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Hematological parameters, serum biochemistry and antioxidant capacity of broiler chickens fed diet supplemented with two sources of antioxidants as feed additives

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Abstract: *Hematological parameters, serum biochemistry and antioxidant capacity of 125 broilers (4 weeks of age) were assessed in other to study the effect of Zingiberofficinale (Ginger), Piper nigrum (Black Pepper) and Vitamin C as feed additives in their diet. Ginger, Black pepper and Vitamin C powder were administered at the level of 50g/10kg of feed. The broilers were randomly allotted to five treatments:T1 (no antioxidant), T2 (0.5% ginger powder), T3 (0.5% black pepper powder), T4 (0.25% ginger+ 0.25% black pepper), T5 (0.5% Vitamin C) in a completely randomized design. The results obtained showed that PCV (%), Hb(g/dl), MCV(fl), MCH (pg), MCHC (%), RBC($x10^6/mm^3$)and platelets ($x10^3/\mu l$) were not significantly ($P>0.05$) different across the treatment groups. Total white blood cell ($x10^6/mm^3$) of T2 (8.22) and T3 (8.25) were significantly ($P<0.05$) higher than the other treatment groups, and results ranged from the highest which was T3 (8.25) to the lowest T4 (7.72),although monocytes count of T1 was significantly ($P<0.05$) higher compared to other groups with T2 having the lowest value of 0%. Serum glucose (mg/dl) of T2(186.50) and T4(184.50) were not significantly different but both were significantly ($P<0.05$) higher than the other treatment groups. Total cholesterol (mg/dl) of T1 (130.00) was ($P<0.05$) higher other treatment groups with T5 having the lowest result. T4 had the highest level of triglyceride of 46mg/dl while T2 had the lowest value of 32.33mg/dl. Low density lipoprotein was highest in T1 (83.08mg/dl) and T2 had the lowest level of low density lipoprotein of 64.77mg/dl. The results of high density lipoprotein showed no significant difference among the treatment groups but ranged from the highest in T2 (35mg/dl) to the lowest in T4 (23.50mg/dl). The total antioxidant capacity in T5 (40.20µmol/l) was significantly ($P<0.05$) higher compared to the other treatment groups. The level of vitamin C (mg/dl) was significantly ($P<0.05$) higher in T3(1.160) compared to other treatment groups.In conclusion, inclusion of ginger and blackpepper as sole agents may maintain or regulate Total white blood count in the blood, increase High density lipoprotein and reduce low density lipoprotein and triglyceride.*

Keywords: *Physiology Broiler, Ginger, Black pepper, Vitamin C, Feed*

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I. Introduction

The immune system of the animal is based on natural and adaptive immunity. The natural immunity is dependent on the efficient function of phagocytic cells namely neutrophils and macrophages which use free radicals as a weapon to kill pathogen, however, when escape from phagosome the same free radicals become dangerous and can damage all sort of biological molecule compromising phagocyte function and damaging adaptive immunity. Phagocytes also produce so called communication molecules (eicosanoids, cytokines, etc.) which are used for effective communications between various immune cells. Adaptive immunity is based on activity of B- and T-lymphocytes which are producing antibodies. As one could realise that communication between immune cells is a crucial factor of immunocompetence. If we imagine that immune system is an army fighting against invaders (microorganisms, viruses, etc) then we would expect them to have something like mobile phones to receive and send signals to each other. Remarkably enough, major immune cells (macrophages, neutrophyls, T- and B-lymphocytes) have on their surface something like “mobile phones” called receptors. Those receptors are extremely sensitive to communicating molecules, but they are also sensitive to free radicals and can be easily damaged. In such a situation without proper communication all those huge armies of immune cells would become useless. They also can start fighting each other as well as eventually destroying immunocompetence. If we imagine that immune cells are soldiers using chemical weapon to kill enemy, then special ammunition protecting them from their own weapon would be crucial for effective battle. In the case of immune cells such ammunition is represented by natural antioxidants with Se-GSH-Px being a major defence. Based on the presented model it is clear that antioxidant defence is a crucial factor of immune defence in the body (Surai, 2002). Animal health depends on many factors and recently it has been appreciated that diet plays a pivotal role in health maintenance and prevention of various diseases. Among many dietary factors, antioxidants have a special place being major players in the battle for animal survival, maintenance of animal health, productive and reproductive performance. This is largely because of the detrimental effects of free radicals and toxic products of their metabolism on various metabolic processes. Free radicals are highly unstable, very reactive and are capable of damaging molecules such as DNA, proteins, lipids or carbohydrates. Damage to DNA is associated with mutations, translation errors, and disruption of protein synthesis (Surai*et al.,* 2003).

