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Effects of Blends of Phytobiotic Additives of *Nigella satval* L., *Trigonella foenum-graecum* L. and *Curcuma longa* on Serum Biochemical Profile of Broiler Chicks

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Abstract: A study was conducted to compare the effectiveness of blends of different phytobiotic additives on biochemical indices. A total of 270-day-old unsexed broiler chicks (Cobb 500) were randomly allocated to six treatments with three replicates of 15 chicks each reared for 49 days. The experimental diets were: basal diet (T0), positive control basal diet+oxytetracycline at 0.035% (T1), basal diet + a blend of black cumin seed and fenugreek (T2), basal diet + a blend of black cumin seed and turmeric powder (T3), basal diet + a blend of fenugreek seed and turmeric powder (T4) and basal diet +a blend of black cumin seed + fenugreek seed + turmeric powder (T5). Serum total protein was significantly ($P<0.05$) higher in birds fed T3 diets as compared to T0 and T1 diet fed birds. While, birds fed in T5, T4 and T3 test diets had showed an enhanced ($P<0.05$) hypoglycemic and hypocholesterolemic effects than the birds in T1 and T0 diets. The value in the concentration of the liver enzymes Asparate aminotransferase (AST) and Alanine aminotransferase (ALT) were not showed a significant difference ($P>0.05$) among treatments. However, Alkaline phosphtase (ALP) concentration was lowered ($P<0.05$) in birds fed diets of T5, T1, T4 and T2. Therefore, blends of phytobiotics showed improved hypoglycemic, hypocholestremic and hepatoprotective ability making them capable of substituting synthetic antibiotics and imparting positive effects on the health of broilers. Therefore, it is recommended that the three phytobiotics should be used in combined forms in order to utilize their beneficial biochemical links on serum biochemical profile of broiler chicks.

Key words: Antibiotics · Biochemical · Broiler · Blends · Phytobiotic

INTRODUCTION

Serum biochemical profiling has been used in several species of domestic livestock and birds to monitor health and to detect subclinical diseases. Biochemical status is a reflection of many factors such as sex, age, breed, diet, management and stress [1]. The routine uses of sub-therapeutic levels of antibiotics often referred to as antibiotic growth promotors (AGP) in poultry feed have been a common practice for more than 50 years to prevent potential diseases as well as to robust gut health, increase meat yield and improve feed efficiency [2]. The aggravated utilization of antibiotics has led to the increased drug resistance of pathogens and the accumulation of antibiotic residues in animal products and the environment [3, 4]. This situation requires most countries in the world to restrict or limit the use of antibiotics in poultry feeds and launch "antibiotic-free" labeled feeds [5]. A practical approach to overcome this gap is the use of medicinal plants. Medicinal plants also known as phytogenic/phytobiotic additives are often very complex mixtures composed of bioactive components, which include several groups of plant products depending on the origin and purpose of

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production. The positive effects of medicinal plant or phytogene/ phytobiotic additives can be expected primarily through beneficial effects on the health of individuals by improving the immune system [6]. Some medicinal plants, such as black cumin, turmeric and fenugreek have been found to have an anti-inflammatory, antioxidant, anti-microbial, anti-protozoa, immune-modulatory effects, hypoglycemia, hypocholesterolemia, hepatoprotective, hepatostimulatory, analgesic, antipyretic, antivenin, antiulcer and anti-carcinogenic actions [7-10]. Fenugreek seed extracts have a unique feature of anti-hyperthyroidism and properties of anti-sterility and anti-androgenic effects [11, 12]. Even though phytobiotics provide a potential alternative to the antibiotic usage, more research on their synergetic or interaction effects is needed to understand the best form of utilization in poultry production, since the interaction of two or more agents to produce a combined effect greater than the sum of their individual effects [13]. Thus, the purpose of this study was to evaluate the effectiveness of *Nigella satval* L, *Trigonella foenum-graecum* L. and *Curcuma longa* L. blend as phytobiotic additives on the biochemical indices of broiler chicken.

MATERIALS AND METHODS

Study Area: The experiment was conducted in the Poultry Farm of College of Veterinary Medicine Addis Ababa University, Bishoftu, Ethiopia. The area is situated at 47 km East of Addis Ababa at an altitude of 1900 m above sea level, latitude of 844`N and longitude of 38° 57`E. The average (25 years) annual rainfall is 851 mm with an average minimum and maximum temperature of 8.9°C and 26°C, respectively. The average relative humidity is 58.6% [14].

Experimental Ration and Treatments: Three medicinal plants, namely *Nigella sativa* L. (Black cumin), *Trigonella foenum graecum* L. (Fenugreek) and *Curicculum longa* (Turmeric) were purchased from the vicinity local markets to incorporate in the diets of broiler chicken as phytobiotic feed additives. The black cumin and fenugreek seed were washed with tap water and sun dried under shade. The dried turmeric rhizome, black cumin and fenugreek seeds were coarsely ground using manual mill and stored in polyethylene bag until required for the formulation of experimental rations.

