Nigerian J. Anim. Sci. 2022, 24 (1): 181-189

Effects of supplementing broiler feed with coconut shell charcoal on their serum biochemical and haematological parameters, total coliform and bacterial counts

Enyenihi G. E., Ogbuzuru E. N. and Ojukwu M. E.

Department of Animal Science, University of Uyo, Uyo, Akwa Ibom State

Corresponding Author: *gloryenyenihi@yahoo.com,* ***Phone Number:*** *08028259884*

Target Audience: *Animal scientists and farmers*

Abstract

This study was conducted to determine the effects of supplementing broiler feeds with different levels of Coconut Shell Charcoal (CSC) on the serum biochemistry, haematological parameters and gut microbial count (Total coliform count; TCC and Total Bacterial Count; TBC). The experiment was conducted with a total of 90 unsexed Day-Old broiler chicks of Ross 308 strains and it lasted for fifty-six days. The chicks were randomly allotted to six different treatments (T1, T2, T3, T4, T5 and T6, containing 0%, 2%, 4%, 6%, 8% and 0% CSC respectively) with each having 15 birds and each treatment further divided into three replicates of five (5) birds in a Completely Randomized Design (CRD). While the T1 and T6 did not have coconut shell charcoal, the treatment 6 was administered antibiotics when necessary (this would serve as a marker in the measure of blood parameters and microbial load). The result from the analysis of these data showed that the supplementation of the feed with charcoal, significantly affected (P<0.05) only a few of the serum (Aspartate Aminotransferase, Alanine Aminotransferase) and haematological parameters (Platelets). However, there was a significant difference (P<0.05) in the TCC and TBC but the counts were higher in the treatments with charcoal than the control. Therefore, irrespective of the fact that the use of coconut shell charcoal at these levels was not detrimental to the birds with respect to haematology and serum biochemical parameters, its use at these levels (2%, 4%, 6%, and 8%) is not recommended since these levels were suspected to increase the gut bacterial and coliform count of the broiler birds. The total white blood cell was highest in the treatment six which entails that administering antibiotics was a better way of boosting immunity than the coconut shell charcoal.

Keywords: *Broilers, Haematology, gut health, total bacterial count*

Description of problem

More emphasis than ever is placed on global food security,[1], owing to the generally expected global human population projected to increase to 10 billion people by 2050 [2]. There is the need for adequate and nutritious food supply for the teeming human population, through sustainable production systems.

Poultry meat offer considerable potential in bridging the gap between supply and demand for animal protein especially in developing countries like Nigeria [3]. However, in the country, the livestock industry is faced with a lot of challenges amongst which are; inadequate nutrition, high cost of feed, poor-quality feed etc. [3]. The problem as relating to feed quality can be attributed to the available feed ingredients, the method of feed processing, storage etc.

Sustainable poultry meat and egg production is important to provide safe and quality protein sources in human nutrition

worldwide, hence the gut health becomes very important. The gastrointestinal (GI) tract of chicken harbor diverse and complex micro biota that plays a vital role in digestion and absorption of nutrients, immune system development and pathogen exclusion. However, the integrity, functionality, and health of the chicken gut depend on many factors including the environment, feed, and the GI microbiota.

Attempts have been made to reduce the risk of mycotoxin in poultry feed which are secondary metabolites of fungi, especially by species of Aspergillus, Penicillium, and Alternaria. The addition of mycotoxins binders to contaminated diets has been considered the most promising dietary approach to reduce effects of mycotoxins [4]. The theory is that the binder decontaminates mycotoxins in the feed by binding them strongly enough to prevent toxic interaction with the consuming animals and prevent mycotoxin absorption across the digestive tract. Inclusion of biochar in poultry nutrition has also been reported to rapidly decrease the incidence of diarrhea, eliminate allergies and ameliorate the detrimental effects of mycotoxins in feed [5]. A recent approach has been the addition of non-nutritive adsorptive material to the diet in order to reduce the absorption of mycotoxins from the gastro intestinal tract (GIT).

