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Effect Of Dietary Supplementation of Ginger and Black Pepper Powder on Haematological Parameters of Cobb-500 Broiler Chicken

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ABSTRACT
An experiment was conducted to investigate the status of blood serum biochemical of broiler chickens in response to the diet supplemented with ginger and black pepper powder. Two hundred day-old Cobb 500 Strain broiler chickens were used for the experiment. The birds were randomly allotted into five treatments each replicated four times with 10 birds per replication. Grounded black pepper and ginger were added in the basal feed with different proportion, T1 (control); T2 (basal diet+0.02% ginger); T3 (basal diet+0.02% black pepper), T4 (basal diet+0.01% ginger+0.01% black pepper) and T5 (basal diet+0.02% ginger+0.02% black pepper. At 42days of rearing the effect of T5 treatment significantly resulted ($p<0.05$) increased in total protein, albumin and hemoglobin concentration and decreased in triglyceride, cholesterol, very low-density lipoprotein, and calcium concentration. Administration of 0.02% ginger and 0.02% black pepper to broiler chickens improve blood profile, boosted their immunity as well as improved their health status. Therefore, the inclusion of 0.02% of black pepper and ginger could be beneficial to improve the performance of broiler.

Keywords: Ginger, Black Pepper powder, Blood Profile, Immunity

INTRODUCTION

1.1 Background

Poultry is the epitome of an emerging economy in Nepal in recent decades. It has become one of the major national agriculture industries (Bhattarai, 2005). According to the FAOSTAT(2014) GDP contribution by the poultry industry is 3.5% and the investment in the industry is NRs 22 billion with a growth rate of 17-18%. There are 21956 commercial poultry farms and 128 hatcheries (Nepal Commercial Poultry Survey, CBS, 2015) with the total broiler meat production of 110689545 kg. Nepal ranks 112th in chicken meat production in the world and 92nd in egg production (FAOSTAT, 2014). The production of broiler is 1170537 per week (FAOSTAT, 2014). The broiler is the most produced commercial poultry in Nepal for meat production.

According to Parajuli (2008), 46 percent of the total bird population is of indigenous or local poultry scattered throughout the country, mostly in rural areas and the remaining 54 percent of the poultry population is reared by organized commercial poultry farms confined mostly to large peri-urban areas. To meet the urban demand for meat and egg, commercial poultry production is rising rapidly by more than three times in current years (from 1985 to 2014)(Acharya, andKaphle2015). The blooming poultry industry requires a surplus amount of poultry feed. The feed is the major component of the total cost of the poultry venture as 80% of the total expenditure is on procurement of the feed (Borazjanizadeh et al., 2011). The production of commercial poultry feed was 646845 t in 2010/1011 (FAOSTAT, 2014) which has increased abruptly in the last 6 years. The commercial poultry feed aims to attain maximum live weight, especially in the broilers.

Feed additives are a group of nutrient and non-nutrient compounds that helps in improving the efficiency of feed utilization and thus reducing the high cost of feed.Antibiotics in poultry are generally administered to the entire flock and are used for the treatment of disease (therapy), disease prevention (metaphylaxis), and growth promotion (Poole and Sheffield, 2013). In the past, antibiotics were the most routinely used feed additives. However, nowadays use of antibiotics is not only limited but their use in livestock and poultry industry also has been banned in many countries due to reasons like alteration of natural gut microbiota and drug resistance in bacteria and humans. It is thus necessary to replace them with other alternatives without adversely affecting the performance of birds accordingly natural growth promoters such as prebiotics, probiotics, synbiotics, enzymes, plant extracts, etc., can be used to feed the broilers (Borazjanizadeh et al.,2011).Available evidences indicates that phytogenic feed additives may add to the set of non antibiotics growth promoters for use in livestock, such as organic acids and probiotics (Windisch et al., 2008).