In nature there are thousands of compounds possessing antioxidant properties. There are both fat-soluble (vitamin E and carotenoids, etc.) and water-soluble (ascorbic acid, glutathione, bilirubin, etc.), they can be synthesized in the body (ascorbic acid, glutathione) or are delivered with food/feed (vitamin E, carotenoids, Se etc.). The antioxidant enzymes that are synthesised in the body require metal co-factors. For example selenium in the form of selenocysteine is at the active site of several families of enzymes such as the glutathione peroxidases (GSH-Px) and thioredoxin reductase (TR). Zinc, copper and manganese are integral parts of another antioxidant enzyme family called superoxide dismutases (SOD); and iron is an essential part of the antioxidant enzyme called catalase. Only when these metals are delivered with the diet in sufficient amounts can the body synthesize the antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and allows damage to biological molecules and membranes. It is important to realize that all antioxidants in the body function in concert to provide antioxidant defence. Studies have also revealed that oxidative stress caused by transportation in hot humid tropical condition can bring about haematological derangements (Polycarp, 2016). With these knowledge of antioxidant effect in the animal body, it is imperative to evaluate the effects of these natural sources of antioxidant (Ginger and Black Pepper) on the immune system, health status as well as total antioxidant capacity of the animals fed these antioxidant sources.

II. Materials And Methods

A total of 125 broiler chicks were brooded with coal heat source for 4weeks and were fed a 24% protein broiler starter commercial ration *ad libitum*. The diet was changed to commercial finisher and the experimental treatment was incorporated into it and was fed from 4weeks of age till 9th week of age. The birds were randomly allocated to five (5) treatments having 25 birds per treatment in a Completely Randomized Design (CRD). Body weight and feed consumption were determined by measurement weekly and feed conversion ratio was also calculated. The birds were vaccinated against Newcastle and Gumboro diseases according to schedule and other medications when necessary. The birds were provided feed and water *ad libitum* throughout the period of the experiment.

Experimental Feed additives

Fresh matured ginger roots and dried black pepper were purchased in a commercial market in Benin, Edo State, Nigeria. The ginger bulbs were washed in clean water, sliced, dried and milled into a ginger powder and the dried black pepper was milled into pepper powder before being incorporated into the formulated diet. The vitamin C (10mg) was obtained from a pharmaceutical store and was ground into powder.

Thus we have the following treatments:
Treatment 1: Control group – (No antioxidant).
Treatment 2: Ginger powder – (*Zingiberofficinale*).
Treatment 3: Black pepper – (*Piper nigrum*).
Treatment 4: Black pepper+Ginger powder– (25g Black pepper and 25g Ginger*).*
Treatment 5: Vitamin C– (*ascorbic acid*)

Each treatment diet was supplemented at 50g/10kg feed. The proximate analysis of the experimental diet is shown in Table 1

Table 1 Proximate analysis of test diet included the test ingredients, ginger, blackpepper and vitamin C.

