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Effect of Phytogenic Feed Mixture on Blood Hematology, Metabolites, Enzymes and Lipids in Broilers

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ABSTRACT

Phytogenic feed additives have been used traditionally in medicine preparations and in the preparation of culinary food dishes. However, in recent years it has attracted researchers for their use as feed additives in broilers. This study was organized to access the effect of dietary supplementation of phytogenic mixture containing powder of *Allium sativum* bulbs, *Ocimum sanctum* leaves and *Nigella sativa* seeds in 1:1:1 ratio. For this study, three comparable groups were formed viz. control (non supplementation), T_1 (supplementation @0.25%) and T_2 (supplementation @0.50%). A total of 48 broiler chicks were randomly distributed in three groups, each group containing 16 chicks of same strain and body weight. The experiment lasted for a period of 28 days. Statistical analysis of data revealed a significant ($P<0.001$) change in blood lipid profile of three groups. T_1 and T_2 groups had significantly lower ($P<0.001$) levels of triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol and Non-HDL cholesterols ($P = 0.05$). However, a non-significant ($P>0.05$) difference among three groups on blood hematology (Hemoglobin, WBC, RBC, PCV and Platelet count) was observed. Study showed that there was statistically similar ($P>0.05$) concentrations of blood metabolites (Glucose, Total protein, Albumin, Globulin) and statistically similar ($P>0.05$) levels of blood enzyme (Bilirubin, serum AST, serum ALT and serum ALP) among three groups. Based on the findings of this study, conclusively, it can be said that dietary supplementation of phytogenic mixture containing powder of *Allium sativum* bulbs, *Ocimum sanctum* leaves and *Nigella sativa* seeds in 1:1:1 ratio may favorably lower the levels of blood lipid without adversely affecting the normal physiology of broilers.

HIGHLIGHTS

- Phytogenic mixture improves physiological working of broilers.
- Phytogenic mixture feeding lowered blood cholesterol in broilers.
- Phytogenic mixture didn't affect hematology, metabolites and enzymes levels.

Keywords: Broilers, blood lipid, blood hematology, blood metabolites, blood enzymes, phytogenic mixture

As people are getting aware about the importance of quality protein which may biologically become available to their body easily. Many people are shifting to non-vegetarian foods. Consumption of meat has increased under Indian conditions and according to the survey of comptroller general of India, more than two third of youths of India consume meat (Singh *et al*., 2021a; Singh *et al*., 2021). Poultry meat has been seen as fastest growing and consumed meat around the world as it is cheaper, provides quality meat, among several other attributes (Singh *et al*., 2021b). However, consumption of meat in

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India is around 5 kg per person whereas; consumption of meat in United States of America and Australia is around 100kg per person annually (Ritchie and Roser, 2017; Nagar *et al*., 2020; Nagar *et al*., 2021). Cultivable land for agriculture is limited for production of food grains and other nutrient providing sources (Arora, 2019; Vianna *et al.,* 2022). Production of poultry requires a high amount of concentrates feeding which directly competes for the resources available for production of food items for human (Mengesha, 2012; Thakur *et al*., 2020a; Thakur *et al*., 2020b). Hence, researchers are continuously engaged in finding economical feed additives for broilers which may enhance broiler production (Amsathkumar *et al*., 2019). With the ban of antibiotic feed additives in broiler feed has pushed researchers for alternative feed additives which promote broiler production (Ayalew *et al*., 2022). Among such alternative feed additive the emerging and economical feed additives is phytogenic mixture as is it environment friendly, non-toxic, doesn't have residual effects (Amsathkumar *et al*., 2019).

Phytogenic feed additives may be defined as ingredients which are derived from plant parts such as leaves, bulbs, roots, etc. incorporated into the animal feed to enhance animal's performances by the virtue of their ability to improve digestibility, nutrient assimilation and destroying pathogens found in animal digestive tracts and additionally they improve the quality products from the animals fed with phytogenic feed mixture (Kamel, 2001; Balunas and Kinghorn, 2005; Windisch *et al*, 2008). Among several phytogenic feed additives, *Allium sativum, Ocimum sanctum* and *Nigella sativa* have gained much attention from scientific community for their growth and health promoting attributes in broilers as they as economical and easily available throughout the world (Bihari *et al*., 2010; Abu-Al-Basal, 2011; Khan *et al.,* 2012; Palani *et al*., 2014; Puvaca *et al*., 2015; Prajapat *et al*., 2018). Several studies suggest that combination of two or more phytogenic ingredients synergistically improves the performance of poultry birds (Amsathkumar *et al*., 2019, Ali *et al*., 2014).

