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Haematological and biochemical profile of broiler chickens fed diets containing ginger and black pepper additives

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Target Audience: *Animal scientist, Poultry farmers and Researchers*

Abstract

This study was carried out to examine the haemato-biochemical characteristics of broiler chickens fed diets containing ginger (Zingiber officinale L) and black pepper (Piper guineense Schum & Thonn) additives. A total of 240 day old unsexed Anak-2000 broiler chicks were allotted to four treatments (T1, T2. T3 and T4) in a completely randomized design. (T1) was the control with no additive, (T2) had 0.5% of ginger powder, (T3) had 0.5% black pepper powder, while (T4) had a mixture of 0.25% ginger and 0.25% black pepper. From the results obtained, the values of packed cell volume (34.53%) and red blood cell (3.60 x $10^6/mm^3$) was significantly ($p<0.05$) highest in the ginger treatment group. Aspartate aminotransferase and Alanine aminotransferase had significantly ($p<0.05$) lower values (270.23u/l and 65.00u/l respectively) in the ginger treatment compared to the control which had the highest (518.45u/l and 79.67u/l respectively). The treated groups also had significantly ($p<0.05$) decreased values of total cholesterol, low-density cholesterol and very-low-density lipoprotein compared to the control. As observed from this study, the addition of ginger and black pepper generally improved the blood parameters assessed in contrast to the control diet. Therefore, it can be concluded that, they hold health-promoting potentials in broiler production.

Keywords*: Ginger; Black pepper; Haematology; Serum biochemistry; Lipid profile; Broiler chickens.*

Description of Problem

It has been shown that data from blood profiles could be utilized in the improvement of chicken stocks (1). Such data can help in the diagnosis of specific poultry pathogens and might serve as basic knowledge for studies in immunology and comparative avian pathology (2). In general, the results of haematology and serum analysis are usually used to assess the health status of an animal. Haematological and serum biochemistry parameters are good indicators of the physiological status of animals and their changes are important in assessing the response of such animals to various physiological situations (3). Furthermore, changes in haematological parameters are often used to assess stress in animals due to environmental, nutritional and/or pathological factors (4).

One of the health-promoting effects of phytochemicals from plants is thought to arise from their protective effects of counteracting reactive oxygen species, as well as their antimicrobial action. Several studies suggest that plants rich in antioxidants, for example, play a protective role in health and against diseases, and their consumption lowers health risks. The potential of these medicinal plants may be related to the concentration of phenolic substances (flavonoids, hydrolyzable tannins, proanthocyanidins, phenolic acids, phenolic terpenes) and some vitamins (E, C, and A) (5). The advent of phytogenic feed additives such as herbs and spices particularly in swine and poultry production improved flavor and palatability, thereby enhancing productive performance (6). Today herbs and spices are extensively studied because of their potent properties and used as alternative non-antibiotic growth promoters (7).

Ginger (*Zingiber officinale*), a widely used herb and food spice is a major constituent of most Nigerian cuisine. The medicinal value of ginger has been linked to its antioxidant potential that arises from the oleoresins which are present in it. The oleoresins in ginger have also been credited for various pharmacological effects such as antimicrobial, anti-inflammatory, antioxidant, anti-hypercholesterolemic, anti-hyperglycemic, and antispasmodic (8).

Black pepper is rich in phytochemicals like glutathione peroxidase and glucose-6-phosphate dehydrogenase (9). It has also been documented that piperine, which is the major active compound in black pepper, can increase the absorption of selenium, vitamin B complex, beta-carotene, and curcumin as well as other nutrients (10). Additionally, it is an active alkaloid that modulates benzopyrene metabolism through cytochrome P450 which is essential for metabolism and transport of xenobiotics and metabolites (11). It also enhances thermogenesis of lipid (12), and increases the flow of digestive juice (13).

This study was therefore carried out to evaluate the effects of ginger, black pepper, and their combination on haematologic and serologic profiles of broiler chickens.

