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GROWTH PERFORMANCE, HEAMATOLOGY AND SERUM BIOCHEMISTRY OF BROILER CHICKS FED DIETS CONTAINING DIFFERENT CLAY SOURCES

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ABSTRACT
The study was conducted to determine the effect of diets containing different clay sources on the growth performance, heamatology and serum biochemistry of broiler chicks. One hundred and twenty broilers were allocated to five treatments diets containing different clay sources having three replicates of eight broilers in a completely randomized design. Data were collected on body weight, feed intake, total protein, albumin, glucose, total cholesterol, urea, low-density lipoprotein, high-density lipoprotein, triglyceride, aspartate transaminase and alanine transaminase, hemoglobin, red blood cell, white blood cell, lymphocytes, and packed cell volume. The total weight gain, average daily weight gain, total feed intake, average daily feed intake, feed conversion ratio and feed efficiency ratio were calculated. Results showed that growth performance parameters were not significantly ($p > 0.05$) different at the starter phase but serum biochemistry and haematological parameters except red blood cells were significantly ($p < 0.05$) different. The T3 diets containing clay formed by sprinter termites improved the serum biochemistry as well as haematological indices of broiler better than the other sources of clay used in this experiment. This implies that the clay in T3 had the greatest capacity to bind to the toxins contained in the feed probably due to the high mineral contents.

Key words: clay minerals, growth, broiler birds, blood profile, toxin binder

INTRODUCTION
There is a need to satisfy the nutritional requirements of broilers since feed is an integral part of the food chain and it plays an important role in the overall growth and development of broilers as well as in the quality and quantity of livestock products such as milk, meat and eggs (Guerre, 2016; Makkar, 2016).

The poultry sector is considered to be the fastest growing sector in the livestock subsector. The expansion is driven by a very strong demand for poultry products in the entire world at all income levels. Large percentage of the world population depends on livestock for their livelihood. This has significantly contributed to the improving human nutrition, provided food such as (eggs and meat) with better quality nutrients; generated revenue for women by improving their capacity to cope with shocks and reducing economic vulnerability; provided farm yard manure for vegetable garden and crop production. The socio-cultural and religious functions of village poultry production for smallholder livelihoods are also highly recognized apart from its economic or nutritional benefit (FAO, 2013). Chicken meat and eggs are considered the best quality protein and have a very high demand throughout the world. Poor nutrition or inadequate nutrition is associated with poverty in Africa and some part of Asia. Poultry products are widely available and relatively inexpensive to the extent that they can help to meet the shortfalls in essential nutrients among impoverished people. Poultry products that are rich in all essential nutrients can help to reduce metabolic diseases associated with deficiencies in critical dietary minerals, vitamins and amino acids.

However, the presence of mycotoxins in feed ingredients remains one of the greatest challenges that poultry farmers and the entire feed industry face (Li *et al*., 2014). Mycotoxins can cause several toxic effects in poultry animals. Mycotoxins can suppress the immunity of poultry species with their action mechanism based on enzyme inhibition to reduce protein synthesis and consequently the immune response. The combined effect of several mycotoxins can be more severe than when the animal suffers from the effect of one mycotoxin. The mycotoxins that affect the immune system of poultry are mainly Aflatoxins: (AFBI, AFB2, AFG1, AFG2). Ochratoxin A: (OTA) and Citrinin. Trichothecenes: Type A – Toxin T-2 Diacetoxyscirpenol (DAS), Fumonisins (FB1, FB2,). These mycotoxins can cause reduction in feed intake, body weight, egg production including oral lesions, increased gizzard weight and liver injury. Mycotoxins can also cause immunosuppression in poultry. Immunosuppression may affect the health and consequently the performances of the animals. There might be increased mortality, uneven growth, decreased body weight, higher feed conversion, higher medication costs and

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higher rate of condemnations at slaughter. Immunosuppressed birds can develop long and complicated vaccine reactions, and can be more easily susceptible to respiratory diseases with secondary bacterial infection. The use of antibiotics growth promoters is no longer encouraged due to the numerous health complications associated with it (Dim *et al*., 2018).

The presence of mycotoxins in animal products such as meat, egg and milk may have negative effects on human health. Pitt and Miller (2017) had described factors that influence the production of mycotoxins to include agronomic practices and climatic conditions such as temperature and humidity. According to Mannaa and Kim (2017), contamination can actually occur during crop growth, storage and transportation.