Feed additives are commonly described as a non-nutrient substance that accelerates growth, efficiency of feed utilization, beneficial for health or metabolism of the animals (Church and pond, 1988). Ginger as a natural growth promoter can be a potential alternative for common artificial growth promoters such as antibiotics (Demir et al., 2003). Vincent (2011) reported that ginger has been used as medicinal herb in a veriety of cultures around the world. Ginger is widely used in many countries as a food spice and as a herbal remedy (Chrubasik et al, 2005).Ginger is consumed as a delicacy, medicine, or spice.Ginger may act as a pro-nutrient because of the immense active ingredients it has been reported to contain such as gingerol, gingerdione, and gingerdiol that also have strong antioxidant activity (Kikuzaki and Nakatani, 1996).Weiner (1999) reported that in a series of experiments with rats, scientists from Japan discovered that extracts of Ginger inhibited gastric lesions by up to 97%. Herbs Hands Healing (2011) reported that ginger contains volatile oils like borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine, zingiberol (gingerol, zingerone, and shogaol) and resin.Ginger contains about 12 antioxidant constituents, the joint actions of which have been considered as being more powerful than vitamin C (Herbs Hands Healing 2011).The finding of the Preliminary research revealed the existence of nine compounds in ginger that may bind to serotonin receptors which may influence gastrointestinal function. Research conducted *in-vitro* in rats shows that ginger extract might control the number of free radicals and the peroxidation of lipids (Al-Amin et al., 2006) and have anti-diabetic properties (Morakinyo et al.,2011).

Black pepper (*Piper nigrum*) is a well-known spice due to its pungent quality (Hassan et al., 2007). Black pepper is a member of the family Piperaceae. Efficient compounds of pepper are cupsaeesin, cupsisin, and cupsantine. Black pepper is found to improve digestibility (Moorthy et al., 2009). Piperine is one of the compounds of black pepper that has antiache effect (Mahady et al., 2008). The bioactive molecule piperine has major pharmacological impacts on the nervous and neuromuscular systems (Sarica et al., 1995). It can dramatically increase the absorption of selenium, vitamin B complex, beta carotene,

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and curcumin as well as other nutrients (Khalaf et al., 2008; Tazi et al., 2014).Piperine enhances the thermogenesis of lipid and accelerates energy metabolism in the body and also increases the serotonin and endorphin production in the brain (Al-Kassie et al., 2011).Ultra structural studies with piperine showed an increase in microvilli length with a prominent increase in free ribosomes and ribosomes on the endoplasmic reticulum in enterocytes, suggesting that synthesis or turnover of cytoskeletal components or membrane proteins may be involved in the observed effect(Khajuria et al.,2002).Several researchers reported increased in body weight and a decrease in feed efficiency when these herbal plants were fed in broilers' diet (Greathead, 1999; Iqbal et al., 2011). Undoubtedly ginger and black pepper which are considered as herbal plants have a wide range of potential uses. Under this context, an experiment was done to explore the uses of ginger and black pepper on the performance and health parameters of broilers.

Objectives

To analyze the effect of ginger and black pepper added diet-fed broiler to the important blood parameters that could equally be valued from human health perspectives.

MATERIALS AND METHODS

Site selection

The experiment was conducted from 15th November 2021 to 27th December 2021 in a broiler farm, Bhairahawa, Rupandehi, Nepal in the winter season (15ºC to 30ºC). The site was situated at 4 km South from Bhairahawa, Nepal located at 27º 29' Latitude, 83º 27' Longitude and 105 m above the sea level.

Experimental birds

A total of 200, day-old Cobb500 broiler chicks were purchased from a private hatchery, RaptiHatchery Pvt. Ltd, Dang. The chicks were brooded in a deep litter for 7 days and were fed commercial broiler starter ration. After 7 days, birds were shifted to deep litter housing systems for experimental purposes.

Experimental design

A total of 200, unsexed 7 days old chicks of broiler were randomly divided into five treatment groups each with four replication having 10 chicks with similar body weight by using a Completely Randomized Design (CRD). Chicks were randomly assigned to 20 pens.

Procurement of the materials

Standard broiler starter B0, grower B1 and finisher B2feed ingredients were purchased from Buddha feed industries at Banghusari, Rupandehi. The experimental basal diet was formulated to meet the standard of NARC (1994). The nutrient requirement was met by using maize, wheat, soya bean meal, rice polish, rapeseed de-oiled cake, vegetable oil and a smaller amount of minerals and vitamins.