Materials and Methods

Experimental location and animal management

The experiment was conducted at the poultry unit of the Teaching and Research Farm of the Faculty of Agriculture, Ambrose Alli University, Ekpoma, Edo State, Nigeria. A total number of 240 Anak-2000 strain broiler chicks were used for the experiment. The chicks were managed in a deep litter system and after brooding for 2 weeks, they were transferred into individual pens measuring $20ft^2$. Feed and water were provided *ad libitum* with appropriate routine medications and vaccinations.

Preparation of test ingredients and experimental diets

The dried ginger and black pepper were obtained from the local market and milled into powder. The milled ginger and black pepper were weighed and used in formulating four balanced broiler starter and finisher diets according to the recommendations of Olomu (14), as shown in Table 1. The experimental treatments had the additives as: Treatment 1 (T1) was the control with no additive; treatment 2 (T2) had ginger powder at 0.5% (5g/1kg of feed); treatment 3 (T3) had black pepper powder at 0.5% (5g/1kg of feed); while treatment 4 (T4) had a mixture of 0.25% ginger and 0.25% black pepper (2.5g each/1kg feed). The feeding trial began from the third week and lasted till the eighth week.

Experimental design and data collection

The experimental design used was a completely randomized design (CRD). After brooding, the birds were randomly allotted to the four (4) experimental groups with 60 birds each, replicated 4 times to give 15 birds per replicate. The treatment effect was estimated by comparing the means of the assessed parameters in the treated groups against the control.

At eight weeks old, four birds were randomly chosen from each treatment, isolated, tagged and starved overnight. They were bled via the jugular vein at slaughter and blood samples were collected. The first set of samples was taken in labeled sterile universal bottles containing ethylene diamine tetra-acetic acid (EDTA), and others in heparin bottles without anti-coagulant. Blood samples in EDTA-containing bottles were used to analyze for full blood count (red blood cells, haemoglobin, packed cell volume, platelets, lymphocytes, and white blood cells) with the help of an automatic haematology analyzer. Serum was obtained from blood samples in heparin bottles by centrifugation (4000 rpm for 5 min at 20°C). Commercially available kits were used to analyze the serum parameters on a biochemical autoanalyzer. The concentration of the biochemical constituents was calculated according to the manufacturer's instructions. Lipid profile studies were carried out by employing a chemistry auto analyzing kit, using the appropriate enzymatic methods. The Friedewald (15) formula stated below was used in estimating the values of very-low-density lipoprotein (VLDL) and low-density lipoprotein.

VLDL = plasma triglyceride / 5
LDL = TC – (VLDL + HDL)
Where:
TC = Total cholesterol
VLDL = Very low-density lipoprotein
HDL = High density lipoprotein

Data generated were subjected to a one-way analysis of variance (ANOVA), with the help of the General Linear Model procedure of the Statistical Analysis System (SAS) (16). Where significant treatment effects were observed, differences between treatments means were compared by Duncan's multiple range test (DMRT) as outlined by Steel and Torrie (17). The level of statistical significance was pre-set at $p< 0.05$.

Table 1: composition of experimental broiler starter and finisher diets

	Starter diets				Finisher diets			
Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
Maize	55.20	55.20	55.20	55.20	54.00	54.00	54.00	54.00
Soybean meal	28.00	28.00	28.00	28.00	20.80	20.80	20.80	20.80
Palm kernel cake	9.00	8.50	8.50	8.50	12.40	12.40	12.40	12.40
Wheat bran	-	-	-	-	7.00	6.50	6.50	6.50
Fish meal	4.00	4.00	4.00	4.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	2.00	2.00	2.00	2.00
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin/mineral								
Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Ginger	-	0.50	-	0.25	-	0.50	-	0.25
Black pepper	-	-	0.50	0.25	-	-	0.50	0.25
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated composition								
Crude protein (%)	23.10	23.04	23.00	23.04	20.62	20.56	20.52	20.56
Metabolizable energy (Kcal/Kg)	3240.04	3232.54	3214.94	3232.54	3088.15	3080.65	3063.05	3080.65

