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Alternanthera Brasiliana: an Organic Growth Promoter in Poultry

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Abstract

The possible application of aqueous and ethanol leaf-extracts of *Alternanthera brasiliana* as poultry growth promoters were investigated. Standard methods were used to study the vitamin, mineral and phytochemical contents of the leaves. Seventy-two broilers were grouped into nine of eight birds per treatment. Carcass yield, meat qualities and blood components evaluations were done using standard methods. Results revealed the presence of water-soluble vitamins (B_1, B_2, B_3, B_6, B_{12}, C), fat soluble vitamins (A, E, D), minerals and phytochemicals necessary for improving broiler performance. The carcass yield and meat qualities of the test broilers were better than the negative control group. Also, the blood proteins and hematological conditions of the chickens were improved by the extracts better than the negative control group. Communality studies showed that all the blood parameters evaluated were affected by the extracts administered to the broilers. It can therefore, be inferred from the results that leaf-extracts of *A. brasiliana* could replace chemical growth promoters in poultry production, with the aqueous extracts as a preferred choice, having produced better effects than the ethanol counterpart.

1. Introduction

Poultry production has grown to become one of the most important money-making industries in the world. The growing human population has led to a direct proportional increase in the demands for poultry products (FAO, 2002). This advantage witnessed in poultry over other livestock is principally due to the high quality of protein products gotten from it (Adeyemo et al., 2010).

In livestock production, especially poultry, the source of growth promoters fed to the animals is an essential determinant of the quality of the products to be consumed by the human populace (Olarotimi, 2018). For years, antibiotics have been the major growth promoters utilized in poultry production. Its inclusion in poultry diets was remarkable for increasing feed efficiency and growth performance of broilers; reducing morbidity and mortality through combating pathogens in the gastro-intestinal tract (Ronquillo and Hernandez, 2016; Costa et al., 2017; Mehdi et al., 2018).

The overreaching effects of antibiotics in poultry production cannot be overemphasized. Soon after its introduction, the justification for this decision was questioned in many countries across Europe and Asia. It has been stated that the non-therapeutic use of antibiotics in livestock production poses high risk, causing the development of resistance in the broilers and transfer of multiple-resistant bacteria strains to human populace (Milanov et al., 2016), leading to the breakdown of immune system (Basak, 2015). A continued use of antibiotics in poultry production has been reported to result in its accumulation in poultry products which can also be excreted into the environment contaminating the confined aquatic and terrestrial environment close to the poultry site (Carvalho and Santos, 2016; AbdulBasit et al., 2020). This situation has created need to look for other better alternatives.

In this context, amongst other alternatives, researchers have shifted attention to the use of phytogenic growth promoters in poultry production because they are cheap, locally available and less competitive

with the human food supply chain. Phytogenic growth promoters also known as phytobiotics, are broad subset plants-derived bioactive compounds incorporated into the diets to improve livestock productivity through amelioration of feed properties, improvement of nutrient digestibility, absorption and elimination of pathogens in the gut (Prabakar et al., 2016; Gheisar and Kim, 2017; Kikusato, 2021). Their inherent advantages are that they are natural, less toxic and residue free (Olarotimi, 2018), which have rightly positioned them as the ideal growth promoters in poultry production.

A. brasiliana, is a variegated green and yellow, or bronze and green, or red and pinkish brown, perennial plant (Kumar et al., 2011), and belongs to the family of Amarathaceae. In traditional medicine, the plant has been reported to be good fodder which increases milk in cattle, and used in the treatment of cough, diarrhea (Brochado *et al.*, 2003), decreases blood viscosity and reduces hypertension (Christian et al., 2006). Pharmacological studies of the leaves of the plant show that it has anti-oxidant, anti-microbial, anti-viral, anti-inflammatory and anti-nociceptive properties (Kumar et al., 2011; Barua et al., 2012) which could be attributed to the presence of flavonoids and phenols in the plant (Attaugwu and Uvere, 2017).

2. Materials And Methods

2.1 Collection, identification and preparation of plant samples

The leaves of *A. brasiliana* were collected from plants growing in Amaizu community in Afikpo North Local Government Area of Ebonyi State during the raining season. The fresh and clean leaves were air dried under a shed until they were crispy to touch, while retaining their colorations. The leaves were powdered using corona manual grinder. Aqueous and ethanol extractions were carried out. The ethanol extraction was done using the Soxhlet apparatus to obtain about 1.5 kg of extract. The aqueous extract was prepared by macerating 400 g of the powdered leaves in 500 ml of cold distilled water for 2 days. It was then sieved using a muslin cloth and concentrated at reduced temperature. This was repeated until a total of 1.5 kg of the samples were extracted.

2.2 Phytochemical screening

One gram (1 g) of sample was weighed and transferred to a test tube. Fifteen milliliters (15 ml) of ethanol with 10 ml of 50%m/v potassium hydroxide (Hawkins, USA) were added in the test tube. The mixture was allowed to react in a water bath at 60 ^{o}C for 1 h. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel containing 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane (Yufeng, Japan). The mixture was washed three times with 10 ml of 10%v/v ethanol solution. The solution was dried with anhydrous sodium sulfate (Hawkins, USA) and the solvent was evaporated.

The sample was solubilized in 1000 µl of pyridine of which 200 µl was transferred to a vial for analysis. The analysis was performed in a Gas chromatography (BUCK M910) equipped with a flame ionization detector (GC-FID). A RESTEK 15 m MXT-1 column (15 m x 250 m x 0.15 µm) was used. The injector

temperature was 280 ^{o}C with splitless injection of 2 µl of sample and a linear velocity of 30 cm/s. Helium 5.0 pa.s was the carrier gas with a flow rate of 40 ml/min. The oven operated initially at 200 ^{o}C. It was heated to 330 ^{o}C at a rate of 3 ^{o}C/min and was kept at this temperature for 5 min. The detector operated at a temperature of 320 ^{o}C. The concentrations of the phytochemicals were calculated as the ratio between the area and mass of internal standard and the area of the identified phytochemicals. These concentrations were expressed in µg/g.

2.3 Minerals analyses

Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrometer according to the method of APHA 1995 (American Public Health Association). The sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume, to which 5 ml of concentrated nitric acid was added and heated to boil till the volume was reduced to about 15–20 ml, by adding concentrated nitric acid in increments of 5 ml till all the residue was completely dissolved. The mixture was cooled and made up to 100 ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

2.4 Vitamin analysis

A calibration curve for each of vitamin A, E, C and D, at different concentrations were prepared and absorbance obtained using a spectrometer (APEL 3000 UV). Vitamins were determined using standard methods by Kirk and Sawyer (1991).

2.5 Experimental animals

A total of seventy-two (72) day old chicks weighing between 48 g to 50 g were purchased from Rosambro Broilers in Abakaliki, Ebonyi State. The chicks were housed in a battery system, randomly divided into nine (9) groups of eight (8) birds per treatment and left in a good environmental condition. Feed and water were provided *ad libitum* and all required management practices applied as at when due.

2.6 Acute toxicity test

This study was carried out using the up and down method of acute toxicity (OECD, 2008). Nine (9) broilers were randomly selected, weighed and divided into 3 groups of A, B and C. Each group was made up of 3 broilers. Group A received 2.0 g/l aqueous extract of *A. brasiliana*, group B received 2.0 g/l ethanol extract of *A. brasiliana* while group C was given equal volume of distilled water. These were administered orally through mixing with drinking water. The broilers were observed for 48 h for signs of toxicity and mortality.