Samples	Moisture contend	Dry matter	Ash	Fat	Fibre	Protein	NFE
T1	8.356	91.643	8.260	10.260	5.423	19.833	47.866
T2	9.273	89.726	7.750	10.550	4.273	21.00	46.153
T3	9.130	89.870	8.320	10.330	4.423	19.250	47.546
T4	8.066	89.933	8.456	10.460	5.756	18.250	49.010
T 5	8.726	89.273	8.136	10.936	5.823	19.416	41.756

III. Data Collection

At the end of the trial, three birds were randomly selected per replicate and bled through the jugular vein. Five (5ml) of blood samples were collected and 3ml out of it was emptied into Ethylene Diamine Tetra Acetic Acid (EDTA) bottle, for haematological evaluation and the remaining 2ml was emptied in a bottle without anticoagulant to determine serum biochemical parameters and Total Antioxidant Capacity (TAC). Serum was gotten by centrifuging the blood samples at 3000rpm for 5 minutes. The blood samples were transferred to the laboratory for analysis. within 12 hours of collection.

Haematological parameters:

Blood samples collected from the chickens were analysed for the following parameters Total erythrocyte count (TEC), Haemoglobin (Hb), Packed cell volume, Leucocyte count, Lymphocytes $X10^3$, Neutrophils $X10^3$, Monocytes $X10^3$, Eosinophils $X10^3$as described by Lamberg and Rothstein (1977). The following parameters were calculated with standard formulars: Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH)

Determination of serum biochemical parameters

The total protein was determined by the Biuret method. Albumin determination was by densitometer scanning method described by Kohn (1958) where Globulin was calculated as Total Protein concentration – Albumin concentration. Lipid profile was determined with the use of Lipid Analysing machine which uses the oxidation method of lipids and measuring the quantity of H_2O_2 produced, by reading the absorbance at 500nm

Determination of Total Antioxidant Capacity (TAC) of blood serum.

Serum total antioxidant capacity activities was carried out according to Korecevic, (2001),
serum lipid peroxidation was determined using thiobarbituric acid assay according to Ohkawa*et al.* (1979).

Statistical analysis

The data obtained was subjected to statistical analysis of variance (ANOVA) procedure of GenStat 17^{th} edition at 5% probability level and Duncan multiple range test option of the same statistical software was used to separate the treatment means.

IV. Results And Discussions

The results of the effect of two dietary sources of antioxidant on hematological parameters of broiler chicken are shown in table 2. The TWBC ($\times10^3/mm^3$)of T2 (8.22) and T3 (8.25) were significantly ($P<0.05$) higher than the other treatment groups, and results ranged from the highest which was T3 (8.25) to the lowest T4 (7.72). Heterophiles (%) ranged from T4 (21.00) which was significantly ($P<0.05$) higher than other treatment groups to T5 (11.67). The results of the monocytes (%) of T1 (1.67) was significantly ($P<0.05$) higher compared to other groups with T2 (0.00) having the lowest percentage of monocyte count. The Volume of thrombocytes ($x10^3/\mu l$) was significantly ($P<0.05$) higher in T1 (18333) and T5 (18033) compared to the other treatment groups, where T4 (14333) recorded the lowest volume of thrombocytes.

Although the total white blood cell count was higher in ginger and black pepper sole agent in diet of broiler chicken, both also recorded the lowest monocyte count. Monocytes are the largest member of white blood cells and are capable of traveling to various parts of the body to eliminate harmful matter. This may signify that the increase in white blood cell count in ginger and black pepper diet was not as a result of inflammation or disease which ordinarily should have increased the monocyte count but that the antioxidative property of ginger and black pepper was responsible in maintaining total white blood count. The heterophile count in sole ginger (15±2.49%) and black pepper (14.67±2.49%) diet were within the normal range of chicken (15.6%-32.8%) as reported by Albritton, 1961. Heterophil which attack bacterial and fungal infections were highest in ginger/blackpepper mixture diet (21.00±2.49%)which also recorded lowest total white blood cell count. The heterophil result may not depict the presence of bacterial and fungal infections since heterophil results fell within the range for normal chicken (15.6%-32.8%) as reported by Albritton, 1961.