Diet formulation and mixing

Standard broiler starter, grower and finisher feeds were used in the experiment whereas a certain proportion of ginger and black pepper powder were added as herbal growth promoters. The starter ration was feed for 0-14 days, Grower for 15-29 days and finisher ration was fed for 30-42 days. The composition of ingredients in broiler feed and the calculated nutrient composition in the feed has been given in Table (1) and Table (2) respectively. The dietary treatments used in the experiment were as follows.

T_1 : Control diet (basaldiet)
T_2 : Basal diet + 0.02% ginger
T_3 : Basal diet + 0.02% black pepper
T_4 : Basal diet + 0.01% ginger + 0.01% black pepper
T_5 : Basal diet + 0.02% ginger + 0.02% black pepper.

Table 1.Ingredients used to prepare the basal starter feed, grower and finisher feed.

Ingredients %	Starter (0-14 days)B0	Grower(15-29 days)B1	Finisher(30-42 days)B2
Maize	53%	51.7%	54.9%
Wheat	10	15	15
Soybean meal	31.5	25.3	21.2
DORB	0.7	0	0
Rapeseed DOC	0	3	3
Marble powder	1.625	1.75	1.75
DCP	1.25	1.05	1
Soya lecithin	0.4	0.9	1.8
Salt	0.425	0.31	0.31
Methionine-DL	0.3	0.255	0.22
Lysine	0.265	0.285	0.24
Betane	0.03	0.03	0.03
Enzyme	0.035	0.035	0.035
Soda-bicarbonate	0.15	0.15	0.15
Threonine	0.055	0.06	0.05
Vitamin-A set	0.02	0.02	0.02
Vitamin-B set	0.04	0.04	0.02
Choline chloride	0.05	0.04	0.04
Probiotic	0.05	0.05	0.05
Livertonic	0.05	0.05	0.05
Mineral	0.05	0.05	0.05
Anticoccidiostat	0.02	0.05	0.05
Toxin binder	0.05	0.05	0.1
Total	100	100	100

Vaccination

All the birds used for the experiment were vaccinated with new castle disease vaccine- F1 at the age of 7 days through intraocular route. Similarly, all birds were also vaccinated with a modified live IBD vaccine containing intermediate strain at 14 days and IBD plus vaccine at 21 days. Lasota vaccine on 28th day in drinking water (Table 2).

Table 2.Vaccination schedule

Age	Vaccine	Route
5-7 days	ND Vaccine-F1	Intra-ocular, intra-nasal
14 days	Gumboroo intermediate	Intra-ocular, intra-nasal
21 days	Gumboroo intermediate plus	Drinking water
28 days	ND (Lasota)	Drinking water

Feed composition analysis

Proximate analysis was done for approximating the composition of basal feed(AOAC, 1990).The chemical composition of broiler basal feed samples was identified through proximate analysis (Weende system of feed analysis) at Nutrition lab at Agriculture and Forestry University, Rampur, Chitwan. Moisture %, Crude Protein (CP), Crude Fiber (CF), ether extract (EE), Nitrogen-free extract (NFE), Ash (Minerals) etc. of starter, grower and finisher rations were calculated after proximate analysis.