Six treatment diets of broiler ration containing blends of different phytobiotic additives and control diet was formulated at a lower dosage levels (1%) set for each of the medicinal plants. The treatments are T0 (negative control diet, which was only basal diet formulated from the feed ingredients of maize, wheat bran, soya bean meal and nuge seed cake), T1 (positive control, the basal diet + Oxytetracycline at 0.035%), T2 (basal diet + a blend of black cumin seed and fenugreek seed), T3 (basal diet + a blends of black cumin seed and turmeric powder), T4 (basal diet + a blend of fenugreek seed and turmeric powder) and T5 (basal diet + a blends of black cumin seed + fenugreek seed + turmeric powder) at equal proportion. The rations were formulated to be nearly isocaloric and isonitrogenous with metabolizable Energy (ME) content of 3000 kcal/kg DM and CP content of 22% during the starter phase of 1-28 days of age and ME content of 3200 kcal/kg DM and CP content of 20% during the finisher phase of 29- 49 days of age [15].

Management of Experimental Birds: A total of 270 unsexed day-old Cobb 500 broiler chicks with average initial body weight of 39.0.4±0.45 g were randomly and equally assigned to the six dietary treatments and with three replications per treatments. Before the commencement of the actual experiment, the experimental pens, watering and feeding trough cleaned and disinfected. The birds were vaccinated against Newcastle disease (Hitchner-B1 strain) at 7-days of age through intraocular and (Lasota strain) a booster dose at 22-days of age and Infectious Bursal Disease (Gumboro) at the age of 14 and 24 days and administered through spring drinking water. The chicks were brooded using 250 watt bulbs with gradual height adjustment as source of heat and light in a deep litter house covered with "teff" straw litter material. Feed was offered after weighed and clean tap water was available all the time throughout the experiment.

Measurements: The experiment was lasted for 7 weeks. Blood was collected at the end of the experimental period from two birds in each replication picked up randomly. About 2-3 ml blood samples were collected from the wing vein. The blood was transferred into non-heparinized tubes and later the coagulated blood samples were centrifuged for 15 minutes at 4000 rpm and the clear serum was separated and stored in a deep freezer at -20°C pending for biochemical analysis. Serum total protein and albumin were determined according to Doumas and Witt and Trendelenburg [16, 17]. Globulin concentration was calculated as the difference between total protein and albumin. Albumin/ Globulin g/100 ml (A/G ratio) was also calculated. Total cholesterol was determined according to Watson [18]. The estimation of alanine amino transferase

(ALT) and aspartate amino transferase (AST) followed the proposed optimized formulation of the International Federation of Clinical Chemistry (IFCC) [19], while measurement of alkaline phosphatise (ALP) was as per the German Society for Clinical Chemistry [20]. Sample analysis was conducted using the instrument HumaStar 80 automated chemistry analyzer (HUMAN Gesellschaft fuer Biochemical und Diagnostic GmbH, Germany).

Statistical Analysis: The collected data were subjected to one-way analysis of variance using the general linear models (GLM) procedures of SAS statistical package version 9.3 (SAS, 2010). Duncan's test was used to detect the differences among treatment means [21]. The model used was:

$Y_i = \mu + \alpha_i + \varepsilon_i$.

where: Y_i= the dependent variables for the ith, μ = overall mean effect, α_i= the ith treatment effect, ε_i= the random error variation.

RESULTS

Serum Protein Metabolites: The analyses of biochemical profile due to feed supplementation of blends of medicinal plants of *Nigella satval* L, *Trigonella foenum-graecum* L. and *Curcculum longa* L. as phytobiotics feed additives in broilers are illustrated in Table 1. The serum protein and globulin levels were significantly different (P<0.05) among the treatments and was higher for phytobiotics additive blends of T3; of course the value was similar (P>0.05) with T5 and T2. The serum globulin was also higher (P<0.05) in T3 as compared to the rest of the treatments. However, there were no significant differences (P>0.05) revealed on albumen and A/G ratio on birds fed the different treatment diets.

Serum Carbohydrate Metabolites: The influence of blends of phytobiotics feed supplementation on serum glucose and total cholesterol (Table 1) showed that there were significant differences (P<0.05) among the treatments. The serum glucose and total cholesterol was significantly (P<0.05) reduced in birds supplemented with blends of phytobiotic additives of T4 and T5 than the control T0 and T1. However, there was no significant difference (P>0.05) observed among T0, T1 and T2 birds in serum glucose. Similarly, phytobiotic additives in T4 and T5 fed bird had lower (P<0.05) serum total cholesterol as compared to the control T0 and T1.