Table 2: Haematological parameters of bird fed feed additives

Parameters	Negative Control (T1)	0.5% Ginger (T2)	0.5% BP (T3)	0.25% Ginger + 0.25% BP (T4)	SEM
Packed cell volume (%)	30.08^{b}	34.53^{a}	31.36^{b}	32.10^{ab}	2.10*
Haemoglobin (Hb)(g/dl)	10.30	11.31	10.24	10.44	1.48NS
Mean cell volume (MCV)(fL)	111.88^{c}	130.15^{a}	137.24^{a}	125.13^{b}	3.66*
Mean corpuscular haemoglobin (pg)	41.25	40.10	41.50	41.00	5.75NS
Mean corpuscular haemoglobin concentration (MCHC)(%)	28.71^{c}	30.76^{ab}	31.28^{a}	31.17^{a}	0.55*
Red blood cell (RBC)($\times10^{6}$/mm^{3})	2.15^{c}	3.60^{a}	2.54^{b}	2.55^{b}	0.25*
Platelets ($\times10^{3}$/µl)	12.21	14.48	16.08	14.50	12.06NS
Total white blood cell (x10^{3}/µl)	79.43^{b}	83.23^{a}	82.50^{a}	76.20^{b}	1.69*
Lymphocyte (%)	81.67^{a}	83.00^{a}	83.33^{a}	75.50^{b}	2.50*
Neutrophils (%)	13.13^{bc}	15.40^{b}	15.60^{b}	19.00^{a}	2.10*
Monocytes (%)	5.20^{a}	1.60^{c}	1.07^{c}	5.50^{a}	0.31*

a,b,c; means in the same row with different superscript are significantly different ($p<0.05$)
*BP = black pepper, NS= Not significant, * = significant difference

Results and Discussion

The effect of the feed additives on the haematological parameters of the experimental birds is presented in Table 2. Significant differences ($p<0.05$) were observed in packed cell volume and ranged from 30.08% (T1, control) to 34.53% (T2, diet with ginger). For mean cell volume and mean corpuscular haemoglobin concentration, the control group was significantly ($p<0.05$) different from the feed additive groups. The lowest values (111.88fL and 28.71%) were recorded from the control in both instances, while the highest values (137.24fl and 31.28% respectively) were recorded for the black pepper treated group (T3). Red blood cell was also lowest in the control group (2.15×10^{6}/mm^{3}) and highest in the ginger treated group (3.60×10^{6}/mm^{3}). However, the lowest value (76.20 x10^{3}/µl) of total white blood cells was obtained in T4 (diet with the ginger and black pepper combination), while the highest value (83.23 x10^{3}/µl) was recorded in T2 (diet with ginger).

Packed cell volume (PCV), haemoglobin and mean corpuscular haemoglobin (MCH) are major indices for evaluating circulatory erythrocytes and are significant in the diagnosis of anaemia (18). They also serve as useful indices of the bone marrow capacity to produce red blood cells in mammals (19). The number of erythrocytes (RBC) in chicken influences the overall conditions of the birds (20). Therefore, the numerical increases in PCV, haemoglobin and RBC counts of the birds fed the test ingredients are an indication that the oxygen-carrying capacity of the blood was enhanced. Furthermore, it has been posited that high PCV reading (polycythemia) is an indication of either an increase in the number of red blood cells or a reduction in circulating plasma volume which might be due to a physiological adaptation to high altitudes or pathological response to chronic circulatory or respiratory disease (19). It can also be as a result of iron storage disease, rickets, hypoxic increase in erythropoietin production or non-hypoxic autonomous increase in erythropoietin production (21). As a guide, a PCV value greater than 56% is an indication of dehydration in most birds (22). In the present study, however, no PCV value was above 35%, and the values obtained were within the reference ranges for broilers of 22-35 percent (23) and 25-45 percent (24).

Haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are important blood parameters whose values are used to determine the presence and severity of anaemia (25). A decrease in the haemoglobin, MCV and MCH levels in birds is also an indication that the birds are exposed and poorly dealing with stressors (26). In the present study, no significant differences were observed in both haemoglobin and MCH values. However, the control group had a significantly lower MCV value (111.88fL) as compared to the treated group which ranged from 125.13fL to 137.24fL. It can be inferred therefore that the test additive generally improved the bird's ability to withstand stress. It has also been reported that low mean corpuscular hemoglobin concentration (MCHC) value less than 29.0 g/dl can be attributed to iron and other trace element deficiency (27). In the present study however, only the control group had an MCHC value of approximately 29.00g/dl, which is within the recommended ranges of 24.00g/dl – 31.00g/dl (28). The values obtained from the treated group are within the referenced range of 31.00g/dl – 33.88g/dl (26). Based on the available literature about the vitamin and mineral content of the test ingredients, it can be inferred that the additives improved the availability and utilization of essential trace elements.

Table 3: Serologic profile of experimental birds

Parameters	Negative Control (T1)	0.5% Ginger (T2)	0.5% BP (T3)	0.25% Ginger + 0.25% BP (T4)	SEM
Aspartate aminotransferase (AST)(u/l)	518.45^{a}	270.23^{c}	365.50^{b}	208.25^{d}	41.50*
Alanine aminotransferase (ALT)(u/l)	79.67^{a}	65.00^{c}	72.30^{b}	84.13^{a}	5.05*
Alkaline phosphatase (ALP)(u/l)	137.75^{d}	180.65^{c}	220.60^{b}	309.00^{a}	15.59*
Total bilirubin (mg/dl)	0.32	0.35	0.33	0.28	0.08NS
Direct bilirubin (mg/dl)	0.23	0.17	0.15	0.13	0.11NS
Malondialdehyde (MAD) (µmol/L)	0.03	0.01	0.01	0.01	0.04NS
Metabolites and electrolytes					
Blood urea nitrogen (mg/dl)	6.57^{b}	7.55^{a}	7.52^{a}	7.00^{ab}	0.75*
Sodium (mmol/L)	162.56^{a}	165.00^{a}	162.05^{b}	154.41^{c}	1.45*
Potassium (mmol/L)	4.77^{a}	4.30^{ab}	3.87^{ab}	3.80^{b}	0.28*
Creatinine (mg/dl)	0.42^{b}	0.48^{ab}	0.47^{b}	0.53^{a}	0.03*
Glucose (mg/dl)	201.50^{b}	220.00^{a}	210.87^{ab}	215.25^{a}	9.54*
Total Protein (g/dl)	3.43^{c}	4.00^{b}	4.13^{a}	3.85^{c}	0.07*
Albumin (g/dl)	1.47	1.45	1.47	1.54	0.29NS
Globulin (g/dl)	1.96^{b}	2.55^{a}	2.66^{a}	2.31^{ab}	0.36*
Plasma lipid profile					
Triglyceride (mg/dl)	55.42^{a}	30.30^{c}	40.12^{b}	42.70^{b}	2.51*
Total Cholesterol (mg/dl)	137.11^{a}	103.20^{b}	115.17^{b}	105.90^{b}	13.78*
Low Density Cholesterol (mg/dl)	100.16^{a}	60.00^{b}	75.90^{c}	68.86^{bc}	8.52*
High Density Lipoprotein (mg/dl)	25.87^{c}	37.14^{a}	31.25^{b}	28.50^{bc}	4.05*
Very low-density lipoprotein	11.08^{a}	6.06^{c}	8.02^{b}	8.54^{b}	0.49*

a,b,c; means in the same row with different superscript are significantly different ($p<0.05$)
*BP = black pepper, NS= Not significant, * = significant difference

The birds fed the treated diets also had numerically higher platelets in the blood. It has been observed that certain phytogenic compounds present during the transformation of arachidonic acid to thromboxane can decrease the sensitivity of the platelets to aggregating agents (29). This can allow the inference that the phytogenic quality of the test additives could be potentially useful in improving blood circulation on account of its inhibitory effect on platelet aggregation. This result, however, contradict with the findings of a study that examined the effect of aqueous extract of ginger and reported that there was a drop in the platelet level of broiler chickens in the treated group (30).