Also, in view of the increasing restrictions in most countries on the use of antibiotics in livestock feeds, studies have been carried out on other additives such as probiotics and organic acids [6,7]. Most of these additives are proprietary products and not within the reach of farmers in poor countries. It is therefore necessary to research on alternatives which are locally available. In this light, a recent study in Cameroun [8] showed a curvilinear trend indicating the improvement in feed intake (6.3%), live weight gain (14%) and Feed Conversion Ratio (by 9%) when the feed of Arbor Acres broilers birds were supplanted with coconut shell charcoal. Other studies have shown that activated charcoal (a similar product to biochar) can adsorb toxins from feed [9,10].

Furthermore, carbon is able to adsorb microorganisms, for example *Escherichia coli* [11] and *Salmonella* species [12] and helps in the treatment of *Cryptosporidium parvum* in goat kids and calves. As *Escherichia coli* or *Clostridium perfringens* are some of the most important reasons for antibiotic medication in turkeys, charcoal might therefore improve livestock intestinal overall health [13].

In the production of charcoal, wood and coconut shell are most commonly used. This might be as a result of their hardness, volatile content and relatively high density [14]. The use of coconut shell in this research was therefore to ascertain how this porous carbon substance would effectively affect the health of the broiler with respect to the bacterial count, ultimate pH, serum and haematological parameters of the broiler chicken.

Materials and method

Experimental site

The experiment was carried out in the Poultry Unit of the University of Uyo Teaching and Research Farm, which is located at the Annex campus of the University**.** The area falls within the tropical rainforest zone in Nigeria, with two distinct seasons. The raining season lasts between March and Mid November while the dry season lasts between November and March). It has an average rainfall range of 2200mm to 3500mm. the temperature of the area ranges from 26^0C to 28^0C. Uyo is located

between latitude $4^0$31 and $6^0$30 North and longitude $7^0$30 and $8^0$20 east of the equator [15].

Processing of coconut shell charcoal

The coconut shell charcoal was processed from mature coconut shell that were sourced locally from eateries, women who sell coconut along the roads, followed by sourcing for a metal drum with cover and preparation of the metal drum by perforating the bottom of the drum. The properly washed and dried coconut shell were processed into charcoal by loading them into the metal drum that had perforation at the bottom, preparation of the burning furnace (with wood fuel), cooking the dried coconut shell in the drum for one hundred and twenty four (124) minutes at a very high temperature, cooling of the already black shell after everything has turned charcoal, selection and removal of incompletely burned shells and finally the charcoal was milled using 2mm sieve.

Experimental birds and management

A total of ninety (90) unsexed day-old chick of Ross 308 strains of broiler bought from a vendor in Uyo, were used for the trial. The birds were managed in a deep litter system during the brooding and rearing phases and wood shaving was used as the litter material. The birds were kept for eight (8) weeks with the first four (4) weeks as the starter and the last four weeks as the finisher phases. The chicks were randomly divided into six (6) treatments groups (T1, T2. T3, T4, T5 and T6), with 15 birds each per treatment. Each of the six (6) treatment was further divided into three (3) replicates. Five birds were randomly assigned to each of the replicates in a Completely Randomized Design (CRD).

They were fed formulated feed which had the inclusion of coconut shell charcoal. Although the T1 and T6 did not have CSC, the T6 were administered antibiotic orally when necessary during the rearing stage to ascertain the effect of the CSC on the blood parameters and the coliform and bacterial count on the other parameters. The other ingredients were adjusted to meet the nutrient requirement of both the starter and the finisher phases. The starter composed of the first four weeks while the finisher phase counted from the fifth week. They were grouped into the different experimental groups and fed the experimental diet from day old. The ingredient composition and the calculated chemical composition of both the starter and the finisher diets are presented in Table 1 and 2.

