

Growth response, hematological indices, organ and carcass characteristics of finisher broilers supplemented with blended African black pepper (*Piper guineense*)

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Abstract

This experiment was designed to investigate the effect of blended African Black pepper (ABP) seed on growth response, carcass characteristics and haematological performance of finishing broilers. Sixty day-old chicks of Cobb 500 strain were used for this study which lasted for four weeks. The day old chicks were procured from Ibadan Nigeria. The chicks on arrival were brooded for four weeks using normal starter feed. At four weeks the chicks were randomly assigned to three treatment diets with four replicates in a completely randomized design (CRD). African black pepper seed were purchased from Ogige market in Nsukka town. The finisher diets were supplemented with African black pepper at 0g/kg, 1g/kg and 2g/kg for T₁, T₂ and T₃ respectively. Water and feed were given *ad libitum* to the birds. The result obtained showed that there were significant differences ($p < 0.05$) across the treatment means. In growth response, there were significant weight gain, improved feed conversion ratio and reduced feed cost per/kg weight gain in treatment groups as against the control group. In Organ and Carcass characteristics, there were significant ($p < 0.05$) differences on the heart weight, shank weight and wing weight of the treatment groups while in haematological indices, all the parameters were not significant except in Packed Cell Volume (PCV). The value of $37.0 \pm 0.58\%$ obtained from T₃ was significantly higher the value of $32.0 \pm 0.58\%$ recorded for T₁. In conclusion, the results have revealed that blended ABP seed can be incorporate into the diets of broiler chickens at 1g/kg for improve growth response, feed conversion efficiency and lower feed cost per kg weight gain.

Keywords: Growth; Heamatology; Carcass; Broiler; African black pepper

1. Introduction

Poultry farming is a rapidly growing sector within animal production and is widely regarded as an effective solution to protein deficiency. This growth is attributed to several factors, including its short production cycle, high productivity, and excellent feed conversion efficiency. Poultry farming requires relatively low capital investment while delivering substantial returns compared to other livestock production systems. The industry significantly contributes to improved nutrition and food security, accounting for approximately 9-10% of GDP (FAO, 2016).

The consumption of poultry products has seen a substantial increase, driven by rapid population growth. However, this demand has not been matched by a corresponding rise in poultry production, leading to a pronounced protein deficit (Dogara et al., 2021). As a result, the prices of conventional feed ingredients have escalated beyond the affordability of the average Nigerian, primarily due to intense competition between human consumption and industrial usage (Abdulrahman *et al.*, 2022). This situation has adversely impacted on poultry production and profitability.

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Consequently, these challenges have created supply bottlenecks, leading to a significant rise in the cost of poultry products in Nigeria.

The use of medicinal plant extracts in animal nutrition has demonstrated several beneficial effects, including immune system activation, enhanced feed conversion efficiency, increased feed intake and appetite, stimulation of endogenous digestive enzyme secretion, and antioxidant properties (Egbeyale et al., 2021). Nigeria is endowed with a wide variety of browse plants and herbs that can be utilized as excellent natural alternatives to synthetic antibiotics or growth promoters in animal production.

African black pepper (*Piper guineense*), a member of the Piperaceae family, is a West African spice commonly referred to as Ashanti pepper (Katzner et al., 2015). In Nigeria, it is known as *Uziza* in Igbo and *Iyere* in Yoruba, while other common names include Benin pepper, Guinea pepper, and false cubeb (Katzner et al., 2015). There are over 700 species of this plant distributed across tropical and subtropical regions worldwide (Anyanwu et al., 2014).

P. guineense is valued not only as a spice but also for its medicinal properties. Traditionally, it has been used to treat various ailments. It is a rich source of nutrients and bioactive compounds such as flavonoids, tannins, piperine, and vitamins, which contribute to its aroma, flavor, and preservative qualities (Nwankwo et al., 2014). Its phytochemicals possess numerous therapeutic benefits, including antioxidant, anti-inflammatory, anti-tumor, anti-allergic, anti-carcinogenic, anti-malarial, anti-hypertensive, and antiplatelet properties (Okoye et al., 2013). Additionally, the plant is sometimes processed and consumed as a vegetable (Okigbo et al., 2007). Given its numerous benefits to animal health, this study aims to explore the potential of African black pepper as a natural feed supplement in broiler poultry production.