T2 (diet with ginger) and T3 (diet with black pepper) had higher and significantly different values of total white blood cells than T1 (control) and T4 (diet with ginger and black pepper combination). This agrees with the findings of a study of the effects of ginger and cinnamon supplementation on broiler chickens (31). The ginger and black pepper treatments also showed similar numerical increases in lymphocytes and neutrophils over the control though not significantly different ($p>0.05$) from each other in these cases. It should be noted that lymphocytes T and B and macrophages interaction are essential in developing an immune response (32). Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with moderate counts are capable of generating antibodies in the process of phagocytosis and have a high degree of resistance to diseases (33) and enhance adaptability to local environmental and disease prevalent conditions (34). In general, an overwhelmingly high WBC count in the peripheral blood is often observed in stress, inflammatory conditions due to generalized or localized infections, trauma, toxicities, neoplasms, and so on (35). No significant difference was observed in lymphocyte values obtained in this study (75.50% - 83.33%) between T1 (control), T2 (diet with ginger) and T3 (diet with pepper), but were higher than the referenced ranges of 45-70 percent (25) and 54-73 percent (30).

The serological profile of the experimental birds is presented in Table 3. The group of parameters assessed include enzymes, metabolites and electrolytes, as well as plasma lipid levels. Significantly higher values were observed for aspartate aminotransferase (518.45µ/l) in T1 (control group) and for alkaline phosphatase (309.00µ/l) in T4 (diet with ginger and black pepper combination). In comparison, the least values in AST (208.25u/l) and ALT (137.75u/l) were observed in T4 (combination diet) and T1 (control diet) respectively. The highest value (84.13 µ/l) for ALT was recorded with birds fed T4 (diet with ginger and black pepper combination) and the least value (65.00µ/l) was observed in when birds fed T2 (diet with ginger).

It should be noted that the liver is the center of several digestive, metabolic and productive activities, and as such, is susceptible to a varying degree of chemical and biological damages. Such damages are made obvious by the serum levels of specific enzymes originating from the liver. These enzymes, depending on their levels may cause some disruptions to bodily functions, thereby resulting in poor health and production performance. The activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in the blood are bioindicators of liver function and damage (36). Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress (37). The values of these enzymes in the present study showed significant differences ($p<0.05$) between the control and the treated group. It was observed that induced liver damage using aflatoxin in broilers and layers led to an increase in serum ALT (38). Therefore, the reduction in AST and ALT due to ginger and black pepper treatments can be deduced as an indication of better liver function. This is however contrary to the findings of a study of the effect of turmeric rhizome powder on the activity of some blood enzymes in broiler chickens, which found non-significant differences in these enzymes' levels (39).

Alkaline phosphatase (ALP) is another enzyme produced mainly by the intestinal mucosa, liver, bone, kidney, and placenta; however, the intestinal ALP does not contribute much to serum ALP levels (40). Reduced activity of ALP may be an indication of a slowdown of bone growth (41). Higher serum levels of ALP are observed when there is increased osteoblastic activity, involving the formation and mineralization of bone associated with increased skeletal growth (37). Even though no specific inference could be made from the pattern of ALP values seen in the present study, it might have an association with the increased growth rate of broiler chickens fed ginger and black pepper additives (42). Also, the values of ALP are in agreement with the referenced values of 167ul – 305ul for poultry birds (30).