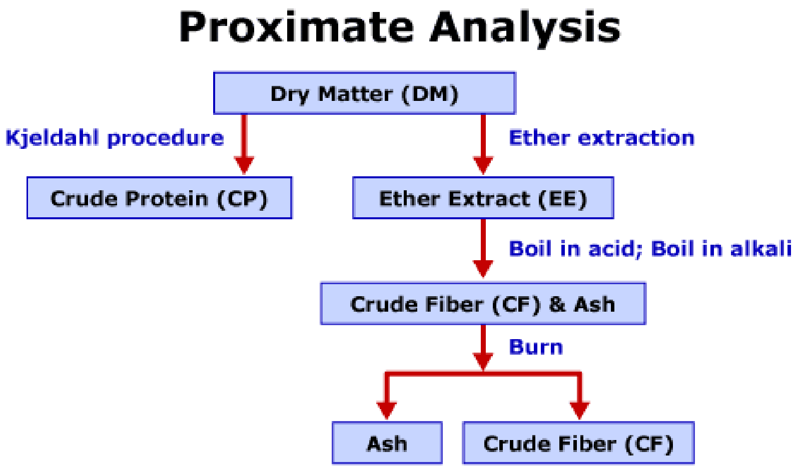

Figure 1. Proximate analysis procedure suggested by AOAC, 1990

Data collection

Layout of the experiment

Table 3.A number of chicks assigned randomly to the respective treatments

Groups	Treatments	Replication				Total chicks
		R_1	R_2	R_3	R_4	
T_1	Basal diet (Control diet)	10	10	10	10	40
T_2	Basal diet + 0.02% ginger	10	10	10	10	40
T_3	Basal diet + 0.02% black pepper	10	10	10	10	40
T_4	Basal diet + 0.01% ginger +0.01% black pepper	10	10	10	10	40
T_5	Basal diet+ 0.02% ginger+ 0.02% black pepper	10	10	10	10	40
Total						200

Blood sampling and analysis

Blood sample of two birds/replication/lot, totaling to eight birds from each treatment on the 6th week was collected from wing vein into Ethylene diaminetetraacetic acid (EDTA) containing and non-EDTA vials. The serum was prepared from the blood sample of the non-EDTA vials. Two lots of blood sampling was done (morning and afternoon). The serum was prepared from a blood sample of the non-EDTA vials. The sample was sent to Lumbini Zonal Hospital for analysis. EDTA containing sample was used for determining hemoglobin by coulter device while serum for lipid profile test for estimating triglycerides, cholesterol, High-density lipoprotein, very low-density lipoprotein, total protein and albumin.

Statistical analysis

The data collected were analyzed using Proc GLM procedure of SAS (9.4). The data were subjected to one-way analysis of variance (ANOVA). Differences between the treatments were set by Least Significance Difference (LSD.

RESULTS

Analysis of feed composition

The chemical analysis (AOAC, 1990) of the basal diet of broiler starter, grower and finisher rations were analyzed at the Department of Animal Nutrition and Fodder Production laboratory for their chemical compositions.

Table 4.Proximate composition of experimental feeds

Nutrient composition	Starter ration (0 -14 days)	Grower (15-29 days)	Finisher ration (30-42 days)
DM	88.68	88.54	88.31
CP%	22.19	21.2	19.16
CF%	3.70	3.5	3.2
Ether extract%	9.18	8.2	7.95
NFE	51.71	53.79	56.24
Ash	1.9	1.85	1.76

DM=Dry matter, CP=Crude protein, CF=Crude fiber, NFE=Nitrogen free extract

Blood parameter analysis

Table 8. Changes in blood parameters in broilers fed a diet with ginger and black pepper in different proportions

Treatments	Tg [mg|dl]	Cholesterol (mg/dl)	SGPT/ALT (u/l)	SGOT/AST (u/l)	HDL (mg/dl)	VLDL (mg/dl)	TP (g/dl)	ALB (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	HB %
T_1	103.8^{a}	172.1^{a}	17.13	123.13	114.8	20.77^{a}	1.69^{c}	1.23^{c}	7.99^{a}	9.91	9.65^{ab}
T_2	83.5^{b}	167.8^{ab}	17.38	115.25	100	16.7^{b}	2.24^{bc}	1.53^{bc}	7.82^{ab}	9.77	10.2^{ab}

T_3	83.75^{b}	140.25^{bc}	18.37	103.75	117.7	16.75^{b}	2.84^{a}	2.06^{a}	7.70^{ab}	9.72	9.175^{b}
T_4	70^{b}	121^{c}	15.63	111.37	110.7	14^{b}	2.64^{ab}	1.95^{a}	7.56^{bc}	9.49	9.32^{ab}
T_5	68.87^{b}	119.5^{c}	15.0	106.25	103.8	13.77^{b}	2.33^{ab}	1.78^{ab}	7.21^{c}	9.61	10.32^{a}
Grand Mean	82.0	144.15	16.70NS	111.9NS	109.45NS	16.40	2.351	1.71	7.65	9.70NS	9.73
F value	5.66	6.57	0.59	0.77	1.09	5.66	4.54	5.53	4.83	1.09	1.81
Probability	<0.0013	<0.0005	0.672	0.551	0.378	<0.001	<0.004	<0.002	<0.003	0.37	0.014
SEM	16.80	27.60	5.03	24.7	20.1	3.36	0.5	0.40	0.38	0.43	1.08
CV%	20.50	19.15	30.17	22.086	18.39	20.50	24.96	23.43	5.03	4.44	11.1