Data collection

At the expiration of the experiment, blood samples were taken from three (3) birds per replicate via jugular venipuncture using a 20-gauge syringe. Blood for serum biochemical analysis was collected. They were taken to the laboratory in less than one hour after collection and analysis was carried out within 72 hours. Blood for haematology analysis was collected in 5ml vials containing potassium Ethylenediamine tetra acetic acid (EDTA), as anti-coagulant and a fresh blood smear made for cytological analysis. The sample was sent to the laboratory in less than one hour after collection and analysis was carried out between 24 and 72 hours of collection.

The content of the gut of the broilers were collected in laboratory bottles and sent to the laboratory for analysis to ascertain the microbial load (TBC and TCC).

Data analysis

Data generated from the haematology, serum biochemical analysis, crop pH,

duodenum pH and bacterial count were subjected to analysis of variance (ANOVA) while significant means were compared using the Duncan New Multiple Range Test (DNMRT) as outlined by [16].

Table 1: Ingredient and Calculated Nutrient Composition of Experimental Broiler Starter Diet

Ingredients (%)	T1 (0% CSC)	T2 (2% CSC)	T3 (4% CSC)	T4(6% CSC)	T5(8% CSC)	T6(0%, antibiotic)
Maize	49.10	46.65	44.19	41.74	39.28	49.10
Soybean meal	29.90	30.35	30.81	31.36	31.72	29.90
Palm kernel cake	8.00	8.00	8.00	8.00	8.00	8.00
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Wheat Offal	4.00	4.00	4.00	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
CSC*	0.00	2.00	4.00	6.00	8.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vit. Premix**	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Proximate Composition (%DM)						
Crude Protein	24.00	24.00	24.00	24.00	24.00	24.00
Ether extract	4.16	4.08	4.00	3.91	3.83	4.16
Crude Fibre	4.62	4.58	4.54	4.51	4.47	4.62
Ash	3.92	3.92	3.91	3.91	3.90	3.92
NFE***	63.30	63.42	63.67	63.67	63.80	63.30
Phosphorus	0.53	0.53	0.52	0.52	0.51	0.53
Calcium	0.24	0.24	0.24	0.24	0.24	0.24
ME****	2819.03	2745.84	2672.54	2599.34	2526.05	2819.03

*Premix supplied per Kg starter diet: vitamin A 15,000 i.u., vitamin D3 13,000 i.u., thiamine 2mg, riboflavin 6mg, pyridoxine 4mg, cobalamine 0.05g, biotin 0.08mg, choline chloride 0.05g,
CSC– Coconut Shell Charcoal; Vit Premix - Vitamin Premix; NFE- Nitrogen Free Extract; ME- Metabolizable Energy

Result and Discussion

Serum biochemical indices of broilers fed CSC

The result of the influence of charcoal supplementation in broiler chicken on the serum Biochemistry is presented in Table 3. The results showed that there were no significant differences ($P>0.05$) in the total ions (except Potassium), total protein, serum pH, Aspartate Aminotransferase (AST) and Glucose parameters, between the control and the other treatments having different levels of CSC. The value of potassium ion in the control was higher and significantly different ($P<0.05$) from T2, T3, T4, and T5. This could have been as a result

of the charcoal. Alanine Aminotransferase (ALT) results showed that there was significant difference (P<0.05) in the value of ALT only in T2 and T5. In the Alkaline phosphatase level (ALP), the control had the highest alkaline phosphatase level and was significantly different (P<0.05) from all the treatments except T5, but the treatments with charcoal had lower value of ALP than the control, thus differing from the report by [3,17] where treatments fed charcoal had higher ALP and linked to charcoal's ability to adsorb toxins. The Value of the Total Bilirubin (TBIL) showed that the highest TBIL value was recorded in the control and there was a significant different (P<0.05) among all treatments containing CSC. The difference in the Conjugated Bilirubin (CBIL) value of the control was only significant (P<0.05) in T2. For the Serum albumin (ALB), the control was only significantly different (P<0.05) from T4. This shows therefore that the coconut shell charcoal supplementation was not significant in majority of the parameters measured.

Table 2: Ingredient and Calculated Nutrient Composition of Experimental Broiler Finisher Diet

Ingredients (%)	T1 (0% CSC)	T2 (2% CSC)	T3(4% CSC)	T4(6% CSC)	T5(8% CSC)	T6(0%, antibiotic)
Maize	56.24	53.79	51.46	48.42	46.42	56.24
Soybean meal	21.76	22.21	22.54	23.64	23.58	21.76
Palm kernel cake	10.00	10.00	10.00	10.00	10.00	10.00
Wheat offal	5.00	5.00	5.00	5.00	5.00	5.00
Fishmeal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
CSC*	0.00	2.00	4.00	6.00	8.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Vit. Premix**	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Proximate composition (% DM)						
Crude Protein	19.9	20.00	19.94	20.17	20.00	19.99
Crude fibre	4.57	4.53	4.50	4.48	4.42	4.57
Ether extract	4.17	4.12	4.04	3.95	3.88	4.17
Ash	3.35	3.34	3.33	3.36	3.32	3.34
Calcium	1.25	1.28	1.28	1.29	1.29	1.28
Phosphorus	1.36	1.36	1.36	1.35	1.34	1.36
NFE***	65.1	65.32	65.55	65.4	65.75	65.1
ME****	2,876.01	2803.81	2730.83	2651.06	2582.56	2876.01

**Premix supplied per Kg finisher diet: vitamin A 10,000 i.u., vitamin D3 12,000 i.u., vitamin E 20 i.u., vitamin K 2.5mg, thiamine 2.0mg, riboflavin 3.0 mg, pyridoxine 4.0mg, niacin 20mg, cobalamin 0.05mg,

CSC– Coconut Shell Charcoal; Vit Premix - Vitamin Premix; NFE- Nitrogen Free Extract; ME- Metabolizable Energy

Table 3: Serum Biochemistry of the Broilers Fed Coconut Shell Charcoal (CSC)

Parameters	T1 (0%)	T2 (2%)	T3 (4%)	T4 (6%)	T5 (8%)	T6 (0%)	SEM
K^+ (mmol/L)	4.80^{ab}	4.10^{d}	4.70^{bc}	4.30^{cd}	4.20^{d}	5.20^{a}	0.15
Cl^- (mmol/L)	98.00^{ab}	96.00^{ab}	90.00^{ab}	102.00^{a}	103.00^{a}	105.00^{a}	2.73
HCO_3 (mmol/L)	17.00^{a}	18.00^{a}	16.00^{a}	18.00^{a}	18.00^{a}	18. 00^{a}	2.05
Na (mmol/L)	122.00	121.00	122.00	121.00	119.00	122.00	2.69
Ca^{++} (mmol/L)	2.30	2.40	2.40	2.40	2.40	2.40	0.19
AST (IU/L)	13.00^{a}	16.00^{a}	7.00^{b}	7.00^{b}	14.00^{a}	6.00^{b}	1.56
ALT (IU/L)	6.00^{b}	12.00^{a}	6.00^{b}	6.00^{b}	10.00^{a}	5.00^{b}	0.91
ALP (IU/L)	12.00^{a}	9.00^{bc}	7.00^{bc}	7.00^{bc}	10.00^{ab}	6.00^{c}	0.91
TBIL (umol/l)	7.20^{a}	5.60^{bc}	5.90^{bc}	6.40^{b}	5.70^{bc}	5.10^{c}	0.26
CBIL (umol/l)	3.20^{a}	2.50^{b}	3.00^{ab}	3.00^{ab}	3.40^{a}	2.40^{b}	0.20
TP (g/l)	36.00^{a}	33.00^{a}	35.00^{ab}	38.00^{a}	32.00^{a}	34.00^{a}	2.45
ALB (g/l)	22.00^{b}	21.00^{b}	22.00^{b}	29.00^{a}	20.00^{b}	18.00^{b}	1.31
Glucose	14.00^{ab}	12.00^{ab}	14.00^{ab}	9.00^{b}	12.00^{ab}	16.00^{a}	1.83
pH	6.00	7.00	6.00	6.50	6.50	7.00	0.76

^{abc}. Means along the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean

key: K^+ - Potassium ion, Cl^- - Chlorine ion, HCO_3 – Bicarbonate, Na^+ - Sodium ion, Ca+ - Calcium ion, AS – Aspertate aminotransferase, AlT – Alanine Amionotransferase, ALP – Alkaline phosphatase level, TBIL – Total Bilirubin count, CBIL – Conjugated Bilirubin count, TP – Total protein, ALB – Albumin.

Haematological indices of broilers fed CSC

Blood analysis is a readily available tool for assessing the clinical and nutritional status of animals on a feeding trial [18]. Usually, animals that have good blood composition tend to possess records of improved performance [19]. The result of the haematology for this experiment is presented in Table 4 below. There was no significant difference ($P>0.05$) in the values of the Lymphocytes (LYM), Neutrophils (NEUT), MID, Red Blood Cell (RBC), Haemoglobin (HB), PCV, MCV, MCH, MCHC. However, there was a significant difference ($P<0.05$) in the platelet values in all the treatments. Equally, there was a significant difference ($P<0.05$) in the Total White Blood Cell (TWC) value of the birds that were fed 0% CSC plus antibiotics (T6). Higher value of TWBC indicates that better defense against foreign bodies and as such Coconut shell charcoal was had lower of such effect. Equally, the control had a higher TWBC value those with coconut shell charcoal except T4 that had 6% CSC.

This result contrasted the findings of [17] and that of [20] who reported significant differences in PCV, Hb concentration and red blood corpuscles values among the experimental birds. The results however conform to the findings of [21], whose study showed that dietary supplementation of 0.3% charcoal did not have significant effect on hematological indices of turkey. [22] reported that bio-charcoals had no significant effect on RBC, WBC, haemoglobin and hematocrit values of broilers fed aflatoxin B1- contaminated diets. The study of [23] also showed that dietary inclusion of activated charcoal (bioachar) did not significantly affect RBC, haemoglobin or hematocrit values of Nile tilapia in a 28-day feeding trial.

Table 4: Haematology of the broilers fed CSC

Parameters	T1 (0%)	T2 (2%)	T3 (4%)	T4 (6%)	T5 (8%)	T6 (0%)	SEM
TWBC	63.23^{b}	53.03^{b}	55.80^{b}	63.86^{b}	59.16^{b}	77.50^{a}	3.72
LYM	90.70	89.43	88.03	88.70	89.40	87.20	1.68
NEUT	1.76^{b}	2.06^{b}	2.33^{b}	2.43^{b}	1.86^{b}	3.80^{a}	0.39
MID	7.53	8.56	9.66	8.86	8.73	8.80	1.44
RBC	2.40	2.43	2.53	2.73	2.60	2.46	0.17
HB	11.56	11.60	12.40	13.00	12.26	11.43	0.74
PCV	30.66	31.00	31.33	34.33	32.00	30.66	1.99
MCV	127.30	127.63	127.63	125.96	126.83	125.23	2.50
MCH	47.13	47.30	47.30	47.23	48.00	45.93	0.97
MCHC	37.13	36.80	37.20	37.73	37.93	37.03	0.59
PLT	63.00^{a}	29.00^{c}	37.33^{bc}	31.33^{c}	30.67^{c}	48.33^{b}	4.46

^{abc}. Means along the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean

key: TWBC – Total white blood cell, LYM – Lymphocytes, NEUT – Neutrophils, RBC - Red Blood cell, HB – Haemoglobin, PCV – Packed Cell Volume, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC- Mean corpuscular Haemoglobin concentration, PLT – Platelet Count.

Total Bacterial Count (TBC) and Total coliform count (TCC)

The results showed that there was a significant difference ($P<0.05$) in TBC with the control having the lowest bacterial count. This shows that charcoal was not effective in the adsorption of bacterial in the gut of the broiler birds. The bacterial count increased with increasing charcoal level. This was against the expected results as charcoal inherently possess adsorptive properties. We suspect this to have been as a result of the method of processing the coconut shell charcoal, air drying after burning may have exposed the charcoal to microorganisms.

Just like in the TBC, there was a significant difference in the TCC but there was no order because the birds fed 6% CSC had zero coliform count and those fed 8% CSC has the second lowest coliform count and lower than the control. The guts coliforms and general bacterial of the birds across the treatments were similar ($P>0.05$). E. coli, Bacillus spp and Proteus spp was present in all the treatments just like in the control. Other microbes found in the treatment were streptococcus spp, Aeromonas spp, Proteus vulgaris, Bacillus licheniforms, Citrobacter spp, Enterobacter spp. Equally Yeast cells were discovered in the gut content of birds fed 2% and 6% charcoal supplementation. These support the studies by [11], that found activated charcoal to strongly adsorb E. coli 0157:H7while adsorption for naturally occurring gut microbes; Enterococcus, Bifidobacterium and Lactobacillus was less strong. But it was contrasting to the findings by [24], that found fewer E. coli and salmonella in the faecal microflora of chickens with activated charcoal and wood vinegar supplementation in their diets.

Table 5: Total coliform count (TCC) and Total bacterial count (TBC) of the broilers fed CSC

Parameters	T1 (0%)	T2 (2%)	T3 (4%)	T4 (6%)	T5 (8%)	T6 (0%)	SEM
TCC (cfu/g)	25.97^{d}	147.40^{b}	550.61^{a}	0.00^{f}	7.63^{e}	40.00^{c}	214.45
TBC (cfu/g)	352.30^{d}	10853.33^{c}	59024.17^{b}	52588.97^{b}	88416.42^{a}	48.82^{e}	48541.41

^{abc}. Means along the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean

key: TCC – Total Coliform Count, TBC – Total Bacterial Count

Conclusion and Application

From the result obtained

1. The dietary supplementation of coconut shell charcoal does not have any detrimental effect on broiler birds.
2. Coconut shell charcoal was not effective in the adsorption of gut microbes at the levels used in this experiment but was even found to increase the gut microbes.

References

1. Onibi G. E, Oluwatoyin E. A, Adebowale N. F., Ayodeji V. A. (2009) Responses of Broiler Chickens in terms of performances and meat quality to garlic (Allium sativum) supplementation. *African Journal of Agricultural Resources*. 4(5):511-517.
2. FAO. How to Feed the World in 2050. Rome: FAO; (2009a). [August 28, 2014]. http://www.fao.org/fileadmin/templates/wsfs/docs/expertpaper/How_to_Feed_the_World_in_2050.pdf
3. Jiya, E.Z., Ijaiya, A.T., Ugochukwu, A., and Tsado, D. (2013). Effect of Activated Coconut Shell Charcoal Meal on Growth Performance and Nutrient Digestibility of Broiler Chickens. *British Journal of Applied Science*. 3 (2): 268-276.
4. Afzal M, Zahid A. (2004) Effect of addition of a mycotoxin detoxifier in poultry feed containing different levels aflatoxin on the performance of broiler. Asian – *Australia Journal of Animal Science*. 17(7):990-994.
5. Marie C. I. (2013). Biochar as a feed supplement. An overview. *International Journal of Livestock Research* 9 (1): 58-72.
6. Ahmad I. (2006) Effects of probiotics on broilers performance. *International Journal of Poultry Science*, 5 (6): 593-597.
7. Murry A.C., Murry A.C. Jr., Hinton A. and Morrison H. (2004). Inhibition of growth of Escherichia coli, Salmonella typhimurium and Clostridia perfringens on chicken feed media by Lactobacillus salivarius and Lactobacillus plantarum. *International Journal of Poultry Science* 3 (9): 603-607
8. Lan, Rui and Li, Si and Zhihui, Zhao and An, Li. (2020). Sodium butyrate as an effective feed additive to improve growth performance and gastrointestinal development in broilers. *Veterinary Medicine and Science.* 6 (3): 1-9.
9. Cooney, W.T., J.S. Butts and L.E. Bacon, 1948. Alfalfa meal in chick rations. Poult. Sci., 27: 828-830.
10. Neuvonen P, J and Olkkola K. T (1988). Oral activated charcoal in the treatment of intoxications. Role of single and repeated doses. *Medicinal Toxicology*. 3: 33-58.
11. Naka K., Watarai S., Tana, Inoue K., Kodama Y., Oguma K., Yasuda T. and Kodama H. (2001). Adsorption effects of activated charcoal on