Much work has been done to examine the effects of dietary supplementation of *Allium sativum, Ocimum sanctum* and *Nigella sativa* individually on blood profile of broilers. However, to the knowledge of authors, no experiment has been structured to observe the combined effect of *Allium sativum, Ocimum sanctum* and *Nigella sativa* mixture on blood profile of broilers. Therefore, the present study was structured with an objective to investigate the effect of phytogenic mixture containing *Allium sativum, Ocimum sanctum* and *Nigella sativa* on blood lipid profile, hematology, metabolites and enzyme concentrations of broilers.

MATERIALS AND METHODS

Site of the study

The present experiment was carried out in small animal laboratory of the Department of Animal Husbandry & Dairying, SHUATS, Prayagraj-211007.

Pre-experimental preparations

Prior to placement of the chicks in the cages, the entire poultry house and its premises were thoroughly cleaned with water and disinfected followed by fumigation with formalin and potassium permanganate ($KMnO_4$ @0.02%). To ensure the maintenance of the bio-safety security of the house, a disinfectant (lime powder) was always provided as a dip at the entrance of the house.

Preparation of phytogenic mixture

Garlic (cloves), Tulsi (leaves) and black cumin (seeds) were purchased from the local market. All three ingredients were procured fresh from the local market. The bulb portion of garlic was peeled followed by slicing into small pieces, fresh Tulsi leaves were washed with clean water and seeds of black cumin seeds were taken together and dried at 40°C for 24 hours in hot air oven. Dried samples were converted to powder using electric blender and then transferred to separate and labeled air tight vessels and kept in a cool dry place away from sunlight till further usage.

Experimental animals and management

A total of 48 straight run Cobb 400 broiler chicks were purchased from a corporate hatchery firm. Chicks were then administered anti-stress (sugared solution and multivitamins) after their arrival and were distributed according to the treatment groups and identified individually by means of wing tags. Battery type cages were used for rearing birds and they were provided with

standard farm managemental practices providing 1 sq. ft/bird space from day-old to four weeks of age. Fresh feed and clean water were served *ad libitum*. Birds were reared under the same environmental conditions. During the brooding, chicks were kept under the maintained temperature at 35°C during the first week, followed by lowering the temperature by 3°C every week till temperature of room was adjusted to 25°C in following days. One bulb of 100 watt was provided in each cage for light and to maintain the temperature in the room. The experimental period was for 28 days from starting week of February 2022 to second week of March 2022.

Experimental design and treatments

The birds were assigned into three comparable treatment groups. Each treatment group had 16 birds comprising 4 replicates in each group with 4 birds per replicate in a complete randomized design. Each group was fed with corresponding experimental diet—

- T_0: Chicks were provided with standard ration as per NRC, 2007 standards, without any supplement.
- T_1: Standard ration supplemented with 0.25% of phytogenic mixture per kg of feed.
- T_2: Standard ration supplemented with 0.50% of phytogenic mixture per kg of feed.

Phytogenic mixture contained Garlic, Tulsi and Black Cumin powder mixed in equal proportions 1:1:1 and then supplemented in the desired amount in respective treatment groups. The broiler starter diet contained 22% crude protein (CP) with 3, 000 ME kcal/kg whereas, the broiler finisher diet had 20% CP and 3, 150 ME kcal/kg in accordance with NRC standards. The feeding and watering was made available *ad-libitum* to the birds.