Research has shown that absorbents such as clay minerals may be an effective tool for the removal of mycotoxins from feed. Clay (Kaolin) is added in the diet of poultry to improve digestion and nutrients absorption, promote the reduction of toxins that cause injuries in the intestinal mucosa thereby increasing the excretion of the pathogen, hence improving the productivity of the poultry (Owen *et al*., 2012). The antimicrobial and antitoxic properties of clay have been shown to improve the appetites and weight gain in chickens. It also promotes a proper digestion, increase food retention time and contribute to improving water retention and reducing the moisture content of droppings. It also enhances organoleptic characteristics. Clay minerals are phyllosilicates, hydrous aluminum with variable amounts of iron, magnesium, alkali metals and alkaline earth metals. There are various clay sources that can contain varying amounts and quality of kaolinite and bentonite. The clay sources may also have varying capacities to bind toxins and subsequently promote growth, feed intake, feed conversion and immune boosting. These include mud daube commonly known as wasp on the walls of houses, clay formed by acrobat ants on the tree trunks, clay formed by sprinter termite and clay formed on hill-top with cap.

Thus, the aim of this present work was to determine the growth performance, heamatological and serum biochemistry of broilers fed diet containing different clay sources.

MATERIALS AND METHODS

Location and Duration of Study

The experiment which lasted for eight weeks was carried out at the Poultry Unit of the Department of Animal Science and Research Farm, Nnamdi Azikiwe University (UNIZIK) Awka, Anambra State, Nigeria. Awka is located in the tropical transitional rainforest-savanna zone of the south-eastern part of Nigeria. The climate of this zone is controlled by the seasonal movement of the intertropical convergence zone, which is determined by two air masses operating in the region of West Africa.

Experimental Diet

Five different diets were formulated by including each clay source at 5% level for the starter phases. The diets were composed of maize, soyabeans meal, fish meal, clay, wheat bran, groundnut cake, lysine, methionine, salt and V/M premix. The clay was collected from different sources including mud daube commonly known as wasp on the walls of houses (cwh), clay formed by sprinter termite (cst), clay formed by acrobat ants on the tree trunks (ctt) and clay formed on hill-top with cap (chc). The composition of Starter diets is presented in Table 1.

Feeding

The birds were fed experimental diets, starting with Sarter mash for the first four weeks. Feed and water were provided ad libitum. The quantity of feed offered to each experimental groups were weighed every morning while left-over of previous day supply was weighed in order to determine their daily feed intake. The birds were brooded for four weeks using 200-Watt bulb, flat plastic feeders and shallow drinkers

Vaccination

The chicks were properly vaccinated against Newcastle disease and infectious bronchitis (LaSota strain and H120 strain on day 7 via drinking water. The Gumboro vaccine was administered on day 14 and Newcastle Kamorof was administered on day 21 following the broiler bird vaccination protocol by the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Table 1: Composition of the experimental diet at starter phase using clay from different sources

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	55.00	50.00	50.00	50.00	50.00
Soyabeans meal	24.00	24.00	24.00	24.00	24.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Clay	-	5.00	5.00	5.00	5.00
Wheat bran	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
V/M premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Housing
The birds were housed in a well-ventilated open-sided pens covered with wire mesh on deep litter with concrete floor covered with wood shavings. All birds would be reared and kept under similar management conditions. The dimensions of the experimental replicate pens were 3.15 ft by 14.55 ft.

Experimental Design
A completely randomized design was used for the experiment. A total of 120-day-old, unsexed broiler chicks were purchased from a commercial hatchery. On arrival, the chicks were weighed individually using a weighing scale. The experimental birds were randomly divided into five dietary treatment groups containing 24 broiler chicks per treatment. Each treatment group was replicated three times with eight birds per replicate.

Data Collection
The initial body weights were determined by weighing individual broilers at the beginning of the experiment, while the weekly body weights were also determined weekly. The daily feed intake was also determined through weigh back mechanism on daily bases. The blood Samples were collected from the jugular vein of three chicks in each replicate using siring. For serum biochemistry, the samples were transferred into non – EDTA tubes and allowed to clot for 1 h at room temperature and then centrifuged at 3000 rpm for 20 min. The samples were analyzed for total protein, albumin, glucose, total cholesterol, urea, low-density lipoprotein (LDP), high-density lipoprotein (HDL), triglyceride, aspartate transaminase, (AST) and alanine transaminase (ALT). During hematological analysis, the samples were transferred into Eppendorf Tubes containing Ethylen Diamine Tetraacetic Acid (EDTA) as an anticoagulant. Hematological parameters measured include hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), Lymphocytes, and packed cell volume.