Table 2: Effect of two dietary sources of antioxidant on hematological parameters of broiler chicken

Parameters	T1	T2	T3	T4	T5	±SEM
PCV (%)	29.70	30.93	28.93	29.40	30.23	2.68
Hb(g/dl)	9.63	9.47	9.17	9.10	9.33	0.81
TWBC($\times10^3/mm^3$)	7.94^b	8.22^a	8.25^a	7.72^b	7.94^b	2.18
Lymphocytes(%)	81.67	83.00	83.33	75.50	85.00	2.55
Heterophiles(%)	14.33^b	15.00^b	14.67^b	21.00^a	11.67^c	2.49
Monocyte(%)	1.67^a	0.00^c	0.33^c	1.33^b	1.33^b	0.29
Eosinophils(%)	2.33	2.00	1.67	2.33	2.00	0.36
MCV(fl)	97.27	95.70	98.97	100.17	100.27	4.1006
MCH(pg)	31.50	30.37	31.30	31.10	30.97	1.5257
MCHC (%)	32.37	31.77	31.54	31.10	30.83	0.5996
RBC($\times10^6/mm^3$)	3.05	3.20	2.94	2.94	3.02	0.2353
Thrombocytes($\times10^3/\mu l$)	18333^a	14667^c	16333^b	14333^c	18033^a	1.7061

T1: Control (No antioxidant), T2: (0.5% Ginger), T3: (0.5% Black pepper), T4: (0.25% Ginger and 0.25% black pepper), and T5: (0.5% Vitamin C) respectively

PCV: Pack cell volume; HB: Haemoglobin;TWBC: Total white blood count ; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RBC: Red blood cell

The total white blood cell result in the study across the treatment group were a little lower than the acceptable ranges for normal chicken, (9.20-28.6 x10^3/mm^3) as recorded by Albritton, 1961, although this could be as a result of breed differences, but none the less, ginger and blackpepper sole agent diets results were closer to the normal range which may mean that the antioxidative property of ginger or blackpepper influenced the immune system response in broiler chicken. This result also corresponds to the report of Ademola*et al.,* (2009) who reported that ginger incorporated into diet influenced white blood cell count among other blood parameters. The result of total white blood cell in ginger/ blackpepper mixture diet which appeared to be the lowest across treatment groups may indicate that the combination of ginger and blackpepper in this inclusion rate reduced the quantity of white blood cell, this indicates that incorporating ginger or blackpepper as sole agents in diets may be safer to the immune system rather than in combination. Thrombocytes count which was lower in ginger diet (T2) and the ginger/blackpepper mixture diet (T4) could depict that ginger which was present in both treatment groups at the inclusion rate could have being responsible for the reduced thrombocytes count which may further result to the risk of bleeding (in-ability of blood to clot). This result implies that care should be taken against over dose or consumption of ginger in large amounts as reports have proved that very minute quantities of ginger, had strong impact on the growth and serum lipid of broilers (Ademola*et al.,* 2009). However, dietary addition of the supplements did notadversely affect the red blood cells, PCV and haemoglobinconcentration of the broiler chickens