Blood hematology and biochemical components

Towards the completion of this study, blood samples were collected aseptically from the wing vein of each bird using disposable syringe into sterilized tubes. Serum samples were obtained from blood samples as per Sitohy *et al*. (2013), Ashour *et al.* (2020)**.** Blood heamatology, including red blood cells (RBC) count, total white blood cells (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), platelet count by standard procedures. Serum samples were analyzed using spectrophotometer for Glucose (G), total protein (TP), albumin (Alb), Globulin (Glb), serum enzymatic activities of alanine amino transferase (ALT) and aspartate amino transferase (AST), total bilirubin, alkaline phosphatase (ALP), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were estimated through commercially available standard diagnostic kits as followed by Sinha *et al.* (2017).

Table 1: Ingredient and nutrient composition of experimental diet (%DM)

Ingredients (%)	Broiler starter (0 – 21 day)	Broiler finisher (22 – 42 days)
Corn	53.53	59.58
Soyabean meal (44 %CP)	38.95	33.33
Monodibasic Phosphate	1.44	1.22
Limestone	1.34	1.37
Vegetable oil	3.85	3.53
Salt	0.40	0.41
DL- Methionine	0.208	0.215
L-Lysine- HCL	0.128	0.196
Choline HCL (60%)	0.06	0.05
Mineral- Vitamins premix	0.01	0.01
Total	100	100
Calculated Nutrients		
Crude protein %	22	20
ME, Kcal/kg	3000	3,150
Calcium %	0.91	0.88
Available phosphorus %	0.40	0.36
Sodium %	0.21	0.22
Chloride %	0.26	0.28
Digestible Lys. %	1.17	1.08
Digestible Met. %	0.48	0.49
Digestible Met + Cys %	0.82	0.78
Digestible Thr, %	0.79	0.73
Choline, mg/kg	1,422	1,312

Statistics and data analysis

All data collected were analysed meticulously for analaysis of variance (ANOVA) using Statistical Analysis System software of IBM (SPSS 22). Microsoft Excel was used for statistics and graphical representations. Duncan

Multiple Range Test was performed for determining the significant differences among different groups. Difference was considered significant when the value of *P* was lower or equal to 0.05.

RESULTS AND DISCUSSION

From the perusal of statistically analyzed data (Table 2), non-significant difference ($P>0.05$) was observed in hematological parameters (Hemoglobin, RBC, WBC, PCV and platelet count), blood metabolites (Glucose, total protein, albumin and globulin) and blood enzymes (Bilirubin, SGOT, SGPT and Alkaline Phosphatase). However, significant ($P<0.001$) difference was reported for blood cholesterol, triglycerides, HDL Cholesterol and LDL in treatment groups T_1 and T_2 birds than control group in this experiment. Nevertheless, T_1 and T_2 groups differed non-significantly ($P<0.05$) for blood cholesterol and LDL cholesterol while Triglycerides and HDL cholesterol values differ significantly ($P<0.05$) in group T_1 and T_2.

Significantly non-different ($P<0.05$) blood metabolites, blood enzymes and hematological parameters in treatment group birds show that the supplementation of phytogenic feed had no adverse effect on the normal physiology of birds. The significant difference ($P<0.001$) recorded in the blood cholesterol, triglycerides, HDL cholesterol and LDL in treatment groups T_1 and T_2 birds as compared to control group. Similar result was reported by Bamidele and Adejumo (2012) who found that the blood cholesterol and HDL cholesterol were significantly reduced in the treatment group. Lowest cholesterol in the treatment group was reported by Borgohain *et al.* (2019) by supplementing garlic powder @ 1 % and 1.5% of in the diet of broilers. Ismail *et al.* (2021) also analyzed and reported that total cholesterol and LDL cholesterol were significantly

Table 2: Least square means of indices of blood hematology, metabolites, enzymes and lipids in broilers