Hepatotoxicity Indicators: The effect of supplementation of blended phytobiotic additives of *Nigella satval* L, *Trigonella foenum-graecum* L. and *Curcculum longa* L. on liver enzyme hepatotoxicity (AST, ALT and ALP) is presented in Table 1. There were significant difference (P<0.05) among the treatments on liver enzyme, ALP concentration. However, there were no significant differences (P>0.05) were revealed on AST and ALT concentration in birds fed different blends of phytobiotics additives. While, the activity of AST observed in the range of 131.57-155.33 with no significant (P>0.05) differences among treatments. While, the activity of ALT tended to decrease from T0 to T3, T1, T5 and T4, respectively. Serum ALP activities were reduced significantly (P<0.05) by supplementing blends of phytobiotic feed additive diets of T1, T4 and T5 than the control diets. The minimum serum ALP activity was recorded from treatment T5 than T0 and T2 diet fed birds (P<0.05).

DISCUSSION

Blends of phytobiotic feed additives effects on biochemical profile defined by black cumin + turmeric mixture as phytobiotic additives resulted in increased serum protein and globulin level than the antibiotics supplemented birds. The results were in agreement with El-Khalek and El-Naggar [22] who reported that addition of black cumin seeds, ginger, thyme and oregano oil mixtures were improved plasma total protein and globulin. Similarly, Yatoo *et al.* [23] also reported that the total protein was increased in the black cumin and fenugreek mixture supplemented birds. In inimitable study El-Bahr and Saad, [24] concluded that dietary supplementation of black cumin seed and turmeric mixture improved the serum protein of fish. As serum protein depends on the availability of dietary protein, these mean that the proteins of the black cumin + turmeric mixture diets were more available to the birds to support the nutritional requirements. The increase in total protein and globulin might be the synergetic effect of the active compounds in black cumin (thymoquinone and thymohydoquinone) and turmeric (curcuminoids and curcumin) which promote protein deposition, maintained a stable colloid osmotic pressure and improve the transportation of metabolic protein in birds [25]. In contrary to the current findings Toghyani *et al*. El-Bahr and Al-Azraqi and Fallah and Mirzaei [26, 27, 25] indicated that plasma total protein was not changed in broiler chicks fed black cumin seeds and turmeric powder.

Table 1: Serum biochemical profiles of broilers fed blends of black cumin, fenugreek seed and turmeric powder

	Treatments							
Parameters	T0	T1	T2	T3	T4	T5	SEM	P value
Total protein (g/dl)	2.50b	2.67b	3.33ab	4.50a	2.83b	3.5ab	0.19	0.02
Albumen (g/dl)	1.49	1.50	2.0	1.67	1.50	1.83	0.09	0.59
Globulin (g/dl)	1.0b	1.17b	1.33b	2.83a	1.33b	1.67b	0.17	0.02
A/G ratio	0.75	0.92	0.80	1.75	1.08	1.13	0.12	0.16
Glucose (mg/dl)	203.67a	180.0ab	185.0ab	161.67bc	135.33c	153.17bc	6.49	0.02
Cholesterol (mg/dl)	153.67a	151.67a	158.50a	136.17ab	114.33b	110.17b	12.37	0.03
AST (IU/L)	131.57	155.53	153.02	153.23	134.52	132.73	8.97	0.94
ALT (IU/L)	13.93	11.89	14.66	13.79	7.88	10.60	1.15	0.56
ALP (IU/L)	1451.77a	1014.13bc	1362.74ab	1078.35abc	1040.52bc	876.11c	57.88	0.02

*a-c Means in a row with different superscripts differ significantly (P <0.05); T0= Control basal diet, T1= Antibiotics, T2= Black cumin + fenugreek, T3= Basal + Black cumin + Turmeric, T4= Fenugreek + Turmeric, T5= Black cumin + Fenugreek + Turmeric. A/G= Albumen to globulin ratio, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline phosphatase

The blood glucose level was reduced in blend of fenugreek seed and turmeric powder better than the antibiotics supplemented birds. However, phytobiotic additives (black cumin + fenugreek + turmeric) mixtures were equivalent to the synthetic antibiotics (oxytetracycline). This is in agreement with Sameer *et al*. [28] who report that low doses of vanadate (0.2 mg/ml) in combination with fenugreek powder was found to be comparable and effective in correcting altered carbohydrate metabolism in rats to the synthetic vanadate (0.6 mg/ml) drug. Comparable to these findings, Al-Kassie *et al*. [29] reported that turmeric was effective in lowering blood glucose level than black cumin seed and their mixtures in broilers. Abou-Elkhair *et al*. [30] reported that supplementation of mixtures of (0.5% of black pepper and turmeric) and (0.5% black pepper, turmeric and 2% coriander seeds) had significantly lowered the blood glucose in broiler birds. Similarly to the present study, El-Bahr and Al-Azraqi [27] reported that the glucose level of diabetic rats (253.3%) has been reduced to 28.6% when rats have been treated with black cumin and turmeric mixture. In agreement with this study, El-Bahr and Saad [24] reported that black cumin + turmeric mixture (5gm/kg diet) were improved blood glucose and other biochemistry profile of Mugil cephalus fish.

