Effect of diet supplemented with two sources of antioxidants as feed additives on growth indices and internal organs of broiler-chicken

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Abstract

Growth indices and the internal organs of 125 broilers (four weeks of age) were assessed in order to study the effect of Zingiber officinale (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 the Average weight gain (g) was significantly ($P<0.05$) higher in T2 (33.31) compared to the other treatment groups. The Wing length (cm) of T1 (9.58), T3 (10.21) and T5 (9.47) were not significantly different from each other but was significantly ($P<0.05$) higher than T2 (8.68) and T5 (7.16). The breast width (cm), chest length (cm), total feed intake(g), average daily feed intake (g) and feed conversion ratio (g) of the broiler birds were not influenced by the dietary antioxidant feed additives. The weights (g) of the Kidney, Liver, Gizzard, Heart, and Spleen in all treatment groups were not significantly different from each other. Effect on kidney function test reveals the results of potassium (Mmol/L) to be significantly ($P<0.05$) higher (4.27) in T1 compared to the other treatment groups, with the lowest value of potassium content in T5 (2.70). Creatinine (mg/dL) content varied among treatment groups with T4 significantly ($P<0.05$) higher (0.32) compared to other treatment groups. Liver function test showed AST (IU/L) content in T4 (190.33) to be significantly ($P<0.05$) higher compared to other treatment groups. ALP (IU/L) was lowest in T1 (127.67) and T5 (320.00) was significantly ($P<0.05$) higher compared to other treatment groups. Ginger supplemented diet increased feed intake and Vitamin C supplemented diet yielded the best feed conversion ratio.

Keywords: Broiler, Ginger, Black pepper, Vitamin C, Feed, Growth.

Introduction

Growth promoters or feed additives are molecules that are added at low rate to animal feeds without changing considerably their composition (Admad *et al.*, 2006). They speed up the growth and consequently increase the body size and weight of animals (Biovet, 2005). Among all growth promoters, the most commonly used are antibiotics, although their use is decreasing towards total extinction (Biovet, 2005). Some growth promoter's act as pro-nutrients because of the role they play in enhancing the physiological and

microbiological status of the animals. Pro-nutrients are substances that could have the same effect as antibiotic feed additives and are defined as micro ingredients included in the formulation of animal feeds with physiological and microbiological functions different from any other nutrient (Biovet, 2005). Many active ingredients from plants are considered as pro-nutrients and are recently been tried in animal feeds (Biovet, 2005). Pro-nutrients are also sometimes referred to as phytogetic feed additives. Phytogetic feed additives are plant-derived products used in animal

feeding to improve their performance. This class of feed additives has recently gained increasing interest, especially for use in swine and poultry. This appears to be strongly driven by a complete ban on most of the antibiotic feed additives within the European Union in 2006 (Windisch *et al.*, 2008).

Zingiber officinale is a perennial plant, commonly known as ginger. Ginger may act as a pro-nutrient because of the vast active ingredients it has been reported to contain. Herbs Hands Healing (2011) reported that ginger contains a protein digesting enzyme (Zingibain) which is believed to improve digestion as well as kill parasites and their eggs. It was also reported to enhance antibacterial and anti-inflammatory actions.

Another natural antioxidant of interest is the black pepper (*Piper nigrum*). Its role in the male reproductive organ and fertility of mice (Kumar *et al.*, 2008) has been well documented. In connection with reproductive function, black pepper extracts effects on human and animals seem to contradict each other as well (Kpomah *et al.*, 2012). Artificial sources of antioxidant also play help in broilers physiological performance. Vitamin C is one of the nutritional factors important in layering hen performance and egg quality. It is mandatory in maintaining normal body metabolic activities and to meet physiological requirements as well as exhibit anti-oxidative or pro-oxidative effects (Mc Dowell, 1989). Synthetic antioxidants such as butylated hydroxyl toluene and butylated hydroxyl lanisole have been approved to control lipid oxidation in foods, but recently cancer restricts their use (Okada *et al.*, 1990). Therefore, the need to research into an alternative to synthetic antioxidants becomes imperative. Antioxidant inclusion

is shown to be very vital to improve growth performance in commercial poultry. It was known that increase of live weight gain of broilers was as a result of the supplementation of herbal extracts rich in antioxidant (Man war *et al.*, 2005). According to Lin *et al.* (2003), dried black pepper has strong antibacterial, anti-ulcer, analgesic, antioxidant and hypocholesterolaemic activities. With this knowledge of antioxidant effect in the animal body, and paucity of information regarding their influence on broilers, it is imperative to evaluate the effects of these natural sources of antioxidant (Ginger and Black Pepper) on the growth and performance of broiler chicken.