Parameters	T_0	T_1	T_2	SEM	Statistical Significance
Blood Hematology					
Haemoglobin (g/dl)	9.89	9.82	9.76	0.09	NS $P = 0.862$
WBC (10^3 /ul)	75.05	74.88	74.82	0.17	NS $P = 0.840$
RBC (10^6/ul)	2.28	2.27	2.24	0.02	NS $P = 0.661$
PCV (%)	30.28	30.04	30.51	0.27	NS $P = 0.778$
Platelet Count (10^3/ cu mm)	10.94	10.88	10.87	0.11	NS $P = 0.968$
Blood Metabolites					
Glucose (mg/dl)	224.12	223.75	224.38	1.19	NS $P = 0.977$
Total Protein (g/dl)	2.78	2.81	2.77	0.02	NS $P = 0.700$
Albumin (gm/dl)	1.35	1.36	1.36	0.02	NS $P = 0.990$
Globulin (gm/dl)	1.38	1.38	1.39	0.01	NS $P = 0.914$
Blood Enzymes					
Bilirubin-Total (mg/dl)	0.29	0.28	0.29	0.01	NS $P = 0.902$
Serum AST/SGOT (U/L)	235.94	235.50	240.25	1.47	NS $P = 0.354$
Serum ALT/SGPT (U/L)	8.50	8.48	8.56	0.07	NS $P = 0.883$
Alkaline Phosphatase (U/L)	2025.44	2026.12	2025.25	9.00	NS $P = 0.999$
Blood lipids					
Cholesterol (mg/dl)	132.31^a	118.19^b	117.69^b	0.67	S $P = 0.000$
Triglycerides (mg/dl)	61.69^a	54.19^b	52.06^c	0.37	S $P = 0.000$
HDL Cholesterol (mg/dl)	90.38^a	71.12^b	74.81^c	0.65	S $P = 0.000$
Non-HDL Cholesterol (mg/dl)	41.94	47.06	42.88	0.89	S $P = 0.05$
LDL Cholesterol (mg/dl)	28.88^a	22.50^b	23.50^b	0.34	S $P = 0.000$

Least Square Means bearing different superscripts differ significantly ($P<0.05$) column wise in same row.

reduced by adding garlic powder in the diet of broilers. The present results were in agreement with Miraghaee *et al.* (2011) who reported that total serum cholesterol and triglycerides were decreased in group fed 1% black cumin seed than control. (Shewita and Taha, 2011; Nasir, 2005; Al-Beitawi and El-Ghousein, 2008; Fadlalla *et al.*, 2008) also reported the decrease in serum total cholesterol and serum triglyceride levels with supplementation of black cumin seed in broilers ration.

In earlier study, Fadlalla *et al.* (2008) also reported the similar findings for blood serum protein, albumin and globulin level which did not get affected significantly by adding 0.3% graded garlic in the treatment group. No significance difference in the blood hematological parameters was found by (Fadlalla *et al.,* 2008; Khatun *et al.,* 2013) in the treatment group as compare to control by supplementing different levels of tulsi and neem extract in drinking water of the birds. This result was similar to our findings in which the blood hematological parameters did not get significantly affected by treatments. However, there are some studies which reported contrary results for hematological parameters (PCV, Hb, RBC, and WBC) which were found to be significantly (P<0.05) affected by lemon grass - garlic extracts (CLGE) augmentation in the broiler's diet (Alam *et al*., 2014). However, (Fadlalla *et al.,* 2008; Alagbe and Oluwafemi, 2019; Jahejo *et al*., 2019) showed that WBC was significantly increased in the treatment groups by supplementation of phytogenic mixture. The reason behind this phenomenon may be concentrations of cholesterol gets altered with balance between dietary inputs and body synthesis. Hence, if any feed material which may change the working of cells of body, it might play a role in changes in the levels of cholesterol. The reduced levels of plasma cholesterol may be due to supplement of one of the ingredients, *Nigella sativa* which may stimulate the cholesterol excretion into the intestine (Khodary *et al*., 1996). El-Alfy *et al*. (1975) investigated *Nigella sativa* oil has cholorelic effect that may elevate bile flow. Nevetheless, bile flow is an emulsifying agent which activates pancreatic lipase and finally contributes in digestion and absorption of fat and fat- soluble vitamins (Crossland, 1980).

CONCLUSION

Based on the findings of this study, it may be concluded that dietary supplementation of phytogenic mixture containing powder of *Allium sativum* bulbs*, Ocimum sanctum* leaves and *Nigella sativa* seeds in 1:1:1 ratio may favorably lower the levels of blood lipids without adversely affecting the normal physiology of broilers. Though, the number of chicks was limited in our study, hence, it is recommended to conduct further studies on large number of birds.

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