The results also showed no significant difference between the experimental groups and the control group for the serum alkaline phosphatase (ALP) activity. This suggested that no significant liver problem will arise from the use of the spices as treatments.

Keywords: Spices; Blood; Chemistry; Total protein; Broiler; Alkaline phosphatase

Introduction

The synthetic drugs in animal feeds can be substituted by the non-traditional feed additives in animal's feeds to avoid its hurtful effect. Therefore, researches for alternative feed supplements which have a positive effect on the human health have been increased such as the extensive and considerable attention to the aromatic plants as growth promotants. Nowadays, aromatic plants and their oil extracts are becoming more important in poultry production as growth promotants [1]. Recently, antimicrobial resistances have existed regarding the prevalence of antibiotic-resistant infections in humans. Total protein measurements can reflect protein synthesis and nutritional status [2].

Kapelański et al. [3] found that, the biochemical parameters of blood related to protein metabolism, such as total protein was higher in pigs group which were characterized by a very high rate of growth compared to slower growing group. This gives an indication to the relation between body weight gain and blood total protein. However, total protein test is a rough measure of all of the proteins in the plasma portion of the blood. Proteins are important building blocks of all cells and tissues; and important for body growth and health. Total protein measures the combined amount of two classes of proteins, albumin and globulins. Total protein measurements can reflect protein synthesis and nutritional status. However, also it can reflect e.g. dehydration, kidney disease, liver disease, and many other conditions. If total protein is abnormal, further tests must be performed to identify which protein fraction is abnormal, so that a specific diagnosis can be made [2].

In this study an attempt was carried out to evaluate the four spices, *Cinnamomum verum*, *Cuminum cyminum*, *Trigonella foenum-graecum* and *Zingiber officinale* as natural sources of botanicals origin as growth promoters to replace the antibiotics in broiler diets. The evaluation includes biochemical changes in serum proteins and the liver enzyme alkaline phosphatase.

Material and Methods

The experimental animals

Two hundred and forty, one day old, white broilers (Cobb – strain) obtained from El' – Garr company and transferred to the Faculty of Animal production. All chicks were subjected to the basal diet Table [1] for one day then divided in to six groups, A, B, C, D, E and F, randomly.

Each group replicated in a four pens, each pen contained ten birds.

The birds were vaccinated against Gumboro, Newcastle and Coccidiosis at 17d old and 24d old.

Feed and water was provided ad libitum, the light was maintained for 24 hours per day naturally and artificially.

The bird fed starter diet for the first three weeks and finisher diet for next three weeks (Table 1). The dietary regime as the following:

Group (A) fed basal diets only and kept as control.

Group (B) fed basal diet plus the antimicrobial (Doxystin) as 0.5%.

Group (C) fed diets plus *C. verum* powder as 2%

Group (D) fed diets plus *C. cyminum* powder as 2%

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Group (E) fed diets plus *T. foenum-graecum* powder as 2%

Group (F) fed diets plus *Z. officinale* powder as 2%.

The preparation of the spices

The spices were purchased from Khartoum local market then cleaned, dried and powdered. The antimicrobial drug used in this treatment was the Doxystin which is produced by Arab Veterinary Industrial Co. (AVICO) Jordan. Each gram of the Doxystin contains: Doxycycline HCl 50 mg and Colistin sulfate as 400 000 IU.

Blood collection: 15 ml of blood samples were collected from the birds at slaughter into clean tubes and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 minutes, collected into plain containers and used for the biochemical estimations.

Biochemical estimations: Serum samples have been collected at 42 day of age for the estimation of total protein, albumin, total globulins and the activity of the enzyme alkaline phosphatase.

The serum total protein concentration was determined using biuret reagent method as described by Cannon [4]. The serum albumin concentration was determined using modified bromocresol green colorimetric method as described by Doumas et al. [5]. Alkaline phosphatase (ALP) activity was determined according to Young [6].

Statistical Analysis

The data were analyzed by one way ANOVA procedure according to SPSS computing software program. Each test was conducted at 5% level of significance.