Materials and methods

Experimental animal, location and management

A total of 125 broiler chicks were brooded with coal heat source for 4 weeks 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 4 weeks of age till 9th week of age. The experiment was conducted at the Poultry unit of the University of Benin Teaching and Research Farm, Benin City, Edo State, Nigeria. The farm lies between latitude 6.50°N of the equator and longitude 5.40 and 6°E of Greenwich meridian, with mean annual temperature of 33.54°C. The area has an average annual rain fall and relative humidity of 2000mm and 72.5% respectively (Google earth, 2016). 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 ginger powder and the dried black pepper was milled into pepper powder before being incorporated into the formulated diet. The vitamin C (10 mg) 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 – (*Zingiber officinale*). 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, black pepper 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
T5	8.726	89.273	8.136	10.936	5.823	19.416	41.756

Wing length, breast width and chest circumference measurement

The wing length and breast width was measured once every week from the 5th week till the 9th week. In taking the measurement a measuring tape was used. In taking the wing length the tape was placed at the beginning of the wing and extended to the tip of the wing, in taking the breast width the tape was place from the joint of one wing to the joint of the other wing. In taking the chest circumference the tape was placed round the chest.

Carcass evaluation

At the end of the experiment, fifteen (15) birds were selected. Diet and water were withdrawn from the broiler chickens overnight 12 hours before being slaughtered. A total of 15 birds were slaughtered (i.e. 3 per treatment pen). The

birds were weighed just before slaughtering, and after slaughtering. The carcass was opened and the organs were weighed (heart, spleen, kidney, liver and the gizzard-with and without its content). The carcass was weighed without the internal organs and again after de-feathering with hot water. All values were recorded.

Liver Function Test (LFT)

The serum for LFT was refrigerated at - 20°C for 8 hours. The enzymes AST (Asparate transaminase) ALP (Alkaline phosphate), ALT (Alanine aminotransferease) were analyzed using the Bechman Synchron LX20 system (2001) which uses an enzymatic rate method to measure enzyme activities in serum. The rate of enzymatic activities is monitored in a b s o r b a n c e o f 3 4 0 n m spectrophotometrically. The absorbance is

directly proportional to the enzyme activity in the sample. The Total and conjugated (direct) bilirubin concentrations were analyzed using the modified Jendrassik-Grof (2008) method which uses the DBI Flex® reagent cartridge.

Kidney Function Test (KFT)

The serum biochemical analyzed for kidney function includes: Urea, Sodium, Potassium, Bicarbonate (HCO_3), Creatinine and chlorine. Urea was analyzed by spectrophotometric measurement of diacetylmonoximere action as described by Wybenga *et al.* (1971), Potassium and Sodium by Flame photometric method as described by Valle and Thiers (1965). Bicarbonate (HCO_3) was determined by titrimetric method as described by Segal (1955). Chlorine was determined by Chloridometric method described by Cotlove (1961) and Creatinine by Spectrophotometric determination of Alkaline Creatinine picrate reaction (Jaffe reaction) as described by Taussky (1954).

Statistical analysis

The data obtained was subjected to statistical analysis of variance (ANOVA) procedure of GenStat 17th edition at 5%

probability level and Duncan multiple range test option of the same statistical software was used to separate the treatment means.

Results

The results of the effect of two sources of antioxidant on the growth indices of broiler chickens are as shown in Table 2. Weight gain of birds on T2 (2099.01g) was significantly ($p < 0.05$) higher than those in other, treatment group. The values for weight gain ranged from 1810.05g in the control to 2099.01g in T2. The values for feed intake ranged from 57.46g in the Control to 63.96g in T2. Vitamin C diet had the best feed conversion ratio of 1.87. The values ranged from 1.87 in control to 2.00 in T4. The values obtained from the breast width (cm) ranged from 8.85 in T5 to 10.15 in the T2. The Wing length (cm) of T1 (9.58), T3 (10.21) and T5 (9.47) were not significantly different from each other but was significantly ($P < 0.05$) higher than T2 (8.68) and T5 (7.16). Values for chest circumference (cm) ranged from 8.15 in T2 to 10.10 in the control.

Table 2: Effect of two sources of dietary antioxidant on growth indices of broiler chickens

Parameters	T1	T2	T3	T4	T5	±SEM
Initial wt(g)	45.13	45.01	45.50	45.13	42.04	1.83
Final wt(g)	1855.18	2144.02	2025.61	1990.29	2027.17	9.85
Wt gain(g)	1810.05 ^c	2099.01 ^a	1980.11 ^b	1945.16 ^b	1985.13 ^b	9.73
AWG(g)	28.73 ^c	33.31 ^a	31.43 ^b	31.51 ^b	31.51 ^b	0.086
Breast wdt (cm)	9.15	10.15	9.20	9.35	8.85	0.760
Wing Lth (cm)	9.58 ^a	8.68 ^{ab}	10.21 ^a	7.16 ^b	9.47 ^a	0.689
Chest cm	10.10	8.15	9.40	8.68	9.95	0.911
TFI (g)	3620.11	4030.09	3793.06	3841.41	3731.55	9.75
ADFL (g)	57.46	63.96	60.20	60.97	59.23	0.11
FCR	2.00	1.92	1.92	2.00	1.87	0.468

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).

Keywords Wt: weight; AWG: Average weight gain. Wdt: width (cm); Lth: length (cm); cm: Circumference (cm); ADFL: Average daily feed intake (g) TFI: Total feed intake (g); FCR: Feed conversion ratio

The results of the effect of two dietary sources of antioxidant on the organ weight of broiler chickens are shown in Table 3. The Kidney weight ranged from 0.55g in T2 to 0.65g in T4. The weight of the liver varied from 2.29g in the control diet to 2.83g in T3. The weight of the gizzard with its content ranged from 2.22g T5 to 2.82g in

T3. The weight of the gizzard without its content ranged from 1.74g T5 to 2.01g in T1. The weight of the heart varied from 0.40g in T2 to 0.51g in T3. The weight of the spleen ranged from 0.29 g in T1 to 0.20 g in T5. The weights (g) of the Kidney, Liver, Gizzard, Heart, and Spleen in all treatment groups were not significantly different from each other.

Table 3: Effect of two dietary sources of antioxidant on the organ weight of broiler chickens

Parameters(g)	T1	T2	T3	T4	T5	±SEM
Kidney	0.60	0.55	0.62	0.65	0.63	0.6975
Liver	2.29	2.73	2.83	2.50	2.58	0.4459
Gizzard wc	2.75	2.73	2.82	2.65	2.75	0.5054
Gizzard woc	2.01	1.84	1.92	1.77	1.74	0.415
Heart	0.45	0.40	0.51	0.48	0.41	0.0833
Spleen	0.29	0.21	0.28	0.24	0.20	0.0473

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); WC: with content, WOC: without content.

The result of the effect of two sources of dietary antioxidant on kidney function test is as shown in Table 4. The urea content ranged from 5.57 mg/dl in the Control to 6.67 mg/dl in T4. The sodium content varied from 142.67mmol/L in the control to 145.00mmol/L in T2. The value recorded for HCO₃ Mmol/L ranged from 21.67 T3 to 27.67 in T4. The chlorine Mmol/L content varied from 105.33 in T5 to 109.67 in T1.

The value obtained for glucose ranged from 162.00mmol/L in T3 to 186.50 Mmol/L in T2. The results of potassium (Mmol/L) was significantly (P<0.05) higher (4.27) in T1 compared to the other treatment groups, with the lowest value of potassium content in T5 (2.70). Creatinine (mg/dl) content varied among treatment groups with T4 significantly (P<0.05) higher (0.32) compared to other treatment groups.

Table 4: Effect of two sources of dietary antioxidant on kidney function test

Parameters	T1	T2	T3	T4	T5	±SEM
Urea(mg/dl)	5.57	6.67	6.67	6.00	6.00	0.730
Sodium (mmol/L)	142.67	145.00	144.67	143.33	144.67	1.814
Potassium (mmol/L)	4.27 ^a	3.80 ^{ab}	3.37 ^{ab}	3.10 ^b	2.70 ^b	0.344
HCO ₃ (mmol/L)	23.33	22.67	21.67	27.67	27.00	2.599
Chlorine (mmol/L)	109.67	108.67	108.67	106.33	105.33	3.451
Creatinine (mg/dl)	0.27 ^b	0.28 ^{ab}	0.27 ^b	0.32 ^a	0.29 ^{ab}	0.013

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), HCO₃ (Bicarbonate). The results on the effect of two dietary sources of antioxidant on Liver

function test are shown in Table 5. Total bilirubin ranged from 0.27mg/dl in T4 to 0.37mg/dl in the control. Conjugated bilirubin varied from 0.13mg/dl in T4 to 0.20mg/dl in the Control. The AST (IU/L) values in T2 and T4 was 174.67 and 190.33

Feed additives on growth indices and internal organs of broiler-chicken

respectively and both were significantly ($P<0.05$) higher than T4. ALP (IU/L) was

lowest in T1 (127.67) and T5 (320.00) was significantly ($P<0.05$) higher compared to other treatment groups.

Table 5: Effect of two sources of dietary antioxidant on liver function test

Parameters	T1	T2	T3	T4	T5	±SEM
Total Bilirubin(mg/dl)	0.37	0.37	0.33	0.27	0.33	0.033
Conjugated Bilirubin (mg/dl)	0.20	0.17	0.17	0.13	0.17	0.030
AST(IU/L)	148.33 ^c	174.67 ^b	136.7 ^c	190.33 ^a	140.67 ^c	4.310
ALT(IU/L)	91.67	75.00	72.33	94.33	89.00	7.262
ALP(IU/L)	127.67 ^c	190.67 ^b	210.67 ^b	297.00 ^b	320.00 ^a	18.159

a,b,c; means in the same row with different superscript are significantly different ($p<0.05$) ALP= Alkaline phosphate, ALT= Alanine amino transferase, AST= Aspartate transaminase.

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)

Discussion

The weight gain of broilers was significantly increased by addition of natural (ginger and black pepper) and synthetic (Vitamin C) sources of antioxidant feed additives. There were tendencies for broilers fed these sources of antioxidants to grow faster than control as numerically higher values of wing length were observed in T3 when compared with the control. All treatments recorded higher values of breast width as compared to Control. T2 had a higher chest circumference and ADFI. Diets with additives recorded higher values for live weight as compared with the Control. T2 and T3 had higher values of bled weight as compared to the Control. These numerical increases observed could be due to the fact that herbal plants (ginger and black pepper) and vitamin C may provide some compounds that help increase digestion and absorption of some nutrients in these experimental diets and also aiding in antibacterial activities which may lead to improved growth of the birds (El-tazi *et al.*, 2014; Hosseini, 2011). Galib *et al.* (2011)

reported that the addition of black pepper in a high activity of piperazine citrate which can affect the flow of digestive juices across the stomach. Also, sometimes addition of this antioxidant could result in reduction in total feed intake as observed with T2 and T3. This may be due to their taste hence reducing feed intake (Hosseini, 2011). Differences were not observed in the organ yield (kidney, liver, gizzard with content, gizzard without content, heart and spleen) of the treatment groups when compared with the control. These results were in accordance with those reported by Al-Kassie *et al.* (2011) and El-tazi *et al.* (2014) where addition of different levels of black pepper had no influence in the edible giblets (liver, heart and gizzard).

Aspartate amino transferase (AST) content in birds on Ginger diet and ginger/black pepper diet were significantly increased compared to the control. Vit. C diet, Ginger diet and black pepper diet recorded lower values of alanine aminotransferase (ALT) when compared with the control. A similar situation was observed by Emadi and Kermanshahi (2007). Fernandez *et al.*,

(1994) induced liver damage using aflatoxin in broilers and layers and observed an increase in serum ALT. Therefore, the reduction in ALT of Vit C, ginger and ginger/black pepper diet can be an indication of better liver function.

Effect on kidney function test reveals the results of serum potassium to be significantly higher (4.27) in T1 compared to the other treatment groups, with the lowest value of potassium content in T5 (2.70). Creatinine content varied among treatment groups with T4 significantly higher (0.32) compared to other treatment groups. Potassium is an essential electrolyte, which is a mineral that the body needs to function correctly. This mineral is particularly important for the nerves and muscles. All muscles need potassium, including the heart. Healthy kidneys flush excess potassium out of the body. In extreme cases, high potassium can cause paralysis and heart problems (<http://www.healthline.com> -retrieved 15/5/2017). The level of Serum Potassium in control group fell within the normal range ($4.60-6.50 \pm 0.41$ Mmol/l) in Chicken as described by Albritton (1952) but was influenced in treatment groups with additives. The reduction in potassium level could affect muscle tone and cause fatigue to the animals (<http://www.healthline.com> -retrieved 15/5/2017), if this be the case, diet supplemented with feed material like soy bean rich in potassium may be added to boost potassium level for the proper functioning of the cells, muscles and the heart. The creatinine levels across treatment groups were lower than the normal range ($0.90-1.85 \pm 0.3$ mg/dl) for normal chicken as described by Albritton (1952). These low levels could signify that the kidney functioned properly in carrying away waste like creatinine out of the body, it could also indicate low muscle mass as

may be caused by low serum potassium and as a result of plant-based diet (www.livestrong.com).

Conclusion

In conclusion, it can be ascertained that ginger and black pepper naturally can be used as growth promoters instead of artificial growth promoters since, increase in the live body weight and feed efficiency was observed. This study goes further to prove that they are better alternative compared to the synthetic growth promoters as there was no observed growth retardation by the ginger and black pepper. It can also be insinuated that ginger or black pepper containing diets respectively improved kidney function by clearing out creatinine in the blood. The low creatinine level reflects low creatine which is normally high in animal protein and when eaten by humans tend to increase creatinine level in their blood system. This reflects that birds fed with diets containing ginger or black pepper will be healthy for human consumption.

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