2.7 Experimental design

Complete randomized design was used in this analysis. Nine (9) treatment groups were created and designated as T_1; T_2; T_3; T_4; T_5; T_6; T_7; T_8; and T_9. The seventy-two (72) experimental broilers were assigned to the treatment groups and each group consisted of eight chicks as illustrated below. Each

treatment group received normal feed and water containing different concentrations of the leaf-extracts. Group 1 (T_1) was given 0.25 g/l ethanol extract of *A. brasiliana*, Group 2 (T_2) was given 0.50 g/l ethanol extract of *A. brasiliana*, Group 3 (T_3) was given 0.75 g/l ethanol extract of *A. brasiliana*, Group 4 (T_4) was given 0.25 g/l aqueous extract of *A. brasiliana*, Group 5 (T_5) was given 0.50 g/l aqueous extract of *A. brasiliana*, Group 6 (T_6) was given 0.75 g/l aqueous extract of *A. brasiliana*, Group 7 (T_7) was given 0.50 g/l Albiovit (a commercial growth promoter) Group 8 (T_8) was given 0.50 g/l B-vit extra (a commercial growth promoter) Group 9 (T_9) was given normal diet without any growth promoter

Groups 1–6 were the study groups. Group 7 and Group 8 served as the positive control groups, while Group 9 served as negative control group. The growth promoters were administered to the chickens through adding them in their drinking water for a complete 8 weeks period.

2.8 Carcass yield and meat quality evaluation

The chickens were fed normal diet (feed + water) plus experimental growth promoters in the morning (08.00 h) *ad libitum* in separate feeders, so the chicks determined their intake of the feed. The diets were offered daily and water was also provided *ad libitum* in plastic containers. All birds were weighed at the start of the experiment before allocating them to the treatments and after 56th day (final day) of the experiment. Also, consumed diets were recorded for calculation of weight gain, feed conversion ratio, and specific growth rate according to methods described by Sarker et al. (2016).

At the end of the experiment, five broilers per treatment (45 broilers in all) were randomly selected, starved of feed for 10 h and sacrificed by cutting the jugular vein to allow proper bleeding. Determination of blood weight was by the difference between slaughter weight and hot carcass weight. The weights were measured using Camry manual weighing scale. The broilers were defeathered by scalding in hot water at 60 ^{o}C and eviscerated to evaluate their carcasses.

The breast meats of the broilers were removed, weighed, and suspended in the deep freezer for 48 h. After 48 h freezing period, the meat was thawed, weighed, and cooked for 30 min after it started to boil. After the cooking period, the meat was allowed to cool and the final weight recorded. The drip loss was measured as the weight loss during suspension of the breast meat in an airtight container over 48 h at 4 ^{o}C. Drip loss was expressed as a percentage relative to the initial weight according to the standard method of Honikel (1987). The cooking loss was calculated by the method of Jama *et al.*(2008) and expressed as:

Cooking loss = (weight of meat after thawing – weight of cooked meat) x 100

Weight of meat after thawing

2.9 Blood component analysis

At the end of the feeding period (after 56 days from the beginning of the experiment), the birds were starved of feed for 10 h before blood samples were collected from five chickens per treatment for

biochemical and hematological analyses. A 2 ml of blood samples was collected from the femoral vein into labeled sterile sample bottles containing no anticoagulants, using a sterilized disposable syringe and needle. This was used to determine the biochemical parameters. Prior to bleeding, a cotton swab soaked in 70% ethanol was used to dilate the vein and to prevent infection. The blood samples without anticoagulants were centrifuged at 500 rpm (revolution per minute) for 3 min in a micro-centrifuge to obtain serum that was free from cell debris.

The serum obtained was analyzed colorimetrically for total protein (TP) by the Biuret method with kits as described by Dawnay et al. (1991). Two grams (2 g) of sample was treated with an equal volume of 1% of sodium hydroxide, followed by a few drops of aqueous copper (II) sulphate. A blue color was formed. The intensity of the blue color formed was proportional to the protein concentration in the plasma or serum. The mixture was incubated at room temperature for 20 min and read at a wavelength of 560 nm using a spectrophotometer.

Albumin (Ab) concentration was determined by the Bromo cresol Green (BCG) method (Peters et al., 1982). The sample was diluted with 0.85% sodium chloride and 2 drops of BCG was added which resulted in a color shift from yellow to green. The concentration of Ab was directly proportional to the intensity of the green color formed. The mixture was incubated at room temperature for 5 min and read with a spectrophotometer at a wavelength of 620 nm.

Globulin (Gb) concentration was calculated as the difference between total protein and albumin concentrations.

An initial 2 ml blood was collected from the drumstick veins of the birds into labelled sterile universal bottles containing Ethylene-Diamine-Tetra-Acetic Acid (EDTA) as anticoagulant. This was used to determine the hematological components within an hour of sample collection. The blood containing EDTA was mixed very well by inverting several times. Then the capillary tube was filled up to two-third the entire length, with one end of it sealed with plasticine. The tube was placed in a hematocrit machine and centrifuged for 5 min at 3000 rpm. PCV was calculated as follow:

$PCV = L_2 \times 100\ L_1$

Where L_1 is the upper layer of the capillary tube after centrifugation L_2 is the lower layer of the capillary tube after centrifugation

Hemoglobin was determined using the sodium lauryl sulphate (SLS) method according to Oshiro et al. (1982). A 25 µl of blood was mixed with 5 ml of a 2.08 mmol/l solution of SLS (buffered to pH of 7.2). The mixture was read with a spectrometer at 539 nm.

The WBC count was determined using Battlemen method of counting WBC. A dilution of the EDTA containing blood was carried out. That is, 1 drop of the sample in 19 drops of Turk's solution. The dilution was allowed to stand for about 5 mins for the red blood cells (RBCs) to lyse. The Neubauer counting

chamber was charged and filled with the dilution. The chamber was allowed to stand for 5 min for the WBCs to distribute evenly. A microscope, focused with x10 and viewed with x40 objective lenses was used to count the WBCs. The counting was done on four large squares. The WBC was calculated as follow:

WBC = N x Df V

Where N is number of WBCs counted Df is dilution factor V is volume of 4 large squares which is given as 4 x 0.1 mm^3

The neutrophils, lymphocytes and eosinophils were determined using methods described by Nakul et al. (2003). On a clean grease free microscopic slide, 0.6 µl of the EDTA containing blood was added. Using a suitable spreader (a slide) 1 cell thick film was made and allowed to air dry on a safe non-contaminating area and then stained with Leishman for about 5–10 mins and then rinsed and allowed to air dry again. It was then taken to the microscope for the differential WBC analysis.

2.10 Statistical analysis

Analysis of variance (ANOVA) for Completely Randomized Design (CRD) was carried out using Statistical Package for Social Sciences (SPSS). Values were presented as mean ± standard deviation (mean ± SEM). Differences between means were separated using the Least Significant Difference (LSD), with a p-value of less than 0.05 ($p < 0.05$) as the level of significance. Factor analysis was done to determine the communalities of the blood components.

3. Results

3.1 Vitamin compositions of the plants

The vitamins analysis of the extracts showed that the plants contained the following vitamins: vitamin A, vitamin E, vitamin C, vitamin D, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_6 and vitamin B_{12} in different proportions, as presented in Table 1. Significant differences ($p < 0.05$) were observed across the extracts of the plant for each of vitamin A, vitamin E, vitamin C, vitamin D and vitamin B_3. Whereas, no significant differences ($p > 0.05$) were observed for vitamin B_1, vitamin B_2, vitamin B_6 and vitamin B_{12}.