Results

The effect of inclusion of 2% dietary powdered spices or 0.5%

Ingredients As percentage	% (1-3wks) Starter Control	% (4-6wks) Finisher Control	% (1-3wks) Starter Spices	% (4-6wks) Finisher Spices
Sorghum	65.1	66.5	63.1	64.5
Groundnut meal	18.7	13.5	18.7	13.5
Sesame meal	10	12.7	10	12
Super concentrate*	5	5	5	5
Lime stone	0.9	0.9	0.9	0.9
Salt	0.25	0.25	0.25	0.25
Lys	0.04	0.06	0.04	0.06
Meth	0.01	0.01	0.01	0.01
Vegetable oil	0	1.08	0	1.8
Spices	0	0	2	2
Total100%	100	100	100	100

*Broiler Super concentrate contains (%): CP 40, CF 1.5, ME 2122Kcal/kg, fat 3, Lysine 13.5, Methionine 5.9, Methionine+cystine 6.25, P 4.6, Ca 6.8, Na 1.5. Vitamins supplied per Kg of diet: Vit. A, 250 000 IU; Vit. D3, 60 000 IU; Vit. E, 800 mg; Vit. K3, 60 mg; Vit. B1, 30mg; Vit. B2, 100 mg; Vit. B6, 50 mg; Vit. B12, 300 mg; Vit. C, 4000 mg; Niacin, 800mg; Folic acid, 30mg; Biotin, 30mg; Choline chloride, 3000mg; Copper, 30 mg; Iron, 100mg; Manganese, 160mg; Zinc, 100mg; Iodine, 1.3mg; Selenium, 5mg; Cobalt, 1.2mg; Fytase enzyme, 15000; Antioxidant.

Table 1: Composition of the basal diet fed to the experimental chicks.

Parameter	Control	Doxystin	<i>C. verum</i>	<i>C. cyminum</i>	<i>T. foenum-graecum</i>	<i>Z. officinale</i>
T.P(g/dl)	2.24 ^a ± 0.35	3.52 ^a ± 0.37	2.45 ^a ± 0.26	3.48 ^a ± 0.31	4.18 ^b ± 0.43	2.87 ^a ± 0.47
Albumin(g/dl)	1.88 ^a ± 0.20	1.77 ^a ± 0.11	1.67 ^a ± 0.31	1.81 ^a ± 0.23	1.50 ^a ± 0.05	1.68 ^a ± 0.08
Globulin(g/dl)	0.36 ^a ± 0.06	1.75 ^{bc} ± 0.30	0.77 ^a ± 0.12	1.67 ^b ± 0.2	2.68 ^c ± 0.20	1.19 ^{ab} ± 0.39
A/G ratio(g/dl)	5.24 ^a ± 0.42	1.01 ^b ± 0.06	2.16 ^b ± 0.12	1.09 ^b ± 0.15	0.56 ^b ± 0.24	1.41 ^b ± 0.68
ALP(U/L)	62.16 ^a ± 9.22	28.23 ^a ± 5.1	64.47 ^a ± 11.0	64.47 ^a ± 11.0	56.87 ^a ± 7.04	52 ^a ± 8.84

^{a,b,c} : Row means with no common superscript differ significantly at (P<0.05)

Table 2: Effect of inclusion of dietary powdered spices and Doxystin on broiler chicks serum proteins and the alkaline phosphatase for 6 weeks. Mean ± SE.

(Doxystin) on broiler chicks' serum total protein is presented in Table [2]. The results showed significant (P<0.05) increase in the serum total protein concentration in *T. foenum-graecum* group compared to the *C. verum*, *Z. officinale* and control groups (Table 2).

But no significant increase observed in the serum total protein concentration of the other experimental groups compared to the control group.

The inclusion of (Doxystin) or powdered spices for 6 weeks in broilers ration showed no significant effect on albumin concentration. Numerically the control group represented the highest concentration level, whereas, *T. foenum-graecum* group represented the lowest value.

The effect of inclusion of 2% dietary powdered spices or 0.5% (Doxystin) on broiler chicks serum globulins are presented in Table [2]. The globulins concentration was significantly higher in *T. foenum-graecum*, (Doxystin) and *C. cyminum* groups compared to the control group, and numerically increased in *C. verum* and *Z. officinale* groups compared to the control group. Also there was a significant (P<0.05) increase observed in globulins concentration in *T. foenum-graecum* group compared to the *C. verum*, *Z. officinale* and *C. cyminum* groups.

The effect of inclusion of 2% dietary powdered spices or 0.5% (Doxystin) on broiler chick's serum albumin/ globulin (A/G) ratio is presented in Table [2]. There was a significant (P<0.05) decrease in serum A/G ratio in the experimental groups compared to the control. But there was no significant difference within the experimental groups for the serum A/G ratio.

The effect of inclusion of 2% dietary powdered spices or 0.5% (Doxystin) on broiler chick's serum alkaline phosphatase (ALP) activity is presented in Table [2]. The results showed no significant difference between the experimental groups and the control group for the serum ALP activity. The group treated with (Doxystin) showed very low ALP activity compared to the control and the spices treated groups, as they showed activity of three folds higher compared to the antibiotic treated group.