Statistical Analysis
Data obtained from this study were statistically analyzed using a One-way analysis of variance (ANOVA) and significant means separated using Duncan's Multiple Range Test. Data analysis was performed using SPSS statistical software.

RESULTS AND DISCUSSION

Growth Performance of Broiler Starter Fed Diets Containing Different Clay Sources
The growth performance of broilers fed diets containing different clay sources at starter stage is presented in Table 2. The results show that there were no significant ($p > 0.05$) differences in all growth performance parameters that were evaluated during the experiment. Sholikin *et al.* (2023) reported that clay minerals had no effect on average daily gain, average daily feed intake and feed conversion ratio of broilers during the starter stage.

Hematological Indices of Broilers at Starter Stage Fed Diets Containing Different Clay Sources
The haematological indices of broilers fed diets containing different clay sources are presented in Table 3. The results show that there were significant ($p < 0.05$) differences among treatments of broilers at starter stage fed these diets in haemoglobin, red blood cell count, white blood cell count, lymphocytes and packed cell volumes.

Table 2: Growth Performance of broilers fed diets containing different clay sources at starter stage

Parameters	T1	T2 (cwh)	T3 (cst)	T4 (ctt)	T5 (chc)	SEM	P value
Initial body weight (g)	50	50	50	50	50	0.00	0.74
Final body weight (g)	933.67	937.67	877.33	862.67	902.67	19.52	0.74
Average weight gain (g)	883.67	887.67	827.33	812.67	852.67	19.52	0.74
Average daily weight gain(g)	31.83	31.70	29.68	29.02	30.45	2.83	0.75
Total feed intake (g)	1790.00	1813.33	1850.00	1753.33	1913.33	35.69	0.74
Average daily feed intake (g)	63.92	64.76	66.07	62.62	68.33	1.27	0.74
Feed conversion ratio	2.05	1.81	2.23	2.17	2.25	0.08	0.39

$^{a-b-c;ab-bc}$ Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM - standard error of mean,
T1 - Control Diet, T2 - Treatment with Clay by Wasp, T3 - treatment with clay by sprinter termite,
T4 - treatment with clay from tree trunk, T5 - treatment with clay from hill top with cap

Table 3: Hematological indices for broiler at starter stage fed diets containing different clay sources

Parameters	T1	T2 (cwh)	T3 (cst)	T4 (ctt)	T5 (chc)	SEM	P value
Hemoglobin (g dl^{-1})	9.43^{a}	9.43^{a}	5.30^{b}	9.07^{a}	8.73^{a}	0.43	0.00
Red blood cell (×10^{6}µ)	2.08	2.36	1.64b	1.98	1.79	0.10	0.13
White blood cell × 10^{3} µl	11.60^{d}	14.82^{b}	5.68^{e}	13.31^{c}	23.35^{a}	1.53	0.00
Lymphocyte (%)	9.29^{d}	13.27^{b}	4.07^{e}	11.87^{c}	21.55^{a}	1.53	0.00
Packed cell volume (%)	27.77^{a}	26.93^{a}	14.50^{c}	26.40^{a}	24.39^{b}	1.31	0.00

$^{a-b-c;ab-bc}$ Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM - standard error of mean,
T1 - Control Diet, T2 - Treatment with Clay by Wasp, T3 - treatment with clay by sprinter termite,
T4 - treatment with clay from tree trunk, T5 - treatment with clay from hill top with cap

Haemoglobin concentration, red blood cell count, white blood cell count, lymphocytes and packed cell volume had the least values in broilers fed T3 diet containing clay formed by sprinter termites (cst). Heamoglobin concentration and red blood cell counts were highest in broilers fed T2 diets. However, the normal range for heamoglobin and red blood cell counts is 7-13 g dl^{-1} and 2.5-3.5 × 10^6 µl, respectively (Bounous and Stedman, 2000). The highest values in T2 of haemoglobin and red blood cell count may be attributable to the kaolin and bentolite values in it. Highest lymphocyte value was attained in T5 but the normal range of lymphocyte in broiler is (45-70%), as reported by Aeangwanich *et al*. (2004). Its highest value may also be as a result of the high concentration of clay minerals in T5. The highest level of packed cell volume was obtained from the broiler fed the control diets. However, the normal range of packed cell volume is between 22 and 35% (Bounous and Stedman, 2000). White blood cell count was highest in T5 but lowest in T3. However, its normal range in broilers is between 12 and 30 × 10^3 µl (Bounous and Stedman, 2000). The low concentration of white blood cells in T3, below the normal range, implies that the clay formed by sprinter termites contains less antigenic substances that are capable of eliciting the production of antibodies in broiler.