2. Materials and methods

2.1. Location of the experimental study

The study was carried out at the poultry unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka is situated within the equatorial rainforest belt of the tropics and falls specifically within the savannah vegetation zone. Nsukka town lies on latitude 6° 24 North and longitude 7° 24 East and has an annual rainfall ranging from 1680mm-1700mm (Breinholt et al., 1981).

2.2. Duration of the experimental study

The experiment lasted for a period of 9 weeks.

2.3. Collection and preparation of materials

The African black pepper was purchased at Ogige market. They were milled and incorporated in the finisher feed in its powdered form.

2.4. Experimental diets

The birds were fed commercial broiler feed from 0 to 4 weeks and the commercial finisher feed from 5 to 9 weeks. The finisher feed was supplemented with the milled African black pepper.

2.5. Experimental birds/ management

A total of 54 day - old broiler chicks were used for this study. Three days before the arrival of the chicks, the pens were washed thoroughly and disinfected. Wood shavings were spread on the floor as absorbent material. The feeders and drinkers were thoroughly washed. Provision for clean water and feeds were made. Charcoal stoves and solar powered lanterns were used as sources of heat and light especially during brooding. Normal vaccinations schedules were strictly followed.

2.6. Experimental treatments

A total of 54 birds were used for the experiment. The birds were randomly divided into three (3) treatment groups and replicated three times with six birds per treatment in a completely randomized design (CRD). The treatments were administered at the finishing stage. The table below shows the amount of African black pepper (ABP) as supplemented in the various treatment groups:

2.7. Parameters to be measured

The following Growth parameters were measured

- Weekly body weight: The weights of the birds were taken on a weekly basis with the aid of a top weighing balance of at least 10kg.
- Weight gain: This was calculated by deducing the difference between the initial and final weights of the broiler broilers from different treatment groups.
- Daily feed intake: This was obtained from the difference between the quantity of feed offered and that of the leftover from the previous day divided by the number of birds per treatment.
- Feed conservation ratio: This was calculated as the ratio of feed intake per bird to that of weight gain per bird.
- Mortality percentage: This was obtained as the number of dead birds in each treatment divided by the initial stock per treatment multiplied by 100.

2.7.1. Linear Body Measurement

The following linear body measurements of the birds were taken and recorded; Shank length, Breast Length, Chest girth, Body length, Head length and kilt length.

2.7.2. Carcass Analysis

At the end of the 9th week, three birds per treatment were randomly selected for carcass analysis. The birds were starved 12 hours ample supply of drinking water prior to slaughter. Each bird was weighed separately, slaughtered and allowed to bleed to death. The slaughtered birds were scalded in hot water for few minutes and feathers were plucked manually and carefully to avoid tearing of the skin. The dressed weight was recorded. The carcass was eviscerated carefully and cut into various parts such as head, shank, thigh, wing, breast, drumsticks and abdominal content and the gastrointestinal tract. Each part was weighed and recorded.

2.7.3. Haematological Indices

At the end of the finisher phase, two birds were randomly selected from each replicate for blood analysis. The blood samples were collected from the wing veins.

Blood Sample Collection: 5ml of blood was collected from the wing veins of the broilers using a small syringe with an anti-coagulant called EDTA

Haematological Determination: Different blood parameters were analyzed at the end of the study. This included the counts of red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), and haemoglobin concentration (Hb) using the method as described by Baker and Silverton, 1985.

2.7.4. Total White Blood Cell Count

The blood sample was mixed properly and then drawn up to a certain mark in a pipette. It was diluted with a specific solution and stained. The white blood cells were counted under a microscope in a known area. Based on the count, the total number of white blood cells per cubic millimeter was calculated.

Since the original dilution of the blood was 1:200,

The number of white cells per cubic millimeter is given by $\frac{n \times 200}{9}$

2.7.5. Packed Cell Volume (PCV)

Blood was collected in a capillary tube and then centrifuged to pack the red blood cells. The height of the red blood cell column was measured and compared to the total blood column. The result of PCV was calculated and expressed as a percentage.

$$\%PCV = \frac{\text{Height of red cell column}}{\text{Height of total blood column}}$$

2.7.6. Haemoglobin Concentration (Hb)

A specific tube was filled with a solution and a small amount of blood. After a few minutes, distilled water was added until the color matched a standard. The amount of solution in the tube was measured, and the Hb concentration was calculated thus;

Calculation: 100% on Sahli scale in gHb/100 ml is 14g

If X is Hb for 100% reading and

Y is the % reading obtained,

Then, Hb in sample tested is $= \frac{X(Y)}{100}$

Red Blood Cell (RBC) Count: To count the number of red blood cells, a clean counting chamber and cover glass were used. Formalin was mixed with trisodium citrate solution to create a diluent. A small amount of blood was diluted with the diluent and loaded into the haemocytometer. After allowing the cells to settle, they were counted in a specific area of the chamber. The chamber was placed on a microscope stage and using X40 objective, the cells lying in 5 of the 0.04 millimeter cube area was counted.

$$\text{Red Blood Cell Count} = \frac{N \times DF \times 106/\text{Litre}}{A \times D}$$

Where

N = no of cell countered

DF = Dilution factor =201

A = Area = 0.2 Millimeter cube

D = Depth = 0.1 Millimeter cube

2.7.7. Experiment design

The experiment was carried out using Completely Randomized Design (CRD) with the statistical model as follows;

$$X_{ij} = \mu + T_i + \sum_{ij}$$

Where X_{ij} = individual observation

μ = population mean

T_i = effect of treatment

\sum_{ij} = experimental error

2.8. Statistical analysis.

All data collected were subjected to statistical analysis of variance (ANOVA) in a completely Randomized Design using Statistical Product and Service Solution (SPSS, 2017). Various means were separated using Duncan Multiple Range Test Procedure as found in the statistical package/software (Duncan, 1955).

3. Results

Table 1: The result of the growth parameters of broiler birds fed varying levels of African black pepper was shown in table 1 above. There was significant difference ($p < 0.05$) on weight gain, daily weight gain, feed conversion ratio, feed cost (₦), feed cost/kg weight gain and specific growth rate. In weight gain of the broiler birds, T_2 had the highest weight gain which was significantly ($p < 0.05$) higher than T_1 and T_3 which were themselves similar. In feed conversion ratio, T_1 (control) had the highest value of 3.75 which was significantly higher than T_3 (2.0g) while T_2 had the lowest value of

2.70 which was significantly lower than other treatment means. In feed cost/kg, T₁ had the highest feed cost/kg weight gain of 1650 ± 5.77 which was significantly ($p < 0.05$) higher than the value of 1478.40 ± 0.23 recorded for T₃ while T₂ had the lowest of 1242 ± 1.16 which was significantly higher than the other treatment groups. However, in SGR, T₂ had the value of 3.01 which was significantly ($p < 0.05$) higher than the other treatment means while T₁ had the lowest value of 2.68 which was significantly lower than the other treatment means. There was no significant difference ($p > 0.05$) in initial body weight, final body weight, feed intake, daily feed intake and feed efficiency ratio.

Table 1 The Growth Performance of broiler birds fed varying levels of African Black Pepper

Parameters	T ₁ (control)	T ₂ (1g) ABP	T ₃ (2g) ABP	P Value
Final weight	2.71 ± 0.32	2.95 ± 0.14	2.72 ± 0.08	0.77 ^{NS}
Weight gain	1.43 ± 0.30^b	1.67 ± 0.17^a	1.52 ± 0.07^b	0.00**
Daily weight gain	51.15 ± 5.73^b	59.87 ± 5.36^a	52.33 ± 1.03^b	0.00**
Feed Intake	4.23 ± 0.06	4.24 ± 0.06	4.09 ± 0.14	0.53 ^{NS}
Daily Feed Intake	151.20 ± 2.37	151.43 ± 2.11	146.33 ± 5.05	0.53 ^{NS}
FCR	3.75 ± 0.19	2.70 ± 0.47	3.08 ± 0.43	0.66 ^{NS}
Feed cost/kg	440 ± 1.16^c	460 ± 2.89^b	480 ± 1.15^a	0.00**
Specific growth rate	2.68 ± 0.01^c	3.01 ± 0.02^a	2.78 ± 0.01^b	0.00**
Feed cost/kg weight gain (₦)	$1,650 \pm 5.77^a$	$1,242 \pm 1.15^c$	1478.40 ± 0.23^b	0.01**

abc Row means with superscript are significantly different ($p < 0.05$). NS means. *= significant; **= highly significant; Not Significant NS = Not significant ($p > 0.05$); FCR = Feed Conversion Ratio

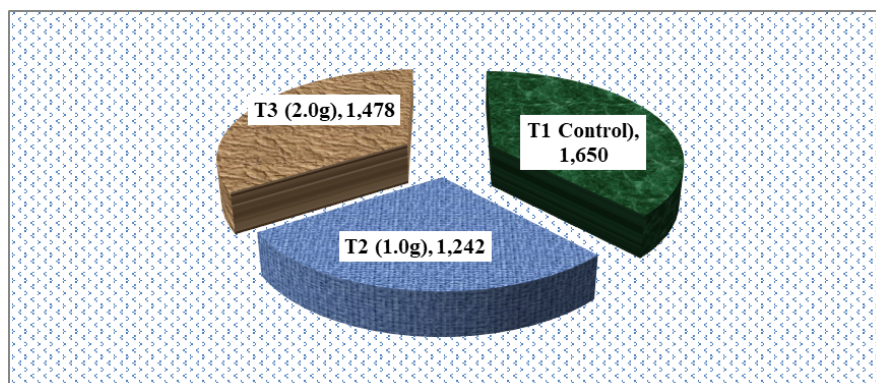


Figure 1 The feed cost/kg weight of the broiler chickens fed diets containing varying levels of African Black pepper (ABP). From the chart T₁ (control) group had the highest feed cost/weight gain of ₦1,650/kg while T₂ had the lowest value of ₦1,242/kg weight gain.

Table 2 The Linear Body Measurement of broiler birds fed varying levels of African Black Pepper

PARAMETERS	T ₁ (control)	T ₂ (1g) ABP	T ₃ (2g) ABP	P Value
SL	5.36 ± 0.25^b	6.67 ± 0.19^a	6.17 ± 0.17^a	0.01**
BL	31.18 ± 0.21	31.87 ± 0.09	31.76 ± 0.26	0.10 ^{NS}
HL	5.2 ± 0.00^b	5.03 ± 0.07^b	5.63 ± 0.03^a	0.00**
BG	7.60 ± 0.08^b	7.80 ± 0.12^b	8.95 ± 0.14^a	0.00**
WL	16.70 ± 0.06^b	19.20 ± 0.40^a	19.10 ± 0.69^a	0.02*
KL	19.08 ± 0.07	18.65 ± 0.26	18.30 ± 0.17	0.06 ^{NS}

^{ab}Mean values in the same row with different superscripts differ significantly ($p < 0.05$). NS means Not significant ($p > 0.05$) SL: Shank length; BL: Breast length; HL: Head length; WL: Wing length; BG : Breast girth; KL: Keel length.

Table 2: The Result of the linear body weight of broiler birds fed varying levels of African black pepper was shown in table 2 above. The shank length, head length, breast girth, and wing length were significantly ($p < 0.05$) affected by the treatment. In shank length, T₂ and T₃ were themselves similar but significantly ($p < 0.05$) higher than T₁ (control). In head length, T₃ was significantly higher than T₁ (control) while T₂ had the value of 5.02cm which was significantly lower than the other treatment means. However, in breast girth, T₃ was significantly higher than T₂ while T₁ was significantly lower than the other treatment means. In wing length, T₂ and T₃ had the highest wing length of 19.20cm and 19.10cm respectively which were themselves similar ($p > 0.05$) but significantly ($p < 0.05$) different from the value of 16.70cm recorded for T₁ (control group).

Table 3 Carcass Characteristics of broiler birds fed varying levels of African Black Pepper

Parameters	T1(control)	T2(1g) ABP	T3(2g) ABP	P Value
Live Weight(kg)	2.71±0.46	2.95±0.29	2.75±0.03	0.86 ^{NS}
Dressed Weight (kg)	2.11 ± 0.16	2.38 ± 0.38	2.15 ± 0.58	0.74 ^{NS}
Dressed %	77.86 ± 0.57 ^b	80.68 ± 0.54 ^a	78.18 ± 0.57 ^b	0.02*
Liver weight (g)	68.67 ± 7.79	63.67 ± 6.07	52.67 ± 5.40	0.43 ^{NS}
Heart weight (g)	10.0 ± 1.73 ^b	9.66 ± 0.88 ^b	16.0 ± 0.58 ^a	0.02*
Spleen (g)	5.33 ± 0.88	5.0 ± 0.58	4.67 ± 0.33	0.77 ^{NS}
Gizzard (g)	56.0 ± 1.39	51.0 ± 1.16	42.0 ± 1.04	0.36 ^{NS}
Shank (g)	43.67 ± 1.04 ^c	69.33± 0.33 ^a	54.0 ± 1.51 ^b	0.03*
Head (g)	53.67 ± 2.48	55.0 ± 2.60	54.67 ± 1.76	0.97 ^{NS}
Breast (kg)	0.62 ± 0.90	0.69 ± 0.89	6.73 ± 0.02	0.76 ^{NS}
High (g)	201 ± 6.90	212 ± 2.02	211 ± 0.88	0.17 ^{NS}
Wing (g)	245 ± 4.14 ^a	225 ± 3.56 ^b	233.0 ± 2.88 ^b	0.03*
Drum Stick(g)	200 ± 7.50	195 ± 7.89	182 ± 6.83	0.06 ^{NS}
S. Intestine (g)	171.67 ± 0.49	168 ± 1.15	167.0 ± 0.28	0.38 ^{NS}
L. Intestine (g)	24.0 ± 1.40	25.33 ± 1.17	23.0± 2.3	0.88 ^{NS}

^{a,b,c} Row means with superscript are significantly different ($p < 0.05$). NS means Not Significant. *= significant;

Table 3: The result of the carcass characteristics of broiler birds fed varying levels of African black pepper was shown in table 3 above. There was significant difference ($p < 0.05$) on the dressed percentage, heart weight, shanks and wing weights. In dressed percentage, T₂ (80.68%) was significantly higher than T₃ (78.18%) while T₁ had the value of 77.86% which was significantly ($p > 0.05$) lower than other treatment means. In heart weight, T₃ had the highest weight of 16.0g which was significantly better than T₂ and T₁ which were themselves similar. In shank weight, T₂ had the highest weight of 69.33g which was significantly higher than the value of T₃ (54.0g) while T₁ had the value of 43.67g which was significantly lower than the other treatment means. However, in wing weight, T₁ had the value of 245g which was significantly higher than T₃ while T₂ had the value of 225g which was significantly lower than the other treatment means.

Table 4: The result of the haematological indices of broiler birds fed varying levels of African black pepper was presented in table 4 above. In packed cell volume (PCV), the birds in T₃ (fed 2g ABP) and T₂ (1g ABP) had the highest values of 36.0% and 35% respectively which were themselves similar but significantly different from the value T₁ (32%) significantly different from the value of 32% PCV and T₁ had the least values. There was no significant different on the hemoglobin, red blood cell, white blood cell, monocytes, basophiles, lymphocytes, eosinophil and neutrophils.

Table 4 Haematological parameters of broiler birds fed varying levels of African Black Pepper

Parameters	T ₁ (control)	T ₂ (1g) ABP	T ₃ (2g) ABP	P Value
PCV (%)	32.0 ± 0.58 ^b	35.0 ± 1.15 ^a	36.0 ± 0.58 ^a	0.01**
Haemoglobin (g/dL)	10.0 ± 0.40	10.85 ± 0.03	10.85 ± 0.03	0.23 ^{NS}
RBC (x10 ⁶)	7.0 ± 0.23	10.0 ± 0.16	10.0 ± 0.08	0.54 ^{NS}
WBC (x10mm ³)	86.0 ± 5.47	87.50 ± 6.60	81.0 ± 4.15	0.06 ^{NS}
Neutrophil (%)	21.50 ± 1.44	22.0 ± 1.15	19.50 ± 0.29	0.29 ^{NS}
Lymphocyte (%)	75.0 ± 2.89	75.50 ± 0.29	77.50 ± 0.29	0.57 ^{NS}
Monocyte (%)	2.0 ± 0.87	1.5 ± 0.87	1.5 ± 0.29	0.56 ^{NS}
Eosinophil (%)	0.5 ± 0.29	1.0 ± 0.58	1.0 ± 0.58	0.59 ^{NS}
Basophil (%)	0.50 ± 0.28	0.00 ± 0.00	0.50 ± 0.29	0.29 ^{NS}

^{ab}Mean values in the same row with different superscripts differ significantly ($p < 0.05$). PCV: Key: Packed cell volume; RBC: Red blood cell; WBC: White blood cell. NS means Not Significant. **= highly significant

4. Discussion

4.1. Growth Performance

The growth performance of the broilers showed significant difference ($p > 0.05$) across the different treatments. This is an indication that African black pepper significantly influenced the growth performance of the broiler birds. This work is in total agreement with the work of (Galib *et al.*, 2011; Ndelekwute *et al.*, 2015; Ufele *et al.*, 2020) who reported significant improvement in growth parameters of chickens fed blended black pepper seeds. However, this work disagrees with the findings of (Al- Kassie *et al.*, 2011; Akbarian *et al.*, 2012), who reported no statistical ($p > 0.05$) differences in the growth performance of birds fed diets supplemented with different types, concentrations, or combinations of plant extracts. This may be related to the environmental conditions of the experiment, the inclusion level or dosage, the age of the animals, the processing method used, the active ingredients and its concentrations and the biological activity of the black pepper.