Note: Means in the column with different superscript differ significantly by LSD ($P<0.05$), where CV= Coefficient of Variation, SEM= Standard error of the mean, LSD= Least significant difference

DISCUSSION

In the present study the decreased triglyceride, cholesterol, VLDL, calcium, and increased total proteinand albuminwere found in the blood of broilers fed the diet with ginger and black pepper**.** It was expressed in the findings of diverse studies that plant extract and propolis intake leads to a decrease in the level of plasma cholesterol, glucose and triglycerides concentrations (Lee et al., 2003a;Al-Homidan 2005El-Bagir et al., 2006). This is consistent with the well-observed effect of ginger on lowering blood cholesterol levels (Ferreira et al., 1999;Ravindran, 2000; Soumyanath et al., 2006; Zomrawii et al., 2012) which might be attributed to the ginger possesses anti-hypercholesterolemia activity.This is consistent with the well-observed effect of ginger on lowering blood cholesterol levels (Fuhrman et al., 2000; Andallu et al., 2003). Ginger has antibacterial, anti-inflammatory action and is known to lower blood cholesterol levels in man (Ferreira et al., 1999; Zomrawii et al., 2013). Increasing the levels of garlic powder and applying garlic powder plus α-tocopherol significantly decreased total and low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol in broiler blood (Choi, Park,& Kim, 2010).Amouzmehr&Dastar(2009) indicated that supplementing of herbs extracts (thymus and garlic) to starter and finisher diets had not any significant effect on body weight gain, feed intake and feed conversion ratio. Supplementing of thymus and garlic extracts and sex had no significant effect on blood lipids concentration and hematocrit value. A decrease in cholesterol levels in broilers may be attributed to the ginger possesses antihypercholeste-rolemic activity. The deconjugation of gallbladder acids in the small intestine can affect control of serum cholesterol, while deconjugated acids are not capable to absorb fatty acids as conjugated acids. As a consequence, they prevent the absorption of cholesterol. The pungent compound of *Piper nigrum*especially piperine increases the production of saliva and gastric secretions (Herati and Marjuki, 2011). Also, free gallbladder acids attach to bacteria and fibers and this can increase their excretion.

Babu and Srinivasan (1997) reported that such a cholesterol-lowering effect could be mediated by the stimulation of hepatic cholesterol-7-hydroxylase which converts cholesterol into bile acids, facilitating the biliary cholesterol excretion. Conversion of cholesterol to bile acids is a multiple-step process in which the initial step, 7α-hydroxylation, is the rate-limiting reaction. It is possible that in spice fed animals whose liver microsomal aryl hydroxylase activity is stimulated, cholesterol-7-α-hydroxylase is also similarly activated (Akbarian et al., 2012). (Soudamini et al., 1992) reported that Curcumin administration has also been shown to reduce serum cholesterol in human volunteers, with increase in the HDL cholesterol (under publication), indicating that curcumin may be mobilizing cholesterol from extrahepatic tissues to liver where it is catabolised. Safaa (2007) concluded that either dietary garlic or fenugreek at 2% level have benificial effects on cholesterol metabolism that resulted in a reduction in serum and egg yolk cholesterol, serum LDL-cholesterol concentrations and an increase of serum HDL-cholesterol in laying hens without affecting hen productivity or egg quality.