Discussion

In the present study the effect of inclusion of 2% dietary powdered spices or 0.5% Doxystin for 6 weeks on broiler chicks serum total protein showed significant increase in the serum total protein concentration in *T. foenum-graecum* group compared to the control group (Table 2). Also a significant increase was observed in serum total protein concentration in *T. foenum-graecum* group compared to *C. verum* and *Z. officinale* groups. Whereas the other treated groups increased numerically compared to the control. These results agree with AL-Kassie [7] who reported that, when 200 ppm of essential oil derived from *C. verum* were added to a standard diet of broiler chicks for 42 days; a significant increase in total proteins was observed, compared to a control group. Also treatment results agree with EL-Manylawi [8] and Ali [9], who reported that *C. cyminum* essential oil at levels 100, 150 and 200 mg kg⁻¹ body weight for 8 weeks resulted in increased total protein

values compared with control group. Also it agree with Abbas [10] who found that dietary *T. foenum-graecum* seeds at 3 g/kg of the diet for 42 days, increased numerically the total protein in the chicks. From this case, one can suggest that elevated serum TP level may be due to high level of protein and other nutrients in *T. foenum-graecum* L seeds. This could be supported by the idea of Hoffman [11] who cited that, serum protein levels are sensitive to nutritional influences. Over and above, Tollba et al. [12] found that mixture of volatile oils including thyme, oregano, *C.verum* and capsicum added to the two groups of chicks diets at 1 or 2 g /kg feed in the experimental period which lasted at 12 wks of age, increased significantly total protein as well as albumin and globulin comparing to un-supplemented control group. *Z. officinale* was studied by Nanjundaiah et al. [13] (2009) they found that oral treatment with *Z. officinale* aqueous extract for 14 days showed a numerical increase in serum total protein compared to the control group. This is in line with Zhang [14] who found that the concentration of total protein in serum of ginger-supplemented 5 g/kg of broiler diet was higher at 21 day and was higher at 42 day of age compared with that of control broilers.

In some cases increased serum TP may accompany acute inflammatory states, dehydration, or secondary to certain types of tissue damage [15]. In this study no adverse clinical signs were observed. From findings and observations in this work, it can suggest that elevated serum TP levels in spice treated groups may be due to nutritional potential effect of the treated diets and an increased body weight gain [3].

As presented in Table [2], the inclusion of Doxystin or powdered spices for 6 weeks in broilers ration, there were no any significant effect on serum albumin. Also no abnormalities noticed in health condition of the all birds after application of different treatments. These findings agree with previous studies when these spices were used as treatments and showed no effect on the levels of serum albumin. Ali [9] found that, when rats administrated with *C.verum* orally they showed no significant effect on plasma albumin concentration compared to the rats used as control. Similar findings were reported for *C.cuminum* application to broilers diet which showed results agree with Ali et al. [9] who found that the dietary 0.2% *C.cuminum* did not affect chick's plasma albumin. A study conducted by Hussein [16] also showed that, oral administration of *Z.officinale* ethanolic extract at different concentrations, 200, 400 and 800mg/kg did not affect the serum albumin when compared to control rats.

The effect of inclusion of 2% dietary powdered spices and 0.5% Doxystin on broiler chicks' serum globulins is presented in Table (2). Globulins concentration is significantly higher in *T.foenum graecum*, doxystin and *C.cuminum* compared to the control group, and numerically increased in *C.verum* and *Z.officinale* groups compared to the control group. Also there was a significant increase in globulin concentration of *T.foenum graecum* group compared to the *C.verum*, *Z.officinale* and *C.cuminum* groups. Tollba et al. [12] found that, a mixture of volatile oils including thyme, oregano, *C.verum* and capsicum added to two groups of chicks diets at 1 or 2 g /kg feed in the experimental period which lasted for 12 wks, increased significantly the serum globulin compared to un-supplemented control group. It is believed that the protective effect of *C. verum* is the result of combination between the antimicrobial effect of *C. verum* and the stimulated immune system as a result of *C.verum* administration. The results reported in the group treated with *C.cuminum* in the present work also agrees with EL-Manylawi [8] and Ali [9] who found that, in rabbits treated with *C.cuminum* essential oil at dose 150 mg kg⁻¹ b.wt. for 8 weeks, serum globulin concentration were significantly higher than in the control group, and suggested that

the increase in globulin content may be due to the immune stimulant effect of *C.cuminum*. *T.foenum-graecum* treatment findings in his work, resulted in an effect agrees with Abdel Zaher et al. [17] who showed that, fish fed diet containing different levels as 0.5, 1 and 1.5 % of *T.foenum-graecum* seeds meal, increased the serum globulin significantly with the increase of *T.foenum-graecum* in the dietary levels. The increase in the serum globulin levels is thought to be associated with a stronger innate response in fish [18]. The increase in globulin was suggested previously to indicate that, fish are immunologically strong [19]. In other study, AL-Homidan [20] reported that, the chicks fed *Z. officinale* as 2% (w/w) for 7 weeks of the diet, showed significant increase in the serum globulin concentration compared to control chicks and suggested that the increase of the serum globulin may be due to the immunostimulant effect of *Z. officinale*. This finding agrees with the present work and is in line with Nya and Austin [21] (2008) who used dietary ginger, *Z. officinale*, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout fish. There was proliferation in the number of neutrophils, macrophages and lymphocytes compared with the controls.

In the present study the level of serum A/G ratio showed significant decrease in all groups that received treated diet, compared to the control. But there was no significant difference observed with in the experimental groups. Ratio of A/G was decreased with the addition of experimental additives which may indicates improved immunity for the birds this was implied by the increase in the globulin level compared to albumin which was stated before by Kamel [22] that the increase of serum globulin indicates that birds are immunologically strong and suggested that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects. Also The results obtained in the present work of *C.verum* treatment agrees with Tollba et al. [12] who found that, a mixture of volatile oils including thyme, oregano, *C.verum* and capsicum added to the chicks diets at 1 or 2 g /kg for 12 wks of age, decreased significantly the ratio of A/G comparing to un-supplemented control group. These findings suggested increased globulins levels and improved immunity. *C.cuminum* results agrees with Venkatesh et al. [22] who found that, alcoholic ethanol extract of the *C.cuminum* (50 mg/day/rat) caused a significant decline in A/G ratio. *T.foenum-graecum* treatment resulted also in significant decrease of A/G ratio compared to the control, this in line with Abdel Zaher, Ahmad HM et al. [17] (2009) who found that, 1% and 5% *T.fenum-graecum* in diet exhibited non significant decrease in the value of A/G ratio. The results obtained from *Z.officinale* treatment were similar to AL-Homidan [20] findings who claimed that, the chicks fed 2% (w/w) for 7 weeks *Z.officinale* diet showed that there is a noticeable decrease in the A/G ratio compared to control chicks.

The increase of the activity ALP in serum is mainly due to the leakage of enzymes from the liver cells cytosol into the blood stream, which gives an indication of hepatotoxicity [23]. Generally, the serum ALP level increase as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis, and liver cancer [24]. Thus, it can be used as marker to assess the extent of liver cells damage. In Table 2, the inclusion of 0.2% Doxystin or 2% powdered spices for 6 weeks in broilers ration, no any significant effect on serum alkaline phosphatase (ALP) activity was observed. This exhibited a healthy, non pathological, non-toxic effect of the spices concentration used on the liver. Previous studies agreed with the present findings. Tim et al. [25] studied twenty-two subjects with pre-diabetes metabolic syndrome and supplemented their diet with either water-soluble *C.verum* extract as (500 mg/d) or a placebo for 12-weeks; he found that there were no effect on the alkaline phosphatase (ALP) activity

between the treated and placebo group values. Also Elhabib et al. [26] found that, rats fed 6% (w/w) for 4 weeks *C. cyminum* diet showed no significant changes in the ALP activity compared to the control group. In other study Kaviarasan and Anuradha [27] investigated the effect of chronic ethanol administration (6 g/kg/day for 60 days) which caused liver damage manifested by elevation of markers of liver dysfunction and ALP activity, the use of *T.foenum-graecum* seed polyphenolic extract restored the levels of ALP activity, and the other liver markers injuries in rats. Similarly AL-Homidan [20] reported that chicks fed 2% (w/w) for 7 weeks *Z. officinale* diet showed no significant change in ALP activity between *Z. officinale* group and control chicks.

It can be concluded that: most of spices treatments applied in this work showed insignificant increase in serum total protein concentration with significant increase of the serum globulins fraction of the total proteins. This effect lowered significantly the A/G ratio. All this suggest good growth rate and improved immunity in the treated broiler chicks. No change on the ALP activity was observed in the groups used spices, which implies no toxic effect to liver cells.

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