Conversely, this work disagreed with the work of Sugiharto *et al.* (2020) who did not observe any significant difference ($p > 0.05$) in weight gain when African black pepper was fed to broiler birds at varying levels but rather had marginal increase in weight gain of the treatment groups over the control group. However, this work is in consonant with the earlier work of Mansoub (2011) who reported significant increase in the body weight gain of broilers fed African black pepper and turmeric at varying inclusions. This was attributed to the components in African black pepper known as piperine, which promotes pancreatic digestive enzymes such as lipase, amylase and proteases and which plays an important role in the digestion process (Shijie *et al.*, 2024). African black pepper equally affects the absorption power of nutrients in food, decreases material transit velocity and increases digestive enzymes. Ogbuewu and Mbajorgu, (2023) also reported that African black pepper may have increased digestion through arousal of the digestive liquids in the stomach and the eradication of infectious bacteria. Thus, this present work in harmony with previous findings of Al-Kassieet *et al.*, 2011 and Valiollahi *et al.* (2013) Abou-Elkhair *et al.* (2014) reported that the addition of a mixture of African black pepper and turmeric powder to broiler diets led daily to body weight gain of broilers during the fattening period of 35 days.

Thus, birds on T₂ performed significantly ($p > 0.05$) better than the birds on T₃ and T₁ (control) diets. This finding is in agreement with work of Abdelrahim *et al.* (2018); Ogwuegbu *et al.* (2021) who reported that spices when incorporated into the diets of broilers at right proportion, results in improved health, better nutrient absorption, increased growth rates as well as improved feed conversion ratio. The reduction in live weight at 2.0% level of the black pepper signifies that at a higher-level the beneficial effects of African black pepper could be retrogressing (Ndelekwute *et al.*, 2015). The negative effect of black pepper at higher levels could be due to the presence of anti-nutritional factors such as terpenoids, tannins and cyanides contained in African black pepper (Ogbuewu and Mbajorgu, 2023) which results to poor growth at high level (Abaza *et al.* 2008).

4.2. Haematological Profile of the broiler birds

The haematological indices of the broiler chickens showed that there were no microbial infections or presence of foreign bodies or parasites in the circulatory system of the experimental birds which implies that the inclusion of African black pepper in the diets of broilers did not negatively impact on the immune system of the broilers. This finding corroborates with the work of Aikpitanyi and Egweh (2020) for broiler chickens fed diets containing ginger and African black pepper respectively. Jiwuba *et al.* (2017) noted that the function of white blood cell is to fight infections, defend the body through phagocytosis against invasion by foreign organisms and produce or transport antibodies and to improve the immune response of an animal. The normal physiological values of WBC obtained in this study may suggest a well-developed immune system of the broilers. However, the earlier work of Shahverdi *et al.* (2013) who reported that chickens fed with black pepper-based diets had significantly lower Hb, PCV, RBC, lymphocyte (L) relative to the control group contradicts the findings from this work. They increased at increasing level of ABP inclusions which is in accordance with the work of Adejumo (2004) who reported that Packed Cell Volume and haemoglobin concentration were positively correlated with the nutritional status of animal and agrees with Uhegbu *et al.*, 2015 that reported that African black pepper significantly increased the haemoglobin level and red blood cell counts of the animals. The higher level of haemoglobin and PCV observed in this study also agreed with the findings of (Tijani *et al.*, 2015). According to Isaac *et al.*, (2013) packed cell volume is involved in the transport of oxygen and absorbed nutrients. Report had it that PCV readily indicates an increase in the number of Red blood cells or reduction in circulatory plasma volume (Chineke *et al.*, 2006). Plant seeds are rich in phytochemicals, vitamins and minerals, hence, may be the cause of increased production of haemoglobin concentration and red blood cells in the animals (Juliani *et al.*, 2013).

5. Conclusions

The work has revealed the beneficial effects of using ABP as phytogenic feed additives on growth performance, feed conversion ratio and cost implications of raising broiler chickens. As observed in this study, ABP had positive effects on growth performance, some carcass traits and haematological indices of broiler birds. This affirms the scientific opinions that natural additive holds potential in improving the overall performance of broiler chickens.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest to be disclosed.

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