Blood calcium level is high(7.99g/dl) at T1 which is significantly ($p<0.01$) different with T5,T4 and statistically similar with T2 and T3.The study results also revealed that the blood calcium was lowest inbroilers fed ginger and black pepper mixture supplemented.Calcium and phosphorus absorption and metabolism are influenced by many factors, such as the levels and ratio of inclusion in the diet, vitamin D3 and its derivatives, phytase and organic acids. Calcium is also necessary for bone formation; blood clotting and functioning of certain enzymes while phosphorus helps to control the acid-alkaline reaction of the blood (Zomrawi et al., 2013). Black pepper and ginger are good sources of vitamin B, C, E and D and they have essentials oils that they can help increase absorption of vitamin D3 and maybe affective in Calcium absorption (Ferreira et al., 1999; Greathead., 1999). Zingiber has magnesium, calcium, and phosphorus which function together in bone formation, muscle contraction, and nerve transmission. The high content of these minerals in ginger makes it a useful candidate for muscle spasms, depression, hypertension, muscle weakness, convulsions, confusion, personality changes, nausea, lack of coordination and gastrointestinal disorders (Yoshikawa et al., 1994). Black pepper contains a good amount of minerals like potassium, calcium, zinc, manganese, iron, and magnesium. Also, active principles in the black pepper may increase gut motility as well as the digestion power by increasing gastro-intestinal enzyme secretions. It has also been found that piperine can increase the absorption of calcium, selenium, B-complex vitamins, beta-carotene, as well as other nutrients from the food (Ravindran,2000; Hosseini, 2011).

Mitruka et al. (1977) stated that the number of erythrocytes (RBC) in chicken is influenced by the conditions of the animal. The Haemoglobin contents of the blood of birds fed the test ingredients is an indication of improved oxygen-carrying capacity of the cells which translated to better availability of nutrients to the birds consequently affecting their well-being. This implies that ginger and pepper could be potentially useful in improving blood circulation (Oleforuh-Okoleh et al., 2015; Habibi et al., 2014). It was proved that there is an increase in body weight and feed conversion ratio with decreasing hematological values of some important blood parameters using ginger or black pepper in broiler diets (Demir et al., 2003; Iqbal et al., 2011).

In this study, the effect of the ginger and black pepper and their combinations at different rates had a significant effect on the blood triglyceride, cholesterol, VLDL, total protein, albumin, calcium, and hemoglobin.The blood triglyceride level was found highest in the control group (103.8 mg/dl) and rather lower (68.87 mg/dl) for T5 (Table 7).The response of the treatment was significant ($p<0.05$) to the cholesterol level (mg/dl).Accordingly, the highest cholesterol level was for T1 (control) which is statistically similar to T2 (addition of 0.02% ginger only).Al-Shuwaili, Ibrahim &Naqi(2015) reported that the adding garlic (5%), ginger (5%), and cinnamon (5%) to the turkey diets decreasing the AST, ALT and glucose levels where as in the present studythe response of the treatment was not significant to the

SGPT/ALT level (u/l), SGOT/AST (u/l), HDL (mg/dl) and Phosphorous level (mg/dl).The response of the treatment was significant to the VLDL level (mg/dl). Accordingly, the highest VLDL level was for T1 (control). The response of the treatment was significant to the total protein and albumin level (g/dl). Similarly,T5 (basal diet with ginger and black pepper containing 0.02% each) possesses the lowest triglyceride, cholesterol, very low-density lipoprotein and calcium which suggest that the use of 0.02% of ginger and 0.02% black pepper in the basal diet improves the blood profile resulting higher weight gain.

CONCLUSIONS

The findings of the experiment revealed that supplementation of a certain proportion of black pepper and ginger improves the performance of broiler when added as feed additives in commercial broiler farms. From the findings of the present study, it can be concluded that supplementation with the basal diet with black pepper 0.02% and ginger 0.02% in feed causes a significant improve in some blood parameter. The serum biochemical analysis revealed that the triglyceride, cholesterol, very low-density lipoprotein, and calcium were decreased however total protein and albumin increased as well as the immunity situation improved. Supplementation of ginger and black pepper (0.02% each) in feed resulted in an improvement in the content of total protein and albumin proving its beneficial use as supplements to the basal diet of broiler chicken. Therefore the inclusion of 0.02% ginger and 0.02% black peppercould be much beneficial to improve the overall performance of broilers.

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