The results of the effect of two dietary sources of antioxidant on lipid profile, serum glucose and Total protein of broiler chicken are shown in table 3. Total cholesterol, tryglyceride, LDL (bad cholesterol) and glucose were influenced by the antioxidative supplements across the treatment groups. Ginger dietary treatments significantly (P<0.05) reduced the concentration of serum triglyceride, total and LDL- cholesterol of broiler chickens. Having a high level of triglycerides, a type of fat (lipid) in the blood, can increase risk of heart disease. The body converts any calories it doesn't need to use right away into triglycerides.Tryglyceride in ginger sole agent diet was reduced to about 32.33mg/dl when compared to control group (41.25mg/dl). Ginger /blackpepper mixture diet influenced tryglyceride level to 46.00mg/dl which was higher than the control. This implies that mixture of ginger and blackpepper may increase serum, total cholesterol, LDL and tryglyceride and so there is need for caution in adding mixture of ginger and black pepper as supplements to broiler diets. Lowerconcentration of the supplements may be appropriate. There was no significant difference between the results of the total Cholesterol of ginger sole agent diet and vitamin c sole agent diet, this may indicate that ginger is highly an active antioxidant as much as Vitamin C in fighting lipid peroxidation which reflected in the reduced total cholesterol in both treatment groups. The results on the effect of ginger as sole agent in broiler diet corresponds to the reports of Ademola*et al.* (2009). HDL (good cholesterol) was not adversely affected across the dietary treatment groups. Although the results were not statistically different among treatment groups, a numerical increase of HDL level of 35.00 mg/dl was recorded in ginger sole agent diets compared to the control group 28.77mg/dl. A knowledge or check on the cholesterol level in circulation in the serum of the chicken is very much important as it affects consumption of birds by humans. The reason resulting from the transfer of cholesterol to human body via the animal consumption. LDL cholesterol makes up the majority of the body's cholesterol. LDL is known as "bad" cholesterol because having high levels can lead to plaque buildup in arteries and result in heart disease and stroke in humans. HDL cholesterol absorbs cholesterol and carries it back to the liver, which flushes it from the body. HDL is known as "good" cholesterol because having high levels can reduce the risk for heart disease and stroke. Triglycerides on the other hand are a type of fat found in the blood that the body uses for energy. The combination of high levels of triglycerides with low HDL cholesterol or high LDL cholesterol can increase risks of heart attack and stroke. Although there were significant differences in the level of glucose across treatment groups, all treatment groups had glucose level within the normal range of 152mg/dl-182mg/dl (Altman and Ditter, 1974) and up to 400mg/dl (www.beautyofbirds.com- retrieved 06/06/2017). In birds, glucose level below 150mg/dl are life threatening (www.beautyofbirds.com- retrieved 06/06/2017). The results of the effect of two dietary sources of antioxidant on total antioxidant capacity, Vitamin C and Malondialdehyde, of broiler chicken are shown in table 4.The total antioxidant capacity in T5 (40.20µmol/l) was significantly (P<0.05) higher compared to the other treatment groups. The level of vitamin C (mg/dl) was significantly (P<0.05) higher in T3(1.160) compared to other treatment groups. Dietary treatment significantly (P<0.05) influenced the level of melondialdehyde across the treatment. All test diets were not significantly different from the control but corresponding numerical reduction in MAD(µmol/l) followed an increase in TAC (µmol/l) although TAC value across dietary treatments showed no significant difference. An increase in Total antioxidant capacity of the body creates a protective system in the body tissues over reactive oxygen and Nitrogen species which are capable of altering the body defence system over diseases.

Table 3: Effect of two sources of dietary antioxidant on lipid profiles, serum glucose and total protein

Parameters	T1	T2	T3	T4	T5	±SEM
Triglyceride(mg/dl)	41.25 ^{b}	32.33 ^{c}	40.33^{b}	46.00 ^{a}	44.25 ^{ab}	2.42
Total CL(mg/dl)	130.00^{a}	115.00^{ab}	125.70^{b}	126.00^{b}	108.00^{ab}	5.67
LDL(mg/dl)	83.08 ^{a}	64.77 ^{c}	70.08 ^{b}	76.33 ^{b}	72.33 ^{b}	5.12
HDL(mg/dl)	28.77	35.00	29.75	23.50	28.00	2.70
Glucose(mg/dl)	167.67^{b} 4.00	186.50^{a}	162.00^{b}	184.50^{a}	170.00^{b}	11.72 0.473
Total P(g/dl)	1.53	4.83	4.07	4.43	4.27	0.269
Albumin(g/dl)	2.47	1.73	1.70	2.07	1.63	0.476
Globulin(g/dl).		3.10	2.37	2.23	2.63	

a,b,c; means in the same row with different superscript are significantly different ($p<0.05$)
T1: Control (No antioxidant), T2: (0.5% Ginger),T3: (0.5% Black pepper),T4: (0.25% Ginger and 0.25% black pepper), and T5: (0.5% Vitamin C) respectively. Keyword: CL: Cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein.