The hypoglycemic effect of the phytobiotic mixtures especially fenugreek + turmeric might be due to the synergetic effects of fenugreek and turmeric active ingredients: trigonelline, the carbohydrate galactomannana, pectin from fenugreek and curcumin from turmeric and from both the amino acid (4-hydroxyisoleucine), which increases the gastric mucosal secretion and some flavonoids possess insulin-like properties and thereby can reduce the blood glucose level. The hypoglycemic effect of black cumin seed mixtures perhaps explained by an insulin-like stimulation of glucose uptake by muscle and adipose tissue [31] or inhibition of intestinal glucose absorption [32].

The reduction of serum cholesterol by supplementation of different blends of phytobiotics (black cumin + fenugreek + turmeric) and (fenugreek + turmeric) were in agreement with El-Khalek and El-Naggar [22] who reported depression in the serum cholesterol level due to the supplementation of black cumin + fenugreek mixtures to the birds. In agreement with our findings, Al-Kassie *et al*. [29] recorded lowering in cholesterol level in the blends of turmeric and cumin at 0.75 and 1%. Black cumin and turmeric mixture restored the cholesterol to normal level in diabetic rats and fish [25- 28]. Similarly, Fallah *et al*. [33] who reported that addition of 1.5% artichoke leaves meal in diet with mentha extract 200 mg/kg in drinking water decrease cholesterol in broilers. On contrary, Abd El-Latif *et al*. [34] who reported rosemary and garlic at 100 and 200 mg/kg increase serum cholesterol in broiler chicken. Whereas, Narimani-Rad *et al*. [35] who reported that pennyroyal, ziziphora and peppermint mixture at 0.5 and 1% and Mohebbifar and Torki [36] ground pits of palm dates with dried garlic and Myandoab and Hosseini-Mansoub [37] Liquorice root extract at 200 ppm with 1% probiotic (*Lactobacillus acidophilus* and *Lactobacillus casei*) was not have any considerable effect on glucose and total cholesterol levels.

The hypocholesterolemic effect of black cumin seeds may relate to the soluble fibers contents and sterols, especially ß-sitosterol which decreases dietary cholesterol absorption and increases bile acid synthesis and degradation [38, 39]. The hypocholesterolemic effect of turmeric related to the altered activity of two effective

enzymes in cholesterol metabolism, HMG-CoA reductase (3-hydroxy 3-methylglutaryl Coenzyme A) and mediated by the stimulation of hepatic cholesterol 7α-hydroxylase [40, 41]. Whereas, fenugreek might have contributed to the hypocholesterolemic effect by inhibiting the bile acid and cholesterol absorption from intestine, thereby, decreasing cholesterol level in blood [42, 43].

Results of liver function enzymes showed that no significant change in level of AST and ALT compared to the negative control and antibiotics supplemented birds. The current findings were in agreement with El-Khalek and El-Naggar [22] who reported that black cumin and other different medicinal plants mixture do not have toxic effect in liver as indicated AST and ALT activities. Similarly, Abd El-Latif et al. [34] who reported rosemary and garlic at 100 and 200 mg/kg had no significant difference on AST level in broiler chicken. The alkaline phosphate (ALP) activities were significantly lowered in birds supplemented with phytobiotic additives blends (black cumin + fenugreek + turmeric) and (fenugreek + turmeric), which were also comparable to the antibiotics drugs. Reductions of these enzymes are important as their accumulation in the liver are related to toxicity and hepatocellular damage. However, the level was within the standard range forwarded by Campbell [44] for the healthy chicken thus indicating the role of phytobiotic feed additives as hepatoprotective agents.

CONCLUSION

In conclusion, the improvement in some biochemical parameters in chickens fed blends of (black cumin + turmeric), (fenugreek + turmeric) as well as mixtures of the three phytobiotic additives (Black cumin + fenugreek + turmeric) revealed the ability of blends of phytobiotics additive to lower blood glucose and cholesterol levels as well as the hepatoprotective ability in broiler chicken; hence can substitute synthetic antibiotics and can impart positive effects on the health of broilers. The results revealed beneficial biochemical links between the three phytobiotics that justify their combined use. Therefore, it is recommended that the three phytobiotics should be used in combined forms in order to utilize their beneficial biochemical links on serum biochemical profile of broiler chicks.

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Declaration of Interest: The authors declare that they have no conflict of interest.

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