Among the analyzed blood metabolites, the control group had the lowest values for blood urea nitrogen (6.57mg/dl), creatinine (0.42mg/dl), glucose (201.50mg/dl), total protein (3.43g/dl) and globulin (1.96g/dl) which were also significantly different ($p<0.05$) from the treated group. Sodium and potassium levels (154.41mmol/l and 3.80mmol/l respectively) were lowest in T4 (diet with ginger and black pepper combination). These values were also significantly different from T1 (control) and T2 (diet with ginger) that had the highest values respectively.

The significant differences ($p<0.05$) in some serum metabolite parameters between the control and the treated diet groups are in agreement with the results of related studies (30; 32). Some other studies have shown that the addition of ginger in broiler diet reduced blood glucose (43; 44), which is in contrast to the present study that showed a significant increase in glucose level in the ginger and combination diets in comparison to the control. This disagreement may be attributed to the fact that blood glucose level is maintained by critical homeostatic mechanisms that depend on the stage of growth, feed consumption, productivity, and environmental changes (45). It is also documented that the active compounds in phytogenics have receptors on the adrenal gland and may affect the nervous system by decreasing adrenocorticotropic hormone (ACTH) secretion (46). This reduction induces stress, which ultimately increases blood glucose concentration and depressed immunity functions (47).

The control group had significantly highest values for triglyceride (55.42mg/dl), total cholesterol (137.11mg/dl), low-density cholesterol (100.16mg/dl) and very-low-density lipoprotein (11.08mg/dl), whereas T2 (diet with ginger) had the lowest values for these parameters. However, T2 (diet with ginger) had the highest value for high-density lipoproteins (37.14mg/dl) and T1 (control) had the lowest (25.87mg/dl).

These results agreed with some previous studies that found that ginger inclusion in the diet of broilers resulted in the reduction of blood serum cholesterol (31; 48). This reduction is attributed to the presence of gingerol and shagol components in ginger which inhibits lipid peroxidation (48; 49). The inhibition of lipid peroxidation during cholesterol synthesis occurs when the key regulatory enzyme, hydroxyethyl glutaryl coenzyme A reductase (HMG-COA) in the liver is inhibited (50). It has been further advanced that a 5 percent inhibition of HMG-COA reductase is capable of lowering serum cholesterol in poultry up to 2 percent (51). The significantly lower values of triglycerides in the treated diet groups in comparison to the control diet is consistent with the result of a previous study which reported that the use of ginger reduces blood triglyceride levels (44). It is believed that the hypertriglyceridemia effects not seen in chickens fed with herbs and spices may be due to active ingredients in these products that leads to a decrease in the activity of lipogenic enzymes in the liver (52). The reported values of total cholesterol, low-density lipoprotein, and very-low-density lipoprotein were significantly higher in the control group. A similar conclusion was drawn by other authors (31; 53). This may probably be due to the possible depression of the hepatic activities of lipogenic and cholesterolemia enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphatase dehydrogenase (54) and 3-hydroxyl-3-methyl-glutaryl-CoA (HMG-CoA) reductase (55) by the active ingredients in both ginger and black pepper. Low-density cholesterol and very-low-density lipoprotein are considered as bad cholesterol because a high LDL level leads to a buildup of cholesterol in arteries. The reduction in the values of these parameters, therefore, adds credence to the fact that the use of ginger and black pepper additives in broiler production can be adjudged as an effective measure of reducing cardiovascular diseases.

Conclusion and Applications

Based on the results obtained

1. Ginger and black pepper additives can be applied singly or in combination in broiler diets with no adverse effects on haematological and serum biochemistry parameters.
2. The positive influence of this additives justifies the practice of using them in broiler production to improve the health and general wellbeing of the flock.
3. The research adds to the credence that ginger and black pepper holds great potential in poultry production. It is expected that this will help researchers and producers face the challenge of raising healthy birds without recourse to chemical-based additives.
4. It is also expected to increase research interest in the utilization, and mechanism of action of natural feed additives.

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