Serum Biochemistry of Broiler Starter Fed Diets Containing Different Clay Sources

The serum biochemistry of broiler at starter stage fed diets containing different clay sources is presented in Table 4. There were significant differences ($p < 0.05$) in all the serum biochemical indices among treatment groups that were fed diets containing different clay sources.

Broilers fed T3 diets had the highest total protein while the lowest total protein was produced from broilers fed T1 diets. This suggest that T3 diets have greater potential of making the protein content in the feed to be released and made available for utilization by the broiler. The total protein value in broiler ranges between 30.00-49.00 g dl^{-1} (Meluzzi *et al*., 1992). The values obtained in this experiment are within the normal range in broiler. Albumin concentration and total cholesterol were also higher in T3. This may be as a result of the greater concentration of essential minerals in T3 diet. The glucose concentration was highest in broilers fed T2 diets and lowest in broilers fed T5 diets. Urea concentration was higher in broilers fed T5 diets and lowest in broilers fed T3 diets. Low density lipid was lowest in broiler fed T3 diets and high-density lipid was also highest in broiler fed T3 diets. The results reported by Bailey *et al*. (2006), Lotfollahian *et al*. (2004), Miles and Henry (2007) and Safaeikatouli *et al*. (2011) disagree with the findings in this experiment which showed a significant ($p < 0.05$) difference between the treatment diet and the control diet on total cholesterol. Triglyceride concentration was equally highest in broiler fed T5 diets and lowest in broiler fed T2 diets. The triglyceride values in the various treatments decreased from the value in the control diet, except for the value obtained in T5 which has a value higher than the value in the control treatment. This agrees with the findings of Safaeikatouli *et al*. (2011) who reported a significant reduction in the concentration of triglyceride in broiler fed diets containing clay.

CONCLUSION

The different clay sources that were used in this experiment did not affect the growth performance parameters but the haematological and serum biochemistry indices were highly affected. Treatment three containing clay formed by sprinter termites, improved the serum biochemistry as well as haematological indices of broiler better than the other sources of clay used in this experiment. This implies that clay in T3 had the greatest capacity to bind toxins contained in the feed due to its mineral content.

Table 4: Serum biochemistry of broiler starter fed diets containing different clay sources

Parameters	T1	T2 (cwh)	T3 (cst)	T4 (ctt)	T5 (chc)	SEM	P value
Total protein (g l^{-1})	32.07^c	33.03^b	37.10^s	32.77^{bc}	32.92^{bc}	0.49	0.00
Albumin (g l^{-1})	14.50^b	14.77^b	16.25^a	14.57^b	14.24^b	0.20	0.00
Glucose (mg dl^{-1})	11.10^b	12.46^a	11.60^{ab}	12.15^a	9.88^c	0.27	0.00
Cholesterol (mg dl^{-1})	2.46^a	1.76^b	2.75^a	1.72^b	1.75^b	0.13	0.00
Urea (mg dl^{-1})	0.83^b	0.58^c	0.55^c	0.76^{bc}	1.16^a	0.63	0.00
LDL (mg dl^{-1})	0.80^a	0.57^b	0.47^a	0.69^c	0.74^c	0.04	0.00
HDL (mg dl^{-1})	2.36^b	2.33^b	3.08^a	1.92^c	1.84^c	0.12	0.00
Triglyceride	0.60^b	0.31^d	0.45^c	0.35^d	0.71^a	0.04	0.00
Aspartate transaminase (µl)	17.08^a	14.70^c	15.99^b	16.67^{ab}	16.86^{ab}	0.26	0.00
Alanine transaminase (µl)	2.65^b	7.14^a	1.84^c	2.45^b	1.44^c	0.55	0.00

$^{a-b-c};^{ab-bc}$ Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM - standard error of mean,
T1 - Control Diet, T2 - Treatment with Clay by Wasp, T3 - treatment with clay by sprinter termite,
T4 - treatment with clay from tree trunk, T5 - treatment with clay from hill top with cap

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