The resultant increase in TAC on test diets could have been responsible for reduced level of MAD which is the end product of lipid peroxidation. This result further explains that lipid peroxidation was at its minimal. Vitamin C in circulation in the animal body was highest in blackpepper sole agent diet followed by ginger sole agent diet. This could imply that ginger and black pepper could be high in vitamin C content.

Table 4: Effect of two sources of dietary antioxidant on total antioxidant capacity, Vitamin C and Malondialdehyde

Parameters	T1	T2	T3	T4	T5	±SEM
TAC(µmol/L)	27.47	36.83	36.13	32.07	40.20	6.3344
MAD(µmol/L)	0.016	0.012	0.008	0.011	0.010	0.0032
Vitamin C(mg/dl)	0.803^{b}	1.130^{ab}	1.160^{a}	1.010^{ab}	0.863^{ab}	0.1003

a,b: means in the same row with different superscript are significantly different ($p<0.05$) T1: Control (No antioxidant), T2: (0.5% Ginger), T3: (0.5% Black pepper),T4: (0.25% Ginger and 0.25% black pepper), and T5: (0.5% Vitamin C) respectively. TAC: Total antioxidant capacity. MAD: Malondialdehyde

V. Conclusion

In conclusion, inclusion of ginger and blackpepper as sole agents may maintain or regulate Total white blood count in the blood. This was observed in the increase of total white blood cell but with decreasing monocyte count and a decrease in Total white blood cell (ginger+blackpepper) but with increasing heterophile count. Mixture of ginger and blackpepper in broiler diet at 0.25% each could affect the Thrombocytes leading to the inability of blood to clot. It was observed that ginger and blackpepper sole agent diets increased High density lipoprotein and reduced low density lipoprotein and triglyceride. Ginger and blackpepper and Vit C sole agents increased TAC in all test treatments compared to control.

Reference

[1] Ademola S.G., Farinu G.O. and BabatundeG.M.(2009). Serum lipid, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. World Journal of Agricultural Science 5(1);99-104

[2] Albritton A.B. (1961). Standard Values in blood. Philadelphia, W.B. Saunders

[3] Altman P.L. and Dittmer P. (1974). Biology Data book, Washington D.C. Fed Am SocExpBiol

[4] Kohn J.(1958). Small-scale membrane Filter electrophoresis and immune-electrophoresis. ClinChimActa 3:450

[5] Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevicand V.Cosic. (2001). Method for the measurement of antioxidant activity in human fluids .JClin Pathol;54:356–361

[6] Lamberg, S, L. Rothestein, R. (1977). Laboratory Manual of hematology and urinanlysis.Avi.publishing company, inc. west povt. Connecticut, USSR.

[7] Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem95: 351-358.

[8] Polycarp, T.N., Obukiwho, E.B and Yussoff, S.M. (2016) Changes in haematological parameters and oxidative stress respose of goats subjected to road transport stress in a hot humid tropical environment. Comp ClinPathol 25:285

[9] Surai P.F. (2002) Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham University Press, Nottingham

[10] Surai P.F., Speake B.K. and Sparks N.H.C. (2003). Comparative Aspects of Lipid Peroxidation and Antioxidant Protection in Avian Semen. In: Male Fertility and Lipid Metabolism, pp. 211-249. [Stephanie DeVriese and Armand Christophe, edotors] Champaign: AOCS Press

[11] www.beautyofbirds.com- retrieved 06/06/2017

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