Table 1
Vitamin composition of leaf-extracts of *A. brasiliana*

	A brasiliana **(Ethanol)**	***A brasiliana*** **(Aqueous)**
Vitamin A (µg/g)	81.719 ± 0.4069^a	70.681 ± 0.3609^b
Vitamin E (µg/g)	55.167 ± 0.2728^a	38.304 ± 0.4692^b
Vitamin C (µg/g)	90.790 ± 0.240^b	105.312 ± 0.4038^a
Vitamin D (µg/g)	27.087 ± 0.373^a	17.291 ± 0.3966^b
Vitamin B_1 (mg/g)	0.191 ± 0.0508^a	0.154 ± 0.0409^a
Vitamin B_2 (mg/g)	0.176 ± 0.0169^a	0.1655 ± 0.0135^a
Vitamin B_3 (mg/g)	2.037 ± 0.0631^a	1.2688 ± 0.0629^b
Vitamin B_6 (mg/g)	0.641 ± 0.042^a	0.790 ± 0.212^a
Vitamin B_{12} (mg/g)	0.140 ± 0.0419^a	0.175 ± 0.076^a
Different letters indicate significant difference across the row ($p < 0.05$), where a > b. Values are presented as mean ± SD		

Table 1

3.2 Mineral compositions of the plants

The results of the mineral compositions of the extracts are shown in Table 2. The study showed that macro elements: calcium, magnesium, potassium, sodium, phosphorus; and trace elements iron, zinc, copper, selenium, molybdenum, manganese and cobalt were present in both the ethanol and aqueous extracts of the plant. Whereas, nickel was absent in the extracts. Significant differences ($p < 0.05$) were observed across all the values obtained.

Table 2
Mineral composition of the aqueous and ethanol leaf-extracts of the plants

Parameters	*A. brasiliana* (aqueous)	*A. brasiliana* (ethanol)
Copper (mg/g)	0.239 ± 0.002^{a}	0.213 ± 0.003^{b}
Magnesium (mg/g)	6.034 ± 0.0302^{a}	1.344 ± 0.059^{b}
Sodium (mg/g)	0.734 ± 0.001^{a}	0.663 ± 0.003^{b}
Potassium (mg/g)	4.222 ± 0.011^{b}	4.675 ± 0.012^{a}
Molybdenum (mg/g)	0.264 ± 0.004^{b}	0.375 ± 0.003^{a}
Calcium (mg/g)	7.288 ± 0.002^{b}	8.180 ± 0.010^{a}
Iron (mg/g)	6.974 ± 0.004^{b}	8.014 ± 0.011^{a}
Selenium (mg/g)	0.382 ± 0.002^{b}	0.824 ± 0.0204^{a}
Phosphorus (mg/g)	0.024 ± 0.002^{a}	0.009 ± 0.002^{b}
Zinc (mg/g)	1.915 ± 0.005^{b}	2.01 ± 0.01^{a}
Cobalt (mg/g)	0.027 ± 0.003^{a}	0.005 ± 0.002^{b}
Manganese (mg/g)	0.297 ± 0.003^{b}	0.356 ± 0.005^{a}
Nickel (mg/g)	NIL	NIL
Arsenic (mg/g)	0.015 ± 0.002^{a}	0.01 ± 0.002^{b}
Different letters indicate significant difference across the row ($p < 0.05$), where a > b. Values are presented as mean ± SD		

Table 2

3.3 Phytochemical compositions of the plants

The results of the phytochemical studies are shown in Table 3. It was observed that flavanones, phenols, oxalate, phytate, sapogenin, kaempferol, steroids, epicatechin, epihendrine, naringenin, and lunamarin were contained in the aqueous and ethanol extracts of the plant in different proportions. Significant differences ($p < 0.05$) were observed across the extracts for all results except for the result of epicatechin.

Table 3
Phytochemical composition of aqueous and ethanol leaf-extracts of *A. brasiliana*

	A. brasiliana (ethanol)	*A. brasiliana* (Aqueous)
Flavan-3-ol (µg/ml)	6.515 ± 0.386[a]	NIL
Flavone (µg/ml)	NIL	4.0441 ± 0.1413[a]
Flavonones (µg/ml)	9.624 ± 0.528[b]	10.891 ± 0.090[a]
Phenol (µg/ml)	8.809 ± 0.172[a]	6.7321 ± 0.053[b]
Oxalate (µg/ml)	4.931 ± 0.421[b]	6.906 ± 0.015[a]
Sapogenin (µg/ml)	9.517 ± 0.285[b]	19.371 ± 0.359[a]
Phytate (µg/ml)	3.974 ± 0.327[a]	3.475 ± 0.396[a]
Tannins (µg/ml)	NIL	14.510 ± 0.399[a]
Kaempferol (µg/ml)	14.069 ± 0.069[b]	17.28 ± 0.4341[a]
Proanthocyanin (µg/ml)	2.709 ± 0.100[a]	NIL
Anthocyanin (µg/ml)	NIL	8.54 ± 0.0798[a]
Steroids (µg/ml)	0.120 ± 0.118[b]	6.932 ± 0.278[a]
Epicatechin (µg/ml)	3.584 ± 0.585[a]	3.808 ± 0.203[a]
Catechin (µg/ml)	NIL	21.845 ± 0.266[a]
Epihendrine (µg/ml)	12.904 ± 0.537[a]	6.439 ± 0.454[b]
Naringenin (µg/ml)	4.246 ± 0.089[b]	5.082 ± 0.188[a]
Naringin (µg/ml)	NIL	4.2296 ± 0.4397[a]
Rutin (µg/ml)	NIL	6.4055 ± 0.4946[a]
Lunamarin (µg/ml)	0.353 ± 0.159[b]	2.633 ± 0.376[a]
Spartein (µg/ml)	NIL	3.2425 ± 0.5235[a]

Different letters indicate significant difference across the row ($p < 0.05$), where a > b. Values are presented as mean ± SD

Table 3

3.4 Acute toxicity test

Oral administration of 2.0 g/l of the leaf-extracts and equal volume of distilled water produced no death or any sign of toxicity after 48 h.

3.5 Carcass yield and meat quality

The results revealed that the weight gain values and specific growth rate values (Fig. 1) obtained in the groups fed the extracts, were not significantly different ($p > 0.05$) from the weight gain and the specific growth rate of the groups fed the control groups. Although, the group fed 0.75 g/l aqueous leaf-extract of *A. brasiliana* had the highest weight gain value and specific growth rate value of 3.085 ± 0.1528 and 7.46 ± 0.086 respectively.

Again, the negative control groups recorded the highest drip loss (Fig. 2) and cooking loss (Fig. 3) of 5.0 ± 0.8824% and 45.0110 ± 1.1492% respectively. These values were significantly higher ($p < 0.05$) than the drip loss and cooking loss values gotten from the groups fed *A. brasiliana* and the positive control groups. Also, the groups fed ethanol extracts had drip loss and cooking loss values that were higher than those observed for the groups fed aqueous extracts. Nevertheless, no significant differences ($p > 0.05$) were observed in the values of all the test groups.

Figure 1; Fig. 2; Fig. 3

3.6 Blood components

Figure 4 shows the results of total blood protein, globulin and albumin. The values of these parameters were dose dependent. The total protein and globulin values increased with reducing concentration of the leaf-extracts The results showed that the negative control group had the least total protein globulin values of 19.32 ± 0.877 g/l and 17.93 ± 0.806 g/l respectively, which was significantly lower ($p < 0.05$) than the values observed for the other groups. Again, the results showed that the group fed 0.25 g/l aqueous leaf-extracts of the plant, had the highest total protein and globulin values of 47.57 ± 0.764 g/l and 44.07 ± 0.735 g/l respectively. These values were significantly higher ($p < 0.05$) than the values obtained for the groups fed ethanol extracts and the positive control groups.

Albumin concentrations were also dose dependent. The groups given aqueous extracts had albumin values that increased with decreasing concentrations, while, the groups fed ethanol leaf-extracts gave albumin values that increased with increasing concentrations. The negative control group gave the least albumin value of 1.39 ± 0.071 g/l, which was significantly lower ($p < 0.05$) than the values for the groups fed aqueous leaf-extracts of the plants, and the positive control group.

Table 4 shows that all the blood components measured were affected by the extracts administered to the chickens. A component plot of blood parameters in rotated space (Fig. 6) show that all the data appear normal and no extreme outliers were apparent. Also, it was observed from Fig. 5 that hemoglobin, packed cell volume, red blood cell count and neutrophil values increased with increasing concentrations, whereas, white blood cell count, eosinophils and lymphocyte values decreased with increasing concentrations of the extracts. The negative control group gave the least packed cell volume, red blood cell count and

hemoglobin values of 15 ± 0.001, 2.05 ± 0.212 x10^9/l and 6.165 ± 0.233 g/l respectively. These values where significantly lower ($P < 0.05$) than the values obtained in the groups fed aqueous extracts, and the positive control groups.

Table 4
Communalities (Principal Component Analysis) of blood parameters.

	Initial	Extraction
Hb	1.000	.892
RBC	1.000	.935
WBC	1.000	.856
Neutrophil	1.000	.756
Eosinophil	1.000	.935
Mesophil	1.000	.901
PCV	1.000	.967
Lymphocyte	1.000	.887
Total protein	1.000	.941
Globulin	1.000	.917
Albumin	1.000	.905

Variables having **high communalities** (r^2 value, -say more than 0.70) contributes more to measuring the underlying principle components (factors). Hence, by implication, all parameters having $r^2 \geq 0.7$ is said to have been highly affected by the treatment across the groups.

Again, the groups fed aqueous extracts had significantly higher ($p < 0.05$) packed cell volume, red blood cell count and hemoglobin values compared to the groups fed ethanol extracts of the plants. The groups fed the leaf-extracts improved the RBC count of the broilers than the commercial growth promoters.

The highest value of total white blood cell count and differential white blood cell counts were obtained in the negative control group. These values in the negative control group were significantly higher ($p < 0.05$) than the values obtained in the groups fed aqueous extracts and the positive control group. Again, it was observed that the groups aqueous extract had significantly lower total white blood cell count ($p < 0.05$) when compared to the groups fed ethanol extracts. Similarly, the positive control groups gave differential white blood cell values that were not significantly different ($p > 0.05$) from the values obtained in the groups fed the leaf-extracts.

Table 4, Fig. 4, Fig. 5, Fig. 6

4. Discussion

It was observed from the study that the extracts contained water soluble vitamins (C and B-complex) and fat-soluble vitamins (A, E, and D) in different proportions. Vitamin A, which was above its minimum dietary requirements in poultry (54µg/100g) (Ogunmodede, 1981) is important for the development of feathers, strong and improved egg production, increase in hatchability and decrease in embryonic mortality (Leeson, 2015). Also, vitamin A is implicated in the improvement of the local immune defences within the gut-associated lymphoid tissue (GALT) of chickens (Dalloul et al., 2002).

Again, the findings of this study show that the vitamin E content of the plants are below the daily requirements of 5–10 mg/kg by National Research Council (1994) in poultry production. Nevertheless, the nutritional requirements of broilers, from 1994 till date, are likely to have changed in response to intense genetic selection that has been observed in poultry over the years (Pompeu et al., 2018). Thus, this study suggests that the extracts can contribute in increasing the level of vitamin E available for broiler intake. An increase in vitamin E intake may lead to the enhancement of the immune responses, regulate platelet aggregation by inhibiting prostaglandin (thromboxane) production as well as play a key role in the regulation of protein metabolism and hormonal production (Pompeu et al., 2018).

Vitamin D which was above its requirement in poultry (5 µg/kg) (National Research Council, 1994) is important for the prevention of bone malformation, as its deficiency can result in rickets, osteoporosis, or poor egg shell quality in laying hens not minding the quantity of the available calcium and phosphorus (Leeson, 2015; Sakkas et al., 2019).

The results revealed that thiamine, riboflavin, niacin, and pyridoxine contents of the plants were lower than 1.0 mg, 1.8 mg, 11 mg and 3.0 mg respectively, required for these vitamins, whereas, cobalamin was higher than the 0.009 mg daily requirement for this vitamin (National Research Council, 1994). Though, some of these vitamins were not as high as cited in National Research Council recommendation, many factors might have contributed to the observed low values such as geographical location of the plants and methods of extraction used. Again, the ingredients utilized in poultry feed production also contain all the necessary nutrients required by the birds. In this light, the presence of these vitamins in the extracts is an indication that this plant adds to the quantity of B-vitamins available for broiler consumption.

The presence of thiamine and niacin implies that broilers treated with this plant will have improved appetite, a healthier nervous system and a higher release of energy from the diets (Leeson, 2015; Attaugwu and Uvere, 2017). Similarly, researchers have pointed out that the presence of cobalamin, riboflavin and pyridoxine in broiler diet is important to maintain and promote growth and good feathering through their activities as co-enzymes in many enzymes systems (Keles, 2010; Leeson, 2015; Pal, 2017; Uraku, 2015).

Again, the findings of this study revealed that the calcium content of the plants where between 7.288 mg/g to 8.180 mg/g. This is consistent with the findings of Mako et al. (2013) who reported 7.8 mg/g of calcium for *A. brasiliana*. Calcium has been reported to be involved in the activation of a large number of

enzymes adenosine triphosphate, succinic dehydrogenase and lipase (Talpur et al., 2012). Leeson (2015) added that a deficiency of extracellular blood calcium in young growing broilers results in abnormal bone development, increased irritability of the nerve tissues and subsequent osteoporosis in laying hens.

In the same vein, the sodium values of the extracts where 0.663 mg/g and 0.734 mg/g for for ethanol and aqueous extracts respectively. These values were in the lines of earlier literature (Mako et al., 2013) that reported 0.855 mg/g for *A. brasiliana*. Reports have shown that sodium and potassium are very essential in poultry production as they are the major determinants of acid-base balance in the cells (National Research Council, 1994). These elements are involved in the transmission of nerve impulses and absorptive processes of monosaccharide, amino acids, pyrimidines and the bile salts (Soetan et al., 2010). Low level of sodium in poultry diet can result in poor growth, poor feed conversion efficiency, soft bones, and increase in oxygen utilization and heat production (Leeson, 2015). Similarly, a deficiency of potassium in poultry diet results in muscle weakness and growth retardation (Balos et al., 2016)

Again, the results revealed that the extracts contain phosphorus which were in line with the 0.022 mg/g for *A. brasiliana* reported by Mako et al. (2013) for the plant. Just like calcium, a deficiency of phosphorus in poultry diet can result in lack of normal skeletal calcification, which may induce rickets in broilers (Leeson, 2015). Research has also shown that the presence of phosphorus in poultry diet is responsible for normal muscle growth, egg formation and functioning of nucleic acids and genetic codes (Soetan et al., 2010; Li et al., 2016).

The trace elements present in the extracts were grossly below the requirements for these elements by the National Research Council (1994). Nevertheless, this result is consistent with the findings Delaporte et al. (2005) and Mako et al. (2013) who reported that the trace elements present in *A. brasiliana* were below the National Research Council (1994) daily requirements for broilers. Nevertheless, the low levels of trace elements in the extracts might not constitute any problem because the main ingredients utilized in the production of poultry diets might have contained all the minimum trace elements sufficient for the poultry needs. Therefore, it could be deduced from the findings of this study, that the presence of these trace elements in the extracts increases the minimum available elements for broiler consumption.

Manganese is an essential trace element necessary for proper functioning of many enzyme systems involved in lipid and carbohydrate metabolism. Soetan et al. (2010) concluded that manganese is required for the synthesis of mucopolysaccharides, such as chondroitin sulphate, to form the matrices of bones and egg shells. Leeson (2015) added that a deficiency of manganese in broiler diet can lead to perosis, poor growth and impaired blood clotting.

Iron is another important trace element for all living cells as it plays important role in oxygen and electron transport, as well as an integral component of myoglobin for the delivery, storage and use of oxygen in the muscle (Lin et al., 2020). Craig et al. (2017) added that Iron is important for the maintenance of meat quality. A deficiency of iron in broiler diets can result in loss of pigmentation of the feathers, severe anemia followed by a reduced packed cell volume (Leeson, 2015).

Copper is essential for the incorporation of iron in hemoglobin, assists in the absorption of iron from the gastrointestinal tract, and helps in the transfer of iron from tissues to the plasma (Soetan et al., 2010). In addition, copper is required for bone formation by promoting the structural integrity of the bone collagen and for normal elastin formation in the cardiovascular system (Samanta et al., 2011). A deficiency of copper in poultry diet can lead to poor feathering, rupture of the aorta and bone disorder (fragile and broken) and causes anemia (Leeson, 2015; Mustafa *et al.*, 2018; Yang et al., 2018).

Zinc was reported by Park et al. (2004) to have a catalytic function in zinc dependent enzymes such as dehydrogenases, oxidoreductases, carbonic anhydrase, DNA and RNA polymerases and also involved in macronutrient metabolisms and cell replication. Also, zinc is essential for growth, bone development and immune functions in chickens (Park et al., 2004; Naz et al., 2016; Zhang et al., 2018). Leeson (2015) reported that a deficiency of zinc in poultry diet can result in stunted growth, poor hatchability, decreased egg production, poor feathering, shortening and thickening of the bones, and in some cases, loss of appetite and death.

Table 3 confirmed that the plants contain alkaloids and polyphenols such as flavonoids, tannins, phenols, saponins, and anthocyanins in different proportions, which is consistent with the reports of Mpiana et al. (2010), Mea et al. (2017) who concluded that these plants contain these phytochemicals. These phytochemicals have important antioxidant, anti-inflammatory, anti-microbial, anti-fungal, anti-viral, immunomodulatory, and other pharmacological properties (Umerie and Ekuma, 2016; Osioma and Hamilton-Amachree, 2017; Ubaoji *et al.*, 2018; Attaugwu and Uvere, 2017)

The water holding capacity (WHC) of broiler meat is measured as the fraction of bound water retained in the muscle (Oko et al., 2012; Oko, 2013; Oko et al., 2013). The groups fed aqueous extracts with lower values of drip losses and cooking losses, are considered to have higher water holding capacities compared to the groups fed ethanol extracts. Thus, the meats of the groups fed aqueous extracts are considered to be of better qualities, because, the lower the drip and cooking losses of meat, the higher the water holding capacity, and the better the meat quality (Oko et al., 2013). In addition, the low cooking loss of the breast meat of broilers fed the extracts suggests that their meats are of high quality because there are limited nutrients losses (especially proteins) into the water during cooking.

Thus, it could be deduced from the results that the plant extracts, especially the aqueous extracts, improve the meat quality of the chicken used in this study. Among the plausible explanations for these findings could be due to the phytochemicals, minerals and vitamins contained in the extracts. Similarly, Yang et al. (2020) reported that broiler drinking water with the inclusion of plant extracts can be used to enhance the oxidative stability, shelf life and meats quality of broilers, which is in good agreement with the reports presented in this study.

Again, it was observed from the results that the extracts improved the weight gain and the specific growth rate of the broilers better than the negative control group, although, the aqueous extracts performed this function better than the ethanol extract. Also, the extracts produced weight gain and specific growth rate values that were not significantly different from the positive control groups. Again, these findings could

be attributed to the pharmacological properties of the phytochemicals, minerals and vitamins contain in the extracts. Reports have shown that feeding broiler chickens with diet containing phytogenic blend leads to improvement in body weight gain and specific growth rate (Hernandez et al., 2004; Mohammadi-Gheisar et al., 2015; Jayanti et al., 2017)

It was observed from this study that the concentrations of blood proteins (total protein and globulin) in the serum of the broilers were significantly increased ($p < 0.05$) by the extracts better than the negative control group. These findings are consistent with the report of Ghazalah and Ali (2008), who suggested that supplementing broiler diets with phytogenic growth promoters (such as rosemary leaves) increases the total protein and globulin levels compared to the control. The findings of this study could be attributed to the crude proteins present in the plants as earlier reported (Mako et al., 2013; Preetha et al., 2018; Arogbodo, 2020).

In addition, the increase in the globulin fraction observed in this study indicates the effective roles of *A. brasiliana* in increasing immunity due to their roles in developing and protecting cells and inhibiting non-enzymatic oxidation (Mpiana et al., 2010; Kumar et al., 2011; Barua et al., 2012; Onoja et al., 2017; Anyasor *et al.*, 2019).

The values recorded by the groups fed aqueous extracts for and packed cell volume were within the normal ranges of 7.5 g/dl to 13.1 g/dl (hemoglobin) and 26.0–45.2% (packed cell volume) reported by Mitruka and Rawnsley (1977) for healthy birds. Again, these observed comparable values recorded for hemoglobin and packed cell volume in the birds fed aqueous extracts and commercial growth promoters indicate nutritional adequacy and improvement / stability of their hematological profile (Oloruntola, 2019).

Finally, the extracts were able to lower the levels of total white cell count and differential white blood cell counts in the chickens compared to the negative control. Olugbemi et al. (2010) opined those increased concentrations of differential white blood cells in the body connotes a threat to normal health, therefore the body builds up its defence against such threat. Thus, the findings of this study suggest that the extracts improved the health status of the chicken through increasing their ability to fight infections and defend their bodies against foreign invasion.

Phytobiotics are potent alternatives to synthetic / chemical growth promoters. The current practice in feeding phytobiotic compounds to poultry seems to justify the assumption that phytogenic growth promoters may have the potential to promote production performance and health, and thus add to the set of non-synthetic growth promoters for poultry production. So, this study was designed to assess the efficacy of *A. brasiliana* as a phytogenic growth promoter in poultry. The study has found that generally, the ethanol and aqueous extracts of *A. brasiliana* are potent alternatives to chemical growth promoters in chicken farming. Nevertheless, the aqueous extract, which is easier to prepare and cost effective, produced better effect than the ethanol extract. Considerably, more work will need to be done for a better understanding of the exact mechanisms of action of these extracts.

Declarations

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Authors contributions Conceptualization: AOO, ETE; Material collation: AOO, ETE; Methodology: AOO, ETE; Statistical analysis: SE, ETE; Resources: AOO, ETE; Supervision: AOO, SE; Writing – Original draft preparation: ETE; Writing – Review and Editing: AOO, ETE, SE

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Ethics and animal welfare Animal care and procedures were performed by following the guidelines of good experimental practices. The Ebonyi State University Ethical Committee granted permission for this research to be conducted.

Consent for participation Informed consent was obtained from all individual participants included in the study.

Consent to publish Not applicable.

Competing interest, The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

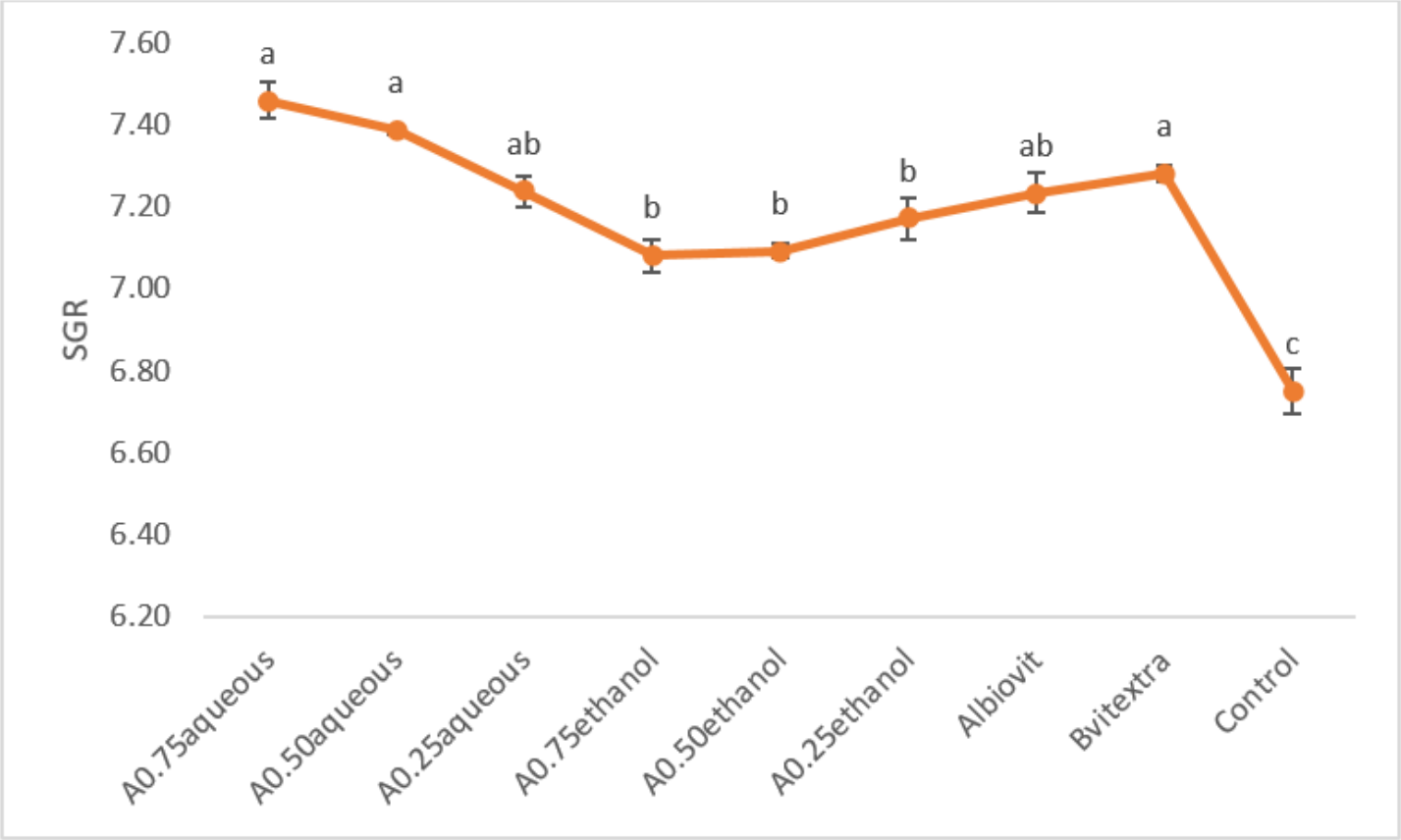

Figure 1

Effects of the leaf-extract on the specific growth rate of the broilers. Different letters indicate significant difference across the groups ($p < 0.05$), where a > b > c. Values are presented as mean±SD and 'A' represents *A. brasiliana*

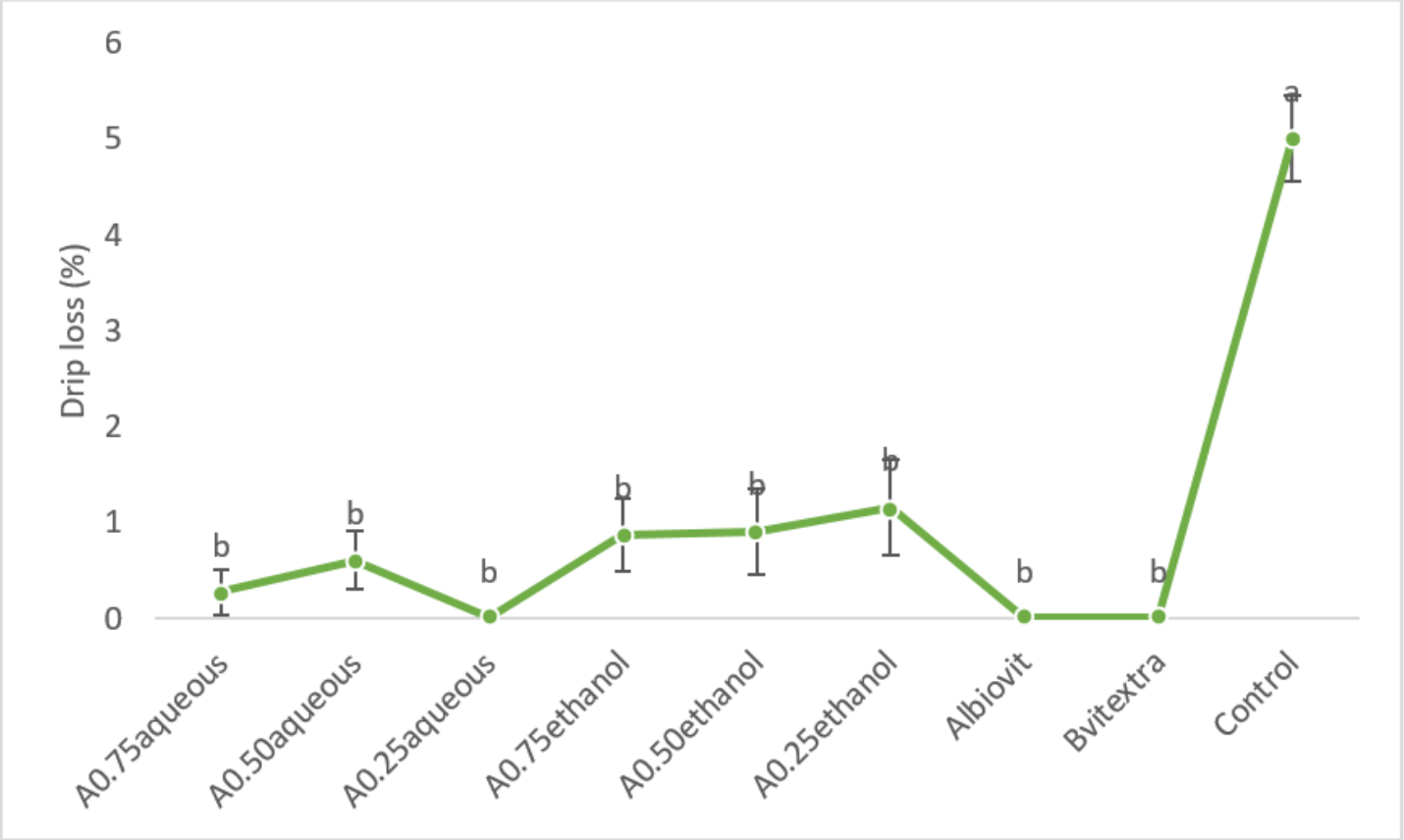

Figure 2

Effects of the leaf-extract on the drip loss of the broilers. Different letters indicate significant difference across the groups ($p < 0.05$), where a > b. Values are presented as mean±SD and 'A' represents *A. brasiliana*

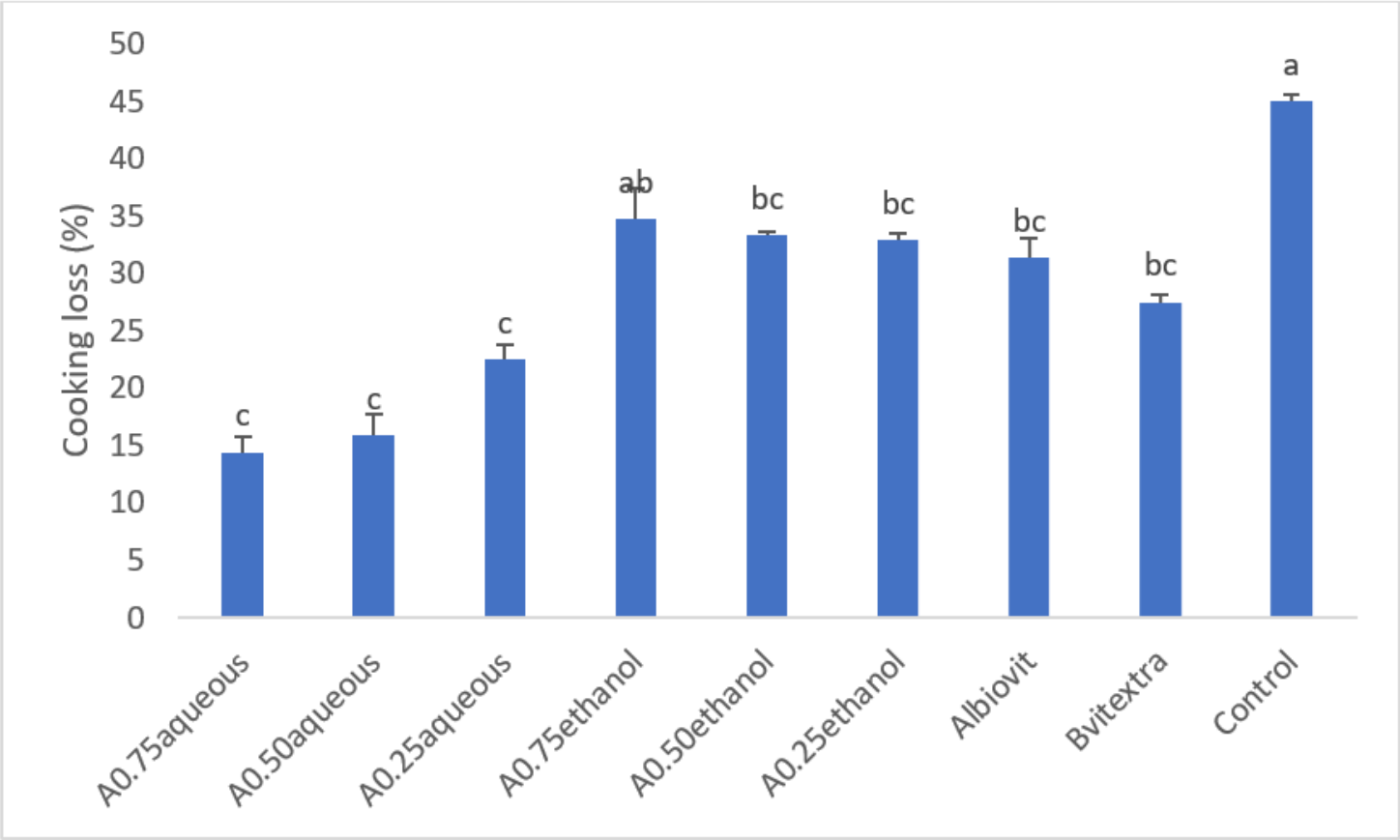

Figure 3

Effects of the leaf-extract on cooking loss of the broilers. Different letters indicate significant difference across the groups ($p < 0.05$), where a > b > c > d. Values are presented as mean±SD and 'A' represents *A. brasiliana*

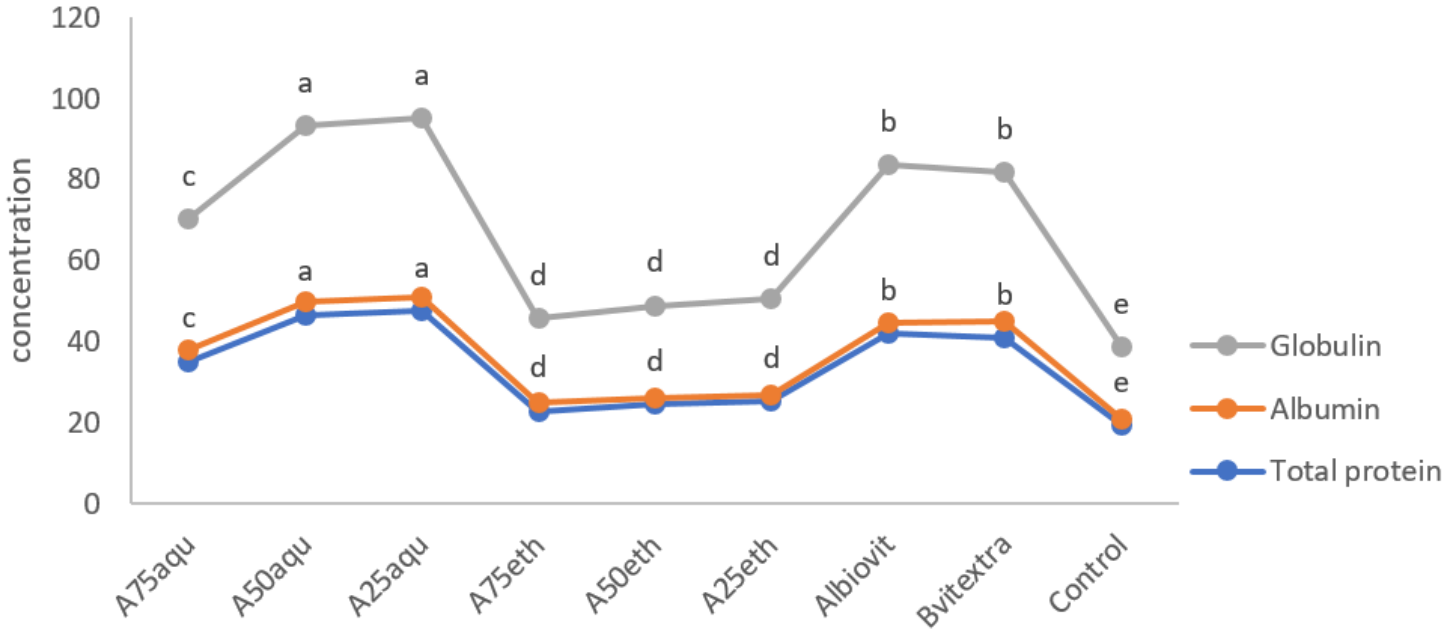

Figure 4

Effects of the leaf-extract on the blood protein concentrations of the broilers. Different letters indicate significant difference across the groups ($p < 0.05$), where a > b > c > d > e. Values are presented as mean±SD and 'A' represents *A. brasiliana*

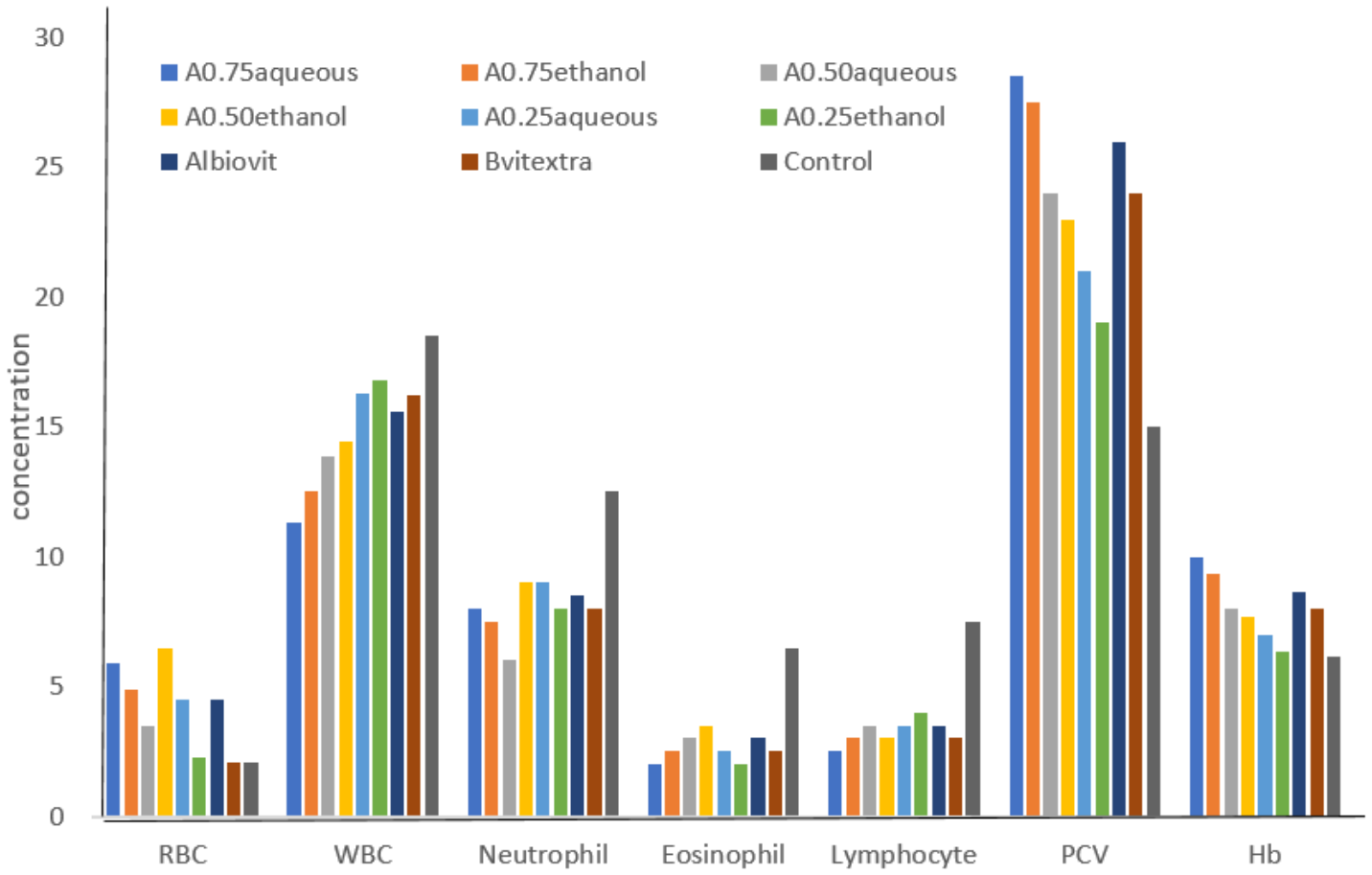

Figure 5

Effects of the aqueous and ethanol leaf-extracts on the hematological parameters of the broilers. 'A' represents *A. brasiliana*

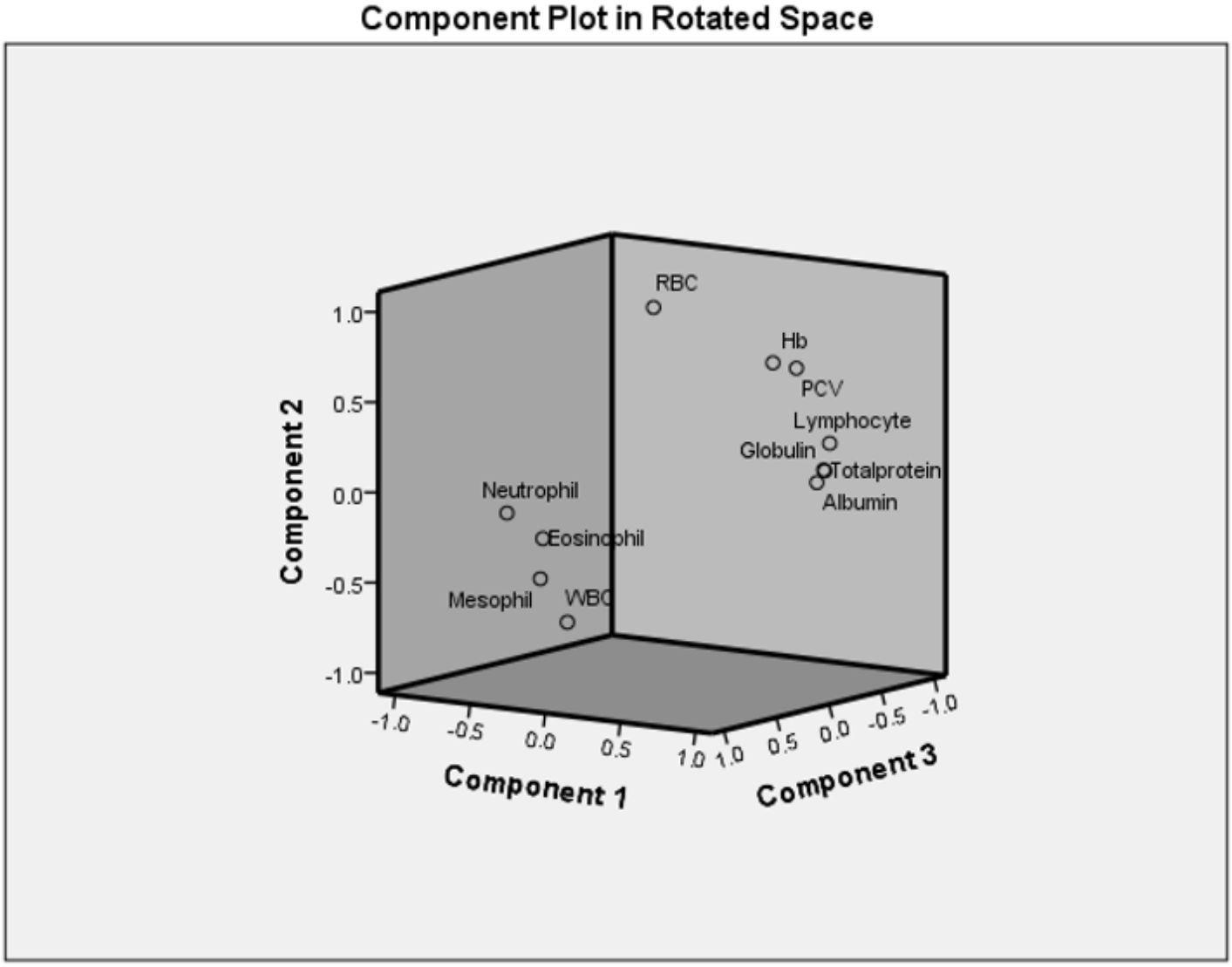

Figure 6

Component plot of blood parameters in rotated space. The data appear normal and no extreme outliers are apparent.