enterohemorrhagic *Escherichia coli. Journal of Veterinary Medical Sciences.* 63(3): 281-285.
12. Watarai, S., Tana, and Koiwa, M. (2008). Feeding activated charcoal from bark containing wood vinegar liquid (nekka-rich) is effective as treatment for cryptosporidiosis in calves. *Journal of dairy science*, 91(4), 1458–1463.
13. Houghes, L., Hermans, P., and Morgan, K. (2008). Risk factors for the use of prescription antibiotics on UK broiler farms. *The Journal of antimicrobial chemotherapy*, 61(4), 947–952. https://doi.org/10.1093/jac/dkn017
14. Zarifah M. S. (2010). To produce the activated carbon from matured palm kernel shell. Bachelor Thesis, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Malaysia.
15. University of Uyo Meteorological station. (2014).
16. Obi I. U. (2002). Statistical Methods of Detecting Differences between Treatment Means and Research Methodology Issues in Laboratory and Field Experiments. AP Express Publishers, Limited, 3 Obollo Road, Nsukka-Nigeria, p. 117
17. Kutlu H. R., Unsal I, Gorgulu I., (2000). Effect of providing directory wood (oak) charcoal to broiler chicks and laying hens. *Animimal Feed Science Tecnol.* Elsevier 90:213-226.
18. Olabanji R. O. Ojebiyi O. O. Tona G. O. and Olaogun O. (2009). Haematological and serum biochemical response of growing rabbits fed diets containing processed mango (Mangifera indica) seed kernel meal. Proceedings of the 14th Annual Conference of Animal Science Association of Nigeria, September 14-17. Pp. 170-273.
19. Isaac, L. J., Abah, G., Akpan, B., and Ekaette, I. U. (2013). Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria: 24-27.
20. Iheukwemere F.C. and Herbert U. (2002). Physiological responses of broiler chickens to quantitative water restrictions: haematology and serum biochemistry. *International Journal of Poultry Science*, 2 (2), 117-119
21. Majeswka T., Mikulski D., and Siwik T. (2009). Silica grit, charcoal and hardwood ash in turkey nutrition. *Journal of Element* 14 (3), 489-500.
22. Kana J. R., Teguia A., Mungfu B. M., and Tchoumboue J. (2010). Growth performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of charcoal from maize cob or seed of canarium. *Tropical Animal Health and Production*, 43 (1),51–56
23. Boonanuntanasarn S. Khaomek P. Pitaksong T. and Hua Y. (2014). The effects of the supplementation of activated charcoal on the growth, health status and fillet composition-odor of Nile tilapia (Oreochromisniloticus) before harvesting. *Aquacult.Int.,* 22, 1417–1436
24. Rattanawut J., Moe S. R., Skobic D., Knezovic Z., Rozic I., Zivkovic M. and Pavlicevic J. (2009). Effects of supplementation with barley and activated charcoal on intake of biochemically diverse Mediterranean Shrubs. Small Ruminant Resources 81:79-84.