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TOXICITY OF COPPER SULPHATE AND BEHAVIORAL LOCOMOTOR RESPONSE OF TILAPIA (*Oreochromis Niloticus*) AND CATFISH (*Clarias Gariepinus*) SPECIES

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ABSTRACT: *Acute toxicity of copper sulphate ($CuSO_4.5H2O$) to tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus) species was investigated using toxicity index of 96 hours LC_{50} and the quantal response determined by the statistical probit analysis method. In response to the lethality of the copper toxicant, behavioral anomalies (locomotor response) of the exposed fish species were studied as indication of toxic effects of the heavy metal. Fish species shows different mortality responses to the varying concentrations of copper studied (50, 60, 70, 80, 100, and 120 mg/l) due to toxicity. Copper was significantly (no overlap in 95% C.L of 96 hrs LC_{50} values) more toxic to Oreochromis niloticus than the catfish. 96 hrs LC_{50} values for Oreochromis niloticus and Clarias gariepinus were revealed to be 58.837 and 70.135 mg/l, respectively. Behavioral changes, mostly locomotor responses (avoidance) were observed among the test animals on exposure to the different concentrations of copper sulphate. There is need to control the use of copper because of its observed toxicity and fish avoidance test shows to be an important predictive and sensitive biomarker in aquatic monitoring and pollution management.*

ORIGINAL ARTICLE

Keywords: *Heavy metal toxicity, 96 hrs LC_{50}, biomarker, dose-response effect.*

INTRODUCTION

Copper is highly toxic in aquatic environments and has effects in fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity (U.S EPA, 1993; Horne and Dunson, 1995). Copper will bio concentrate in many different organs in fish and mollusks. While mammals are not as sensitive to copper toxicity as aquatic organisms, biomagnifications play critical role in their toxicity. Toxicity in mammals include a wide range of animals and effects such as liver cirrhosis, necrosis in kidneys and the brain, gastrointestinal distress, lesions, low blood pressure and fetal mortality (ATSDR, 1990; Kabata-Pendias and Pendias, 1992; Ware, 1983; Vymazal, 1995).

Khangarot et al. (1982) demonstrated that Hg^{2+} was the most toxic (96 hrs LC_{50} -0.023 mg/L) followed by Cu^{2+} (0.034 mg/L), Cr^{6+} (5.97 mg/L), and Zn^{2+} (10.49 mg/L) when the metallic compounds were tested singly against the freshwater Pulmonate snail, *Lumnaea acuminate* in Indonesia. Khunyakari et al. (2001) investigated toxicity of nickel, copper, and zinc in *Poecilia reticulata.* Heavy metal exposure caused increased mucus like secretion over gills, excessive excretion, anorexia and increased fin movement. Copper was found to be the most toxic followed by zinc and nickel.

Oyewo (1998) also tested some prominent metals found in the industrial effluents against five animal species namely; Cypris sp., Mugil sp. Tilapia sp, *Nerita senegalensis*, and *Clibanarius africanus* that normally inhabit the Lagos Lagoon. The author reported that the values on the general order of toxicity of the test metals was Hg, Cu, Mn, and Fe when tested separately against each of the Lagos lagoon species listed above. Consequently, in Nigeria, Oyewo (1998) reported that when heavy metals such as Fe, Mn, Cu, and Hg were tested against some estuarine macrofauna, the order of tolerance of the species were Cypris sp. followed by Mugil sp. Tilapia sp. *Clibanarius afrinuas*, *Nerita senegalensis* and *Tympamotomus fusatus* as the most tolerant species tested in a descending order of sensitivity.

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Behavioral changes represent a higher organizational level of biomarker than any considered so far (Walker *et al*., 2003). One of the early proponents of the value of behavioral toxicology stated that 'the behaviour of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioural parameter is generally more comprehensive than a physiological or biochemical parameter (Walker et al., 2003). Behavioral test that are most advanced are those involving fish. The fish avoidance test is well established in the laboratory as a means of showing effects well below the lethal range. Recent studies include many on the effects of heavy metals. If one compares the lowest observed effect concentration (LOEC) obtained from behavioural studies (avoidance, attractance and fish ventilation) with chronic toxicity studies, one finds that some of the behaviour tests are more sensitive than life cycle or early stage tests (Walker et al., 2003). The perception of motion is important for the survival and reproduction of many animals including fish (Albensi and Powell, 1998). In the laboratory, support for this idea comes from the observation that any fish show a tendency to follow a series of stripes revolving around a circular aquarium (Albensi and Powell, 1998).

Test involving a variety of locomotor behaviors have been insufficiently studied especially with respect to heavy metal lethality to enable a judgment of their sensitivity or utility. In response to the above fact, the present work investigates the lethality (LC_{50}) of the varying concentrations of copper sulphate ($CuSO_4.5H_2O$) and the behavioural locomotor response and changes of the two fish species (*Oreochromis niloticus* and *Clarias gariepinus*) exposed as the most sensitive indication of potential toxic effects.

MATERIALS AND METHODS

Healthy adult fish species (*Oreochromis niloticus* and *Clarias gariepinus*) were obtained from a commercial hatchery and brought to the laboratory within in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for 30-35 minutes for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial feed diet and minced liver trice a day during this period. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

Chemically pure salt of zinc sulphate ($CuSo_4.5H_2O$) dissolved in distilled water, was used as toxicant. The test organisms were subjected to different concentrations (50, 60, 70, 80, 100 and 120 mg/l) of the copper sulphate ($CuSO_4.5H2O$). For the acute bioassay tests, 20 fish were used per concentration. The containers were not aerated at the dosing time. The amount of copper sulphate to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the copper sulphate; keeping all other conditions constant. Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness, and pH) used in the aquaria were periodically determined before the bioassay tests. The water temperature was kept 27 ± 2.0 ^{0}C. In addition, the experimental medium was aerated in order to keep the amount of oxygen not less than 6 mg/l.

All experiments were carried out for a period of 96 hrs period. The number of dead fish were counted every 24 hours and removed from the aquarium as soon as possible. The mortality rate was determined at the end of the 96th hour. No food was given to the fish during the experiments.

Toxicological dose-response data involving quantal response (mortality) following toxicity of copper on the test species, *Oreochromis niloticus* and *Clarias gariepinus* were determined by the use of Finney's Probit Analysis LC_{50} Determination Method (Finney, 1971). Mortality response of the fish species was taken to be when the animals sank to the bottom of the containers and became motionless. The rate of response determined at the end of the 96th hours. The index for toxicity measurement was LC_{50} and deductions were based on the 96 hours LC_{50};
TF (Toxicity factor) = this is used to measure the relative potency ratios =

$$\frac{LC_{50} \text{ of a compound X}}{LC_{50} \text{ of another compound Y}}$$

Significance in 95% confidence limit of the detected 96 hrs LC_{50} values were determined using the Chi-Square technique. The limit of significance was 0.05.

RESULTS AND DISCUSSION

Copper was found toxic to the test fish species with *Oreochromis niloticus* responding higher than *Clarias gariepinus*. Table 1 shows the 96 hours acute toxicity of copper sulphate to *Oreochromis niloticus* and *Clarias gariepinus*, respectively with Figures 1 and 2 displaying the Probit line graphs of the toxicity data for the test freshwater fish species. Various authors in different parts of the world including Nigeria (Khangarot and Ray, 1989; Mackie, 1989; Oyewo, 1998; Khunyakari et al., 2001) have similarly observed and recorded differential toxicity of heavy metal compounds against different test animals. The observed differences in the acting metal (copper) might be due to the physicochemical characteristics of the test medium (Cusimano et al., 1986; Solbe, 1984), species and ages of fishes used and their susceptibility rates to the test chemical, which resulted in their subsequent

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toxicity values. Consequently, the observed toxicity values of copper were shown to be less than those reported by Khangarot et al. (1982).

Table 1 - 96 hours acute toxicity of copper to fish species

Test Animal	96 hrs LC_{50} (mg/l)	96 hrs LC_5 (mg/l)	96 hrs LC_{95} (mg/l)	S.E	T.F
Oreochromis niloticus	58.837	19.627	176.375	1.424	1
Clarias gariepinus	70.135 (58.023-84.771)	21.481 (9.166-31.224	228.989 (157.545-536.575)	1.241	1.19

TF: Toxicity Factor

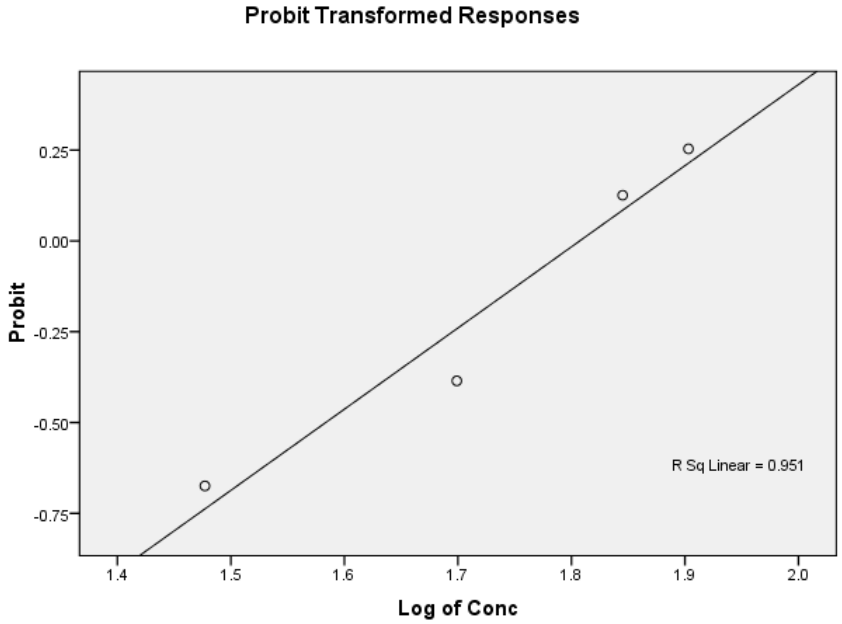

Fig. 1 - Probit line graph of acute toxicity of copper sulphate to tilapia *(Oreochromis niloticus)*

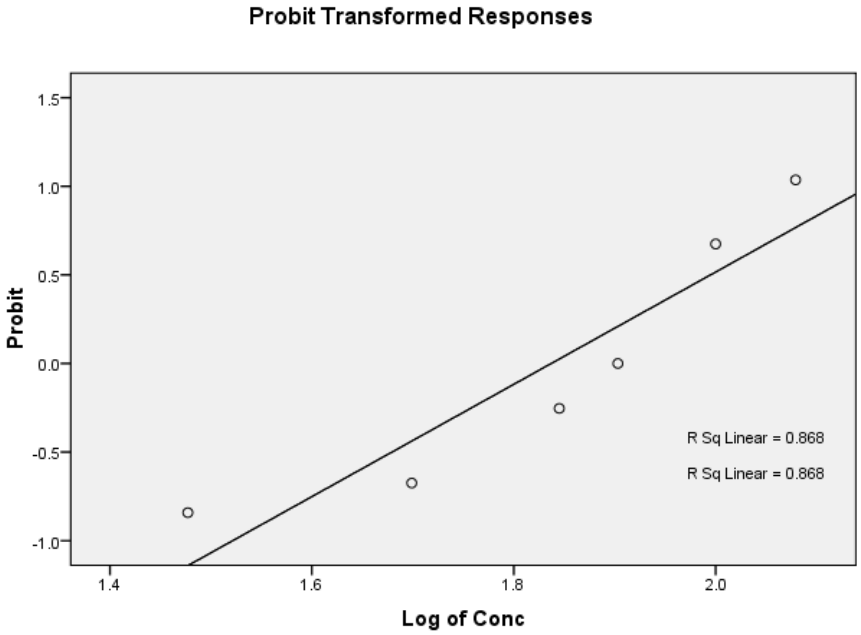

Fig. 2 - Probit line graph of acute toxicity of copper sulphate on catfish (*Clarias gariepinus*)

Copper was significantly (no overlap in 95% C.L of 96 hrs LC_{50} values) more toxic on *Oreochromis niloticus* than the catfish. Tables 2 and 3 portray the parameteric estimates of the Probit analysis, and Chi-Square test for the acute toxicity of the copper sulphate to *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Several workers such as Oyewo (1998) have demonstrated the relatively higher toxicity of the copper repeatedly and

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Otitoloju (2001) against local animal species of which the former author documented Tilapia sp. to be highly sensitive in relation to other aquatic animals employed in the study. Acute (LC_{50}) and sub lethal copper effects on adult fish physiological parameters or copper hazards to invertebrates have been extensively studied and reported by Hogstrand and wood (1996), Svecevicius and Vosyliene (1996), Khangarot (1989) and Eisler (1998). However, the reported LC_{50} values for this metal by the authors were lower than the values obtained in our study. Generally, there was corresponding increase in mortality response of the test fish species with increased exposure and time (Figures 1 and 2).

Table 2 - Parameter estimates of the Probit analyses for *Oreochromis niloticus* and *Clarias gariepinus*

Parameter		Estimate	Std. Error	Z	Sig.	95%Confidence Interval	
						Lower Bound	Upper Bound
Tilapia Species *(Oreochromis niloticus)*							
PROBIT[a]	Conc	3.637	0.796	4.570	0.000	2.077	5.196
	Intercept	-6.350	1.424	-4.460	0.000	-7.774	-4.926
Catfish Species *(Clarias gariepinus)*							
PROBIT[a]	Conc	3.201	0.669	4.783	0.000	1.889	4.513
	Intercept	-5.909	1.241	-4.759	0.000	-7.150	-4.667

[a] PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Table 3 - Chi-Square tests for 96 hrs LC_{50} values of *Oreochromis niloticus* and *Clarias gariepinus*

		Chi-Square	df[a]	Sig.
Tilapia Species *(Oreochromis niloticus)*				
PROBIT	Pearson Goodness-of-Fit Test	7.991	3	0.046[b]
Catfish Species *(Clarias gariepinus)*				
PROBIT	Pearson Goodness-of-Fit Test	3.941	4	0.414[c]

[a] Statistics based on individual cases differ from statistics based on aggregated cases; [b] Since the significance level is less than 0.050, a heterogeneity factor is used in the calculation of confidence limits; [c] Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

The behavioral changes observed among the fish species exposed to various concentrations of copper sulphate are as follow:

Experimental Groups

There was avoidance of the copper sulphate contaminated water through unsteady swimming pattern with jerky movements. Their fins became hard and stretched following high excitability. There was lost of balance and exhaustion. After period of stressful avoidance through various behavioral anomalies, fish remained suspended in vertical position with the mouth up, near the water surface and the tail pointing downward. Finally, they sank to the bottom of the water, became motionless, and did not respond to gentle probing.

Control Groups

There were no observable behavioral changes and death among the fish species during the bioassay. The theoretical spontaneous response rate was zero.

Obviously, the present investigation shows the behavioral anomalies and subsequent death of fish exposed to the heavy metal toxicant (copper sulphate). This could be explained by the fact that the toxic effect is mediated through the perturbed nervous systems, affecting almost all vital activities of the organisms, dopaminergic pathways, and related functions. The effects of pollution on behavior have been reviewed with primary reference to aquatic animals by Atchison et al. (1996), which should be consulted for further information. Neurological impairment has been observed in factory workers exposed to copper dust (ATSDR, 1990).

While emphasizing the importance and acute toxicity of copper to biological systems, there is indication that behavioral changes in fish are an adequate biomarker for pollution monitoring and management of aquatic environment. The employment of other fish species including invertebrates in different aquatic environments is highly recommended to determine their differential sensitivity and applicability in aquatic eco-toxicology and pollution management.

REFERENCES

Albensi BC and Powell JH (1998). The Differential Optomotor Response of the Four-eyed Fish Anableps anableps, Perception, 27 (12): 1475-1483.

To cite this paper: Ezeonyejiaku, C.D., Obiakor, M.O. and Ezenwelu, C.O. 2011. Toxicity of Copper Sulphate and Behavioural Locomotor Response of Tilapia (*Oreochromis niloticus)* and Catfish (*Clarias gariepinus)* Species. *Online J. Anim. Feed Res.,* 1(4): 130-134.
Journal homepage: http://www.ojafr.ir

Atchison GJ, Sandheinrich MB and Bryan MD (1996). Effects of Environmental Stressors on Interspecific Interactions of Aquatic Animals. In Newman, M.C and Jagoe, C.H (eds). Quantitative Ecotoxicology: A Hierarchical Approach. Chelsea, MI: Lewis.

ATSDR (1990). Toxicological Profile for Copper. U.S Public Health Service. Agency for Toxic Substances and Disease Registry, Atlanta, G.A.

Cusimano RF, Brakke DF and Chapman GA (1986). Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences, 43: 1497-1503.

Eisler R (1998). Copper Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. Biological Science Report USGS /BRD/BRS-1997-0002.

Hogstrand C and Wood CM (1996). The Physiology and Toxicology of Zinc in Fish, in Taylor AW, ed., Toxicology of Aquatic Pollution, Physiological, Molecular and Cellular Approaches. University of Cambridge, New York, NY, USA, 61-84.

Horne MT and Dunson WA (1995). Effects of Low pH, Metals, and Water Hardness on Larval Amphibians. Archives of Environmental Contamination and Toxicology, 29: 500-505.

Kabata-Pendias A and Pendias H (1992). Trace Elements in Soils and Plants, 2nd ed. CRC Press, Boca Raton. 365p.

Khangarot BS, Sehgal A and Bhasin MK (1982). Man and Biosphere-Studies on Sikkim Himalayas, Part 1: Acute toxicity of copper and zinc to common carp Cyprinus carpio (Linn.) in soft water, Acta Hydrochimica Hydrobiologica, 11: 667-673.

Khangarot BS and Ray PK (1989). Correlation between heavy metal acute toxicity Values in Daphina magna and fish. Bulletin of Environmental Contamination and Toxicology, 38: 722-726.

Khunyakari RP, Vrushali T, Sharma RN and Tare V (2001). Effects of some Trace Heavy Metals on Poecilia reticulate, Journal of Environmental Biology, 22 (2): 141- 144.

Mackie GL (1989). Tolerances of Five Benthic Invertebrates to Hydrogen Ions and Metals (Cd, Pb, Al). Archives of Environmental Contamination and Toxicology, 18: 1-2.

Otitoloju AA (2001). Joint action toxicity of heavy metals and their bioaccumulation by benthic animals in the Lagos lagoon. Ph.D. Thesis, submitted to the Department of Zoology University of Lagos, 231p.

Oyewo EO (1998). Industrial Sources and Distribution of Heavy Metals in Lagos Lagoon and their Biological Effects on Estuarine animals. Ph.D. Thesis Department of Marine Science. University of Lagos, 274pp.

Solbe JF (1974). The toxicity of zinc sulphate to rainbow trout in very hard water, Water Research, 8: 389-391. Svecevicius G and Vosyliene MZ (1996). Ekolojia (2): 17-21.

US EPA (1993). Wildlife Exposure Factor Handbook. Vol. 1 EPA/600/R-93/187a

Vymazal J (1995). Algae and Element Cycling in Wetlands. Lewis Pub., Boca Raton. 689p.

Walker CH, Hopkin SP, Sibly RM and Peakall DB (2003). Principles of Ecotoxicology, 2nd Edn. Taylor and Francis Group, Fetter Lane, London.

Ware G (1983). Pesticides, Theory and Application. W.H Freeman, New York. 308p.

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FALSE YAM *(Icacina Oliviformis)* LEAF MEAL AS AN INGREDIENT IN THE DIET OF WEANER RABBITS *(Oryctolagus Cuniculus)* TO IMPROVE BLOOD PROFILE

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ABSTRACT: *A 60 day feeding trial was conducted to determine the effect of Icacina oliviformis leaf (IOL) as a feed ingredient on the hematology of weaner rabbits. There were arranged in three treatments with four replicates in a completely randomized design. The control diet (T_0) contained 0% Icacina oliviformis leaf (IOL) while the treatment diets ($T_1$and T_2) contained 5% and 10% IOL, respectively. An amount of 200 g of the experimental diet was given to the animals each day while water was given ad-libitum. Initial blood samples were collected two days earlier before experimental diet was fed. Data were analyzed using Genstat Discovery Edition 3. There were no significant differences (P>0.05) in (Haemoglobin) Hb concentration, PCV, RBC however all the erythrocytes values increased from the initial low values to higher values. The margin of increase was higher for T_1. There were no significant differences (P>0.05) among the treatment means for WBC, Neutrophiles, Eosinophiles, Monocytes counts in the final readings. The hematology values recorded for all the treatments fell within the normal ranges for rabbits. Feeding 5% and 10% IOL to a weaner rabbits led to an increase in erythrocytes values and could be used in feeding without any detrimental effect.*

ORIGINAL ARTICLE

Keywords: *Haematology, Icacina Oliviformis, false yam, terpenes.*

INTRODUCTION

False yam plant is a perennial root crop belonging to the family *Icacinaeae*. In the year 1823 the plant was best known as *Icacina senegalinsis* by Adrein de Juss before later in 1875, the name changed scientifically to *Icacina oliviformis* and described by J. poiret but currently its English name is Icacina (Fay, 1987).

The aerial stems of the plant are highly green; leaves are simple, light green, measures about 5 -10 cm length. The fruit is bright and oval being covered with short hair, a thin layer of white pulp which is approximately 0.2 cm thick surrounding a single spherical seed (NRI, 1987). The Bright–red and plum–seeds are sweet and are usually consumed fresh (Fay, 1987). These seeds after roasting are pounded into flour and stored for use during time of food scarcity (NRI, 1987). Starch is produced from the tuber of Icacina and is used for commercial purpose that includes tapioca (Fay, 1987).

Several millions of people depend on its product as snack, staple and hunger food (Fay, 1987). In Ashanti region of Ghana, the tubers, leaves and stems are reported to be used as medicine (NRI, 1987). There is evidence that the tuber contains some anti-nutritional factors (ANFs) such as, terpenes (Vanhaelen et al., 1986). These anti–nutritional factors reduce its palatability as feed when given to animals (NRI, 1987). Due to the presence of these anti-nutritional factors, increase in the diets of animals, feed intake, growth rate and weight gain retrogresses in mono-gastric species (NRI, 1987).

The production of rabbits at the backyard and commercial level can help alleviate the problems of inadequate protein supply and intake as food. Their small body size and fast growth rate gives them a comparative advantage over ruminant and poultry. In 1994, world's production of rabbit meat was estimated to be 1.5 million tons per annum. This would mean per caput annual consumption of 280 g per person per year (Moreki, 2007).

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The study seeks to investigate the effects of *Icacina oliviformis* leaf meal as an alternative feed ingredient on the haematology of weaner rabbits.

MATERIALS AND METHODS

Study area

The study was carried out at the Livestock unit of the University for Development Studies–Nyankpala campus. Nyankpala is about 18 km west of Tamale in the Tolon–Kumbungu District. It is located in latitude 9° 25´41´´ N and longitude 0°58´42´´W at altitude 183 m above sea level (SARI, 2007). The area is in the Guinea Savanna Zone and characterized by unimodal rainfall pattern. Rains begin in April, rising to a peak in August–September and ending in October or November. Rainfall averages 1060 mm (NAES, 1994). Temperature ranges from as low as 15°C in January when the weather is under the influence of the North East (Harmattan) winds and as high as 42°C around the end of the dry season in March (SARI, 2007).

Experimental animals and design

Completely Randomized design was used in this experiment. There were three treatments with four replicates each. Twelve weaner rabbits which were about eight weeks old were selected randomly from different does (mothers) and were used for the experiment. The dimension of the hutch was 65 cm x 65 cm with wire mesh flooring. Initial blood samples were collected earlier before experimental diets were fed to the animals. Final blood samples were taken at the end of the experiment for analysis.

Experimental diets

Three experimental diets were formulated using the trial and error method and fed to the weaner rabbits twice a day. The control diet (T_0) contained 0% false yam leaf, while the treatment diets T_1 and T_2 contained 5% and 10% IOL respectively. Water was given *ad–libitum.* Table 1 shows the chemical composition and levels of the experimental diet fed to the animals. About 200g of the experimental diet was offered each day.

Table 1 - Composition of experimental diets (%)

Ingredient	Levels of *Icacina oliviformis* leaf (IOL) in diets (%)		
	T0 (0%)	T1 (5%)	T2 (10%)
IOL	-	5	10
Soyabean meal	16	16	16
Sheanut cake	15	15	10
Brewers spent grain	68	63	63
Premix	0.25	0.25	0.25
Dicalcium	0.25	0.25	0.25
Salt	0.5	0.5	0.5
Chemical Composition (%)			
Dry matter	93.2±1.9	93.4±0.8	93.2±2.7
Crude protein (DM)	17.3±13.5	16.1±4.5	16.9±1.4
Ether Extract (DM)	6.4±0.5	7.2±0.9	6.5±0.1
Ash (DM)	9.9±1.4	9.9±0.7	9.9±1.4
Organic matter	83.8±1.9	83.4±0.8	83.2±2.7

Collection and processing of *Icacina oliviformis*

Succulent false yam (*Icacina oliviformis)* leaves were harvested manually from the wild in Nyankpala and sun dried. The dried leaves were milled with hammer grounding mill to a coarse texture. A top pan scale was used to weigh the milled leaves .The processed false yam leaves were bagged and stored for use.

Blood sampling and analysis

The blood was collected in the morning between 7:00 am and 9:00 am using the ear–vine procedure (Radostits et al., 1994). The ear was pricked with a needle. A heparinised capillary tube was used to collect the blood to about 75% of its length. The capillary tube was then sealed with sealant and placed in a labeled container for analysis.

The parameters measured were; Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBC's), White Blood Cells (WBC's) total and White Blood cells differential (Baker and Silverton, 1990)

Statistical analysis

Data was analyzed using ANOVA from Genstat Discovery Edition 3. Means were compared using LSD and results presented in Tables.

RESULTS

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Results on the effects of IOL as a feed ingredient on the haematology of weaner rabbits are presented in table 2. Mean values of PCV increased from lower initial values to higher values after the feeding trial. There was no significant difference (P>0.05) among the three treatments in the final recordings. PCV for T_1 increased by (17.4%) representing the highest increase among the three treatments. Generally, mean values for RBC increased from initial lower values to higher values after the feeding trial for all treatments. Mean values among treatments showed no significant difference (P>0.05) in the final RBC counts. RBC in T_1 increased by 2.3 ^{x} 10^6/µl, the highest among the treatments means.

Treatment means showed no significant difference (P>0.05) in the final Hb concentration. T_0, T_1 and T_2 increased by 1.12 g/dl, 5.85 g/dl and 1.12 g/dl respectively. The final Hb concentration compared favorably with values reported by Njidda et al. (2006).

Mean total WBC values for T_0 decreased by 2.83 ^{x} 10^9L from the initial value of 11.76 ^{x} 10^9Lwhilst T_1 and T_2 had an increase of 2.37 ^{x} 10^9L and 5.37 ^{x} 10^9L, respectively. There was no significant difference (P>0.05) in the final total WBC. The values recorded for total WBC fell within the ranges reported by Archetti et al. (2008) for normal post weaned rabbits. Mean lymphocytes and eosinophiles all increased in T_1 and T_2 compared to T_0. Neutrophiles decreased by 2.6% in T_0 as compared to 6.1% and 9.7% in T_1 and T_2, respectively. However there was no significant difference (P>0.05) among the treatments. Basophiles and Monocytes all decreased from an initial higher value to lower values in T_1 and T_2.

Table 2 - Initial and final haematological values of weaner rabbits fed varying levels of IOL

Parameters	T_0 (means ±SD)	T_1 (means ±SD)	T_2 (means ±SD)	SED
Initial Packed Cell Volume (%)	31.40 ±3.71^{a}	17.32 ±4.34^{b}	29.62 ±2.17^{a}	2.31
Final Packed Cell Volume (%)	38.40 ±3.21	34.80 ±4.34	32.8 ±2.17	3.06
Initial Haemoglobin (g/dl)	11.68 ±3.20^{a}	5.77 ±2.48^{b}	9.80 ±0.72^{a}	1.33
Final Haemoglobin (g/dl)	12.80 ±1.04	11.62 ±2.48	10.92 ±0.72	1.02
Initial Red Blood Cell (x 10^6/µl)	4.10 ±0.48^{a}	2.23 ±0.99^{b}	4.00 ±0.29^{a}	0.28
Final Red Blood Cell (x 10^6/µl)	4.99 ±0.42	4.56 ±0.99	4.28 ±0.29	0.41
Initial Total White Blood Cell (x 10^9l)	11.76 ±2.26^{a}	5.07 ±3.66^{b}	4.47 ±1.07^{b}	0.88
Final Total White Blood Cell (x 10^9l)	8.93 ±2.79	7.44 ±3.66	9.84 ±1.07	1.73
Initial Neutrophiles (%)	36.40 ±5.03^{a}	39.30 ±6.34^{ab}	43.70 ±5.34^{b}	2.45
Final Neutrophiles (%)	33.80 ±3.77	33.20 ±6.34	34.00 ±5.34	3.32
Initial Lymphocytes (%)	58.40 ±5.03^{a}	56.70 ±5.81^{ab}	52.30 ±5.45^{b}	2.62
Final Lymphocytes (%)	48.60 ±5.77^{a}	59.40 ±5.81^{b}	61.80 ±5.45^{b}	3.59
Initial Eosinophiles (%)	5.20 ±1.79^{a}	2.33 ±0.45^{b}	2.33 ±1.64^{b}	1.00
Final Eosinophiles (%)	17.80 ±4.80^{a}	3.20 ±0.45^{b}	4.20 ±1.64^{b}	1.86
Initial Monocytes (%)	0.00 ±0.00^{a}	1.33 ±0.45^{b}	1.00 ±0.00^{a}	0.47
Final Monocytes (%)	0.40 ±0.54	0.20 ±0.45	0.00 ±0.00	0.26
Initial Basophiles (%)	0.00 ±0.00^{a}	0.53 ±0.00^{b}	0.27 ±0.00^{ab}	0.24
Final Basophiles (%)	0.20 ±0.45	0.00 ±0.00	0.00 ±0.00	0.16

DISCUSSION

The inclusion of IOL in the diet led to an increase in the PCV values however the rate of increase decreased with increase in IOL levels in the diet. The decrease with increase in IOL could be attributed to anti-nutritional factors (ATF) that may be present in the IOL. Fay (1987) reported of the presence of terpenes in the tuber and seeds of *Icacina oliviformis*. The final PCV values recorded all fell within the normal range of 33-50% reported by Hillyer (1994).

The lower margin of increase in T_2 is an indication that IOL above 5% inclusion could reduce the RBC counts in rabbits. The RBC values recorded in this study fell within the ranges of 3.5 x10^{12}/l-6.6x10^{12}/l reported by Archetti et al. (2008) for normal post weaned rabbits. Increased RBC's have been associated with high quality dietary protein Hackbath et al. (1983).

High values may conversely mean an increase in the circulation of red blood cells or an increase in plasma volume (Frandson and Spurgeon, 1992). Njidda and Hambagda (2006) indicated that PCV, MCHC and Hb are the most dependable blood indices for assessing the health status of animals. The higher increase in PCV, RBC and Hb values could be attributed to the inclusion of IOL in the diets. Adding IOL to the diet may have improved digestibility making

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the nutrients available to the animals for absorption resulting in the higher increases observed. Rabbits are monogastric herbivores and therefore addition of shrubs to their diet will enhance digestibility.

Icacina oliviformis has been reported to contain some anti-nutritional factors (ATF) (Fay, 1987). This ATF's may have caused some stress and immune response on the animals on the experimental diets leading to a rise in the lymphocyte and eosinophile values.

Conclusion

Feeding 5% and 10% IOL to a weaner rabbits led to an increase in erythrocytes values and there were no negative effects on the animal since all parameters fell within the normal ranges reported for rabbits.

Recommendation

In further research, treatments such as boiling, steaming, soaking is recommended to reduce the ATF's present in the IOL.

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REFERENCES

Anon (1980). Guide to the Care and Use of Experimental Animals. Vol 1. Canadian Council of Animal Care. Ontario, Canada. Pp 185–190.

Archetti I, Tittarelli C, Cerioli M, Brivio R, Grilli G and Lavazza A (2008). Serum chemistry and hematology values in commercial rabbits: preliminary data from Industrial farms in northern Italy, Ethology and Welfare, 9th World Rabbit Congress – June 10-13, 2008 – Verona – Italy.

Baker FJ and Silverton R E (1990). Introduction to Medical Laboratory Technology, 6th edition, Butterworth and Co. (publisher Ltd, London. Pp 305-329

Fay JM (1987). Icacina Oliviformis (Icacinaeae): A close look at an underexploited crop. I. Overview and Ethnobotany. Economic Botany 41(4) pp 512 – 522. The New York Botanical Garden, Bronx, NY 10458.

Frandson RD and Spurgeon TL (1992). Anatomy and Physiology of Farm Animals. 5th Edition, Lea and Febiger, Pennsylvennia. pp. 229-235.

Hackbath H Buron K and Schimansley G (1983). Strain difference in inbred rats: Influence of strain and diet on haematological traits. Laboratory Animal, 17: 7–12.

Hawkney CN and Dennet A (1989). A colour atlas of Comparative Veterinary haematology. Wolfe Medical Publication, England. pp. 192.

Hillyer EV (1994). Pet Rabbits The Veterinary Clinics of North America, Small Animal Practice, 24(1): 25–65.

Moreki JC (2007). Poultry and Rabbits Section, Non-Ruminants Division, Department of Animal, Production, P/Bag 0032, Gaborone, Botswana. p 2.

Natural Research Institute (1987). Root Crops, Crop and Product Digest, 2nd edition. Tropical Development and Research Institute, London. p. 308.

Njidda AA and Hambagda AA (2006). Studies on the haematological parameters and carcass characteristics of weanling rabbits fed sesame seed meal (*Sesamum indicum*) in semi-arid region of Nigeria. Nigerian Journal of Experimental and Applied Biology, 8(1): 81-88.

Njidda AA Igwebuike JU and Isidahomen CE (2006). Haematological parameters and carcass characteristics of weanling rabbits fed different levels of molasses. Global journal of Agricultural sciences, 5(2): 167-172.

Nyankpala Agricultural Extension Service (NAES), CSIR, Nyankpala (1994). Annual Report.

Radostits OM, Blood DC and Gay CC (1994). Veterinary Medicine, A textbook of the diseases of Cattle, Sheep, Pigs, Goats, and Horses. Eighth edition. Baillere Tindall, London, p. 66

SARI (2007). Savannah Agricultural Research Institites, Agro-meteorologcal Station, Nyankpala Station. p 10.

Vanhaelen M, Planchon C, Vanhaelen-Fastre R and On Okoko P (1986). Trepenic constituents from *Icacina senegalensis*. Journal of Natural Products, Volume 50, No. 2.

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TOXICOLOGICAL STUDY OF SINGLE ACTION OF ZINC ON TILAPIA SPECIES (*Oreochromis Niloticus*)

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ORIGINAL ARTICLE

ABSTRACT: *Lethal effects of zinc sulphate ($ZnSO_4.H_2O$), a widespread environmental pollutant was evaluated following exposure at different concentrations of the toxicant to Tilapia species, Oreochromis niloticus based on toxicity index of 96 hrs LC_{50} values. The obtained results were analyzed by the Finney's Probit Analysis LC_{50} Method and 96 hrs LC_{50} value for Oreochromis niloticus was found to be 72.431 mg/l. The work further documented the lower and upper confidence limits for the LC_{50} to be 77.288 mg/l and 67.682 mg/l, respectively. The research showed zinc to be lethal to the test organism and recommends proactive control measures to be put in place to avert possible disaster of zinc poisoning.*

Keywords: *Lethality, heavy metal, single action, pisces, 96 hrs LC_{50}.*

INTRODUCTION

Essential heavy metals are generally considered to be less toxic than non-essential metals (Batley, 1993). Metal such as zinc exhibit aquatic toxicity when present above recommended standard in that they can contaminate surface and groundwater bodies, soil, plant, aquatic life, and man, through bioaccumulation.

There are a numerous studies carried out on the toxicity of zinc sulphate. Malik et al. (1998) studied the effect of zinc toxicity on biochemical composition of muscle and liver of murrel (*Channa punctatus*). Selected specimens of murrel were exposed to a sub-lethal zinc concentration. They had reported that the zinc exposure produced marked changes in the chemical composition of liver and muscle tissues. The metabolism of the fish decreased with the time of exposure and there was a decline in the calorific value of lipid, protein, and glycogen in muscle and liver.

Uviovo and Beatty (1979) investigated effects of chronic exposure to zinc on reproduction in the guppy (*Poecilia reticulata*). They concluded that zinc had no effect on brood time, but the total number of young produced and brood size fell with increasing zinc concentrations (0.36 - 1.7 g Zn/l); dead young with abnormalities were not included in brood size and live young were significantly smaller in the presence of zinc. According to the results, this change in size of young at birth provides a very sensitive parameter of zinc toxicity.

Khunyakari et al. (2001) investigated toxicity of nickel, copper, and zinc in *Poecilia reticulata.* Heavy metal exposure caused increased mucus like secretion over gills, excessive excretion, anorexia and increased fin movement. Copper was found to be the most toxic followed by zinc and nickel.

Pierson (1981) studied effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation of the guppy, *Poecilia reticulata*. The zinc burden increased from 56 to 70 days during the onset of pregnancy. The experiments show that uptake of zinc during logarithmic growth and pregnancy indicates that females actively transfer zinc to the embryo. When guppies exposed to 0.607 mg/l zinc for 134 days, the wet weight of females was reduced by 40%.

It is generally accepted that metal accumulation in tissues of aquatic animals is dependent upon the exposure concentration and period, as well as some factors, such as salinity, temperature, interacting agents and metabolic activities of tissue concerned (Shukla et al., 2007). The mobility as well as the toxicity of zinc to aquatic species is enhanced by the physicochemical characteristics of inhabiting medium, such as temperature, hardness, and pH. Karakoc and Dincer (2003) reported highest accumulation of zinc in kidney tissue of *Oreochromis niloticus* at 15 ^{0}C and 30 ^{0}C for different concentrations, which is followed by gills and liver. In all tissues, zinc accumulation increases with increasing temperature.

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Monitoring pollution limits of heavy metals is important in the aquatic ecosystems, so that approximate measures of the potential hazards can be attained. These measures should give an estimation of the type of effects that could be expected after exposure to heavy metals. Thus, the aim of the research was to assess the mortality response of *Oreochromis niloticus* following different concentrations of zinc exposures using the toxicity scale of 96 hr LC_{50} value.

MATERIALS AND METHODS

Adult *Oreochromis niloticus* were obtained from a commercial hatchery and brought to the laboratory within 30 min in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for 30 - 35 min for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 40 liter capacity.

The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial DANA feed food at least once a day during this period. Acclimated fish were not fed 24 h before the start of the tests. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

Zinc sulphate ($ZnSO_4.H_2O$) in tap water was used in the static bioassays. The test organisms were subjected to different concentrations (50, 60, 70, 80, 100 and 120 mg/l) of the zinc sulphate ($ZnSO_4.H_2O$). For the acute bioassay tests, 20 fish were used per concentration. The containers were not aerated at the dosing time. The amount of zinc sulphate to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the zinc sulphate; keeping all other conditions constant. The mortality rate in the control group did not exceed 10% and 90% of the fish looked healthy throughout the experiment.

The experiment was carried out in a series and 140 *Oreochromis niloticus* species were used. This species was selected for static bioassays because it can be easily cultured and raised under laboratory conditions through a complete life cycle, and it is one of the standard test species used for laboratory toxicity studies.

Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness and pH) using in the aquaria were periodically determined before the bioassay tests (Table 1). The water temperature was kept 27 ± 2.00 ^{0}C. Also the experimental medium was aerated in order to keep the amount of oxygen not less than 6 mg/l.

Table 1 - The temperature, pH, hardness, and oxygen demand (DO) of the aquaria in zinc toxicity tests

Concentration (mg/l)	Temperature (^{0}C)	pH	Hardness (mg/l)	Dissolved Oxygen (mg/l)
50	27.3	7.50	225	6.8
60	28.2	7.65	225	6.0
70	28.1	7.70	215	6.7
80	27.2	7.70	220	6.0
100	25.9	7.63	225	6.2
120	26.8	7.70	235	6.5
Control group	27.7	7.60	230	6.3

All experiments were carried out for a period of 96 hours. The number of dead fish were counted every 24 hours and removed from the aquaria as soon as possible. The mortality rate was determined at the end of the 96th hour. No food was given to the fish during the experiments.

The experiments were carried out with static acute experimental method. In this method the experimental solution and the samples (i.e. fish) are put in a suitable experimental cell (i.e. aquarium) and kept like that for a certain period. Since the decreased amount of oxygen and increased metabolic waste become a problem in long term experiments, the duration of such experiments are usually kept at 96 hours or less. The bioassay system was as described in standardized methods (OECD, 1993; APHA/AWWA/WPCP, 1971).

Assessment of mortality response on zinc exposures of the test species, *Oreochromis niloticus* was determined by the use of Finney's Probit Analysis LC_{50} Determination Method (Finney, 1971). The mortality response of the fish species was taken to be when the animals sank down to the bottom of the containers and became motionless, rate determined at the end of the 96th hour.

RESULTS

The toxicity data revealed 96 hour LC_{50} value for *Oreochromis niloticus* exposed to different zinc concentrations as 72.431. Theoretical Spontaneous Response Rate was zero (for control experiment). Around 95% lower and upper

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confidence limits for the LC_{50} values were 77.288 mg/l and 67.682 mg/l, respectively. These toxic effects increased, as the dose was increased (Table 2 and 4 and Figure 1).

The parameter estimates and the obtained results for the acute 96 hrs toxicity estimated lethal concentration values and their confidence limits are shown in Table 3 and 4, respectively. Figure 1 displays the Probit line graph of acute toxicity of zinc on *Oreochromis niloticus* with Table 5 showing the 96 hrs LC_{50} value for the Tilapia species.

Table 2 - The relationship between the zinc concentration and the mortality rate of *Oreochromis niloticus* for the 96-hour exposure

Conc(Mg/l)	Test Animals	24 hrs	48 hrs	72 hrs	96 hrs
50	20	0	2	2	3
60	20	3	4	5	7
70	20	2	7	7	9
80	20	5	7	10	11
100	20	10	12	13	16
120	20	13	13	15	19
Control	20	0	0	0	0

Table 3 - Parameter Estimates for the Probit Analysis

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	6.390	0.800	7.987	0.000	4.822	7.958
	Intercept	-11.884	1.499	-7.930	0.000	-13.383	-10.386

[a] PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.); Theoretical Spontaneous Response Rate = 0.000

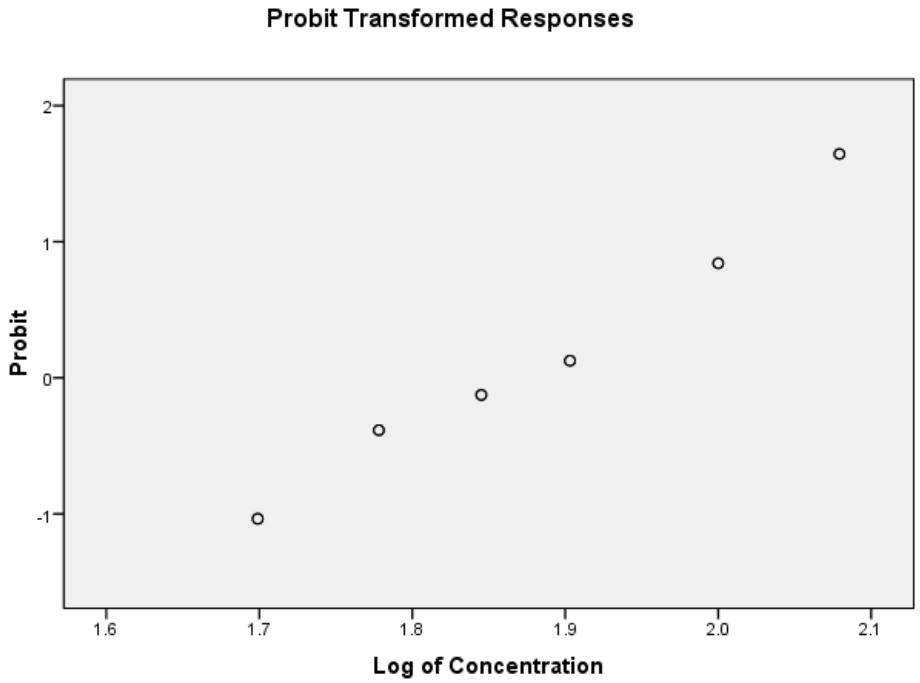

Fig. 1 - Probit line graph of acute toxicity of Zinc on Tilapia (*Oreochromis niloticus*)

DISCUSSION

Gül et al. (2009) found the 96 hrs LC_{50} value for Guppies (*Poecilia reticulate* P., 1859) to be 30.826 mg/l. similarly, Williams and Holdway (2000) examined that the effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatis*). The LC_{50} values of zinc were found to be 0.51, 0.56, and 1.57 mg/l for 24-h, 3-4-day, and 9-10- day-old larval rainbow fish.

Herrera et al. (1995), reported the 96 hrs LC_{50} value of $ZnCl_2$ on *Chanos chanos* as 25 mg/l. Finlayson and Verrue (1982), investigated toxicities of copper, zinc and cadmium mixture to juvenile *chinook salmon*. They found that median lethal concentrations during 4 days (96-hour LC_{50} values) were most variable for zinc (39 to 122 mg/l).

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Table 4 - Estimated lethal concentration values and confidence limits							
		95% Confidence Limits for Concentration			95% Confidence Limits for log (Concentration)[a]		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	0.01	31.321	23.448	37.467	1.496	1.370	1.574
	0.02	34.554	26.677	40.590	1.539	1.426	1.608
	0.03	36.777	28.948	42.711	1.566	1.462	1.631
	0.04	38.542	30.780	44.384	1.586	1.488	1.647
	0.05 (LC_5)	**40.040**	**32.352**	**45.797**	**1.602**	**1.510**	**1.661**
	0.06	41.361	33.752	47.038	1.617	1.528	1.672
	0.07	42.556	35.027	48.156	1.629	1.544	1.683
	0.08	43.654	36.207	49.182	1.640	1.559	1.692
	0.09	44.678	37.314	50.137	1.650	1.572	1.700
	0.10	45.641	38.360	51.034	1.659	1.584	1.708
	0.15	49.856	42.991	54.955	1.698	1.633	1.740
	0.20	53.482	47.026	58.336	1.728	1.672	1.766
	0.25	56.802	50.740	61.459	1.754	1.705	1.789
	0.30	59.959	54.268	64.473	1.778	1.735	1.809
	0.35	63.041	57.687	67.480	1.800	1.761	1.829
	0.40	66.111	61.041	70.564	1.820	1.786	1.849
	0.45	69.224	64.365	73.805	1.840	1.809	1.868
	0.5 (LC_{50})	**72.431**	**67.682**	**77.288**	**1.860**	**1.830**	**1.888**
	0.90	114.946	103.570	134.957	2.060	2.015	2.130
	0.91	117.425	105.435	138.728	2.070	2.023	2.142
	0.92	120.178	107.493	142.952	2.080	2.031	2.155
	0.93	123.280	109.795	147.755	2.091	2.041	2.170
	0.94	126.840	112.417	153.321	2.103	2.051	2.186
	0.95 (LC_{95})	**131.025**	**115.474**	**159.938**	**2.117**	**2.062**	**2.204**

[a] Logarithm base = 10

Table 5 – 96 hrs acute toxicity of Zinc on *Oreochromis niloticus*				
Test Animals	96 hrs LC_{50} (mg/l)	96 hrs LC_5 (mg/l)	96 hrs LC_{95} (mg/l)	S.E
Oreochromis niloticus	72.431 (77.288-67.682)	40.040 (45.797-32.352)	131.025 (159.938-115.474)	1.499

DISCUSSION

Gül et al. (2009) found the 96 hrs LC_{50} value for Guppies (*Poecilia reticulate* P., 1859) to be 30.826 mg/l. similarly, Williams and Holdway (2000) examined that the effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatis*). The LC_{50} values of zinc were found to be 0.51, 0.56, and 1.57 mg/l for 24-h, 3-4-day, and 9-10- day-old larval rainbow fish.

Herrera et al. (1995), reported the 96 hrs LC_{50} value of $ZnCl_2$ on *Chanos chanos* as 25 mg/l. Finlayson and Verrue (1982), investigated toxicities of copper, zinc and cadmium mixture to juvenile *chinook salmon*. They found that median lethal concentrations during 4 days (96-hour LC_{50} values) were most variable for zinc (39 to 122 mg/l).

Khangarot et al. (1981) examined toxicity of interaction zinc-nickel, copper-nickel and zinc-nickel-copper to a freshwater teleost, *Lebistes reticulatus*. The 48-hour median lethal concentrations (LC_{50}) for individual salts were 75 mg/l for Zn^{2+}, 37 mg/l for Ni^{2+} and 2.5 mg/l for Cu^{2+}. Their experiment showed that in the Zn^{2+} – Ni^{2+} mixture, when Ni^{2+} was more in proportion, the toxicity was more than additive. The results indicate that heavy metallic mixtures would pose a greater toxicological danger to fish than the respective individual metals.

Zinc lethality has been widely described and its mixture with certain metals further documented to be synergistic. Joint action toxicity testing with metals involving zinc should be further carried out on different sensitive aquatic organisms to measure deviation from its single component toxicity, since these heavy metals are rarely/ or do not occur in isolation within the environment of influence.

REFERENCES

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Journal homepage: http://www.ojafr.ir

APHA (American Public Health association), AWWA (American Water Works Association), WPCP (Water Pollution Control Program, (1971). Standard Methods for the Examination of Water and Wastewater, Washington D.C.

Batley GE (1993). The current status of trace element speciation in natural waters. In: Trace element speciation in surface waters (Ed.), G.G. Leppard. Plenum Press, New York. Pp. 17-36.

Finney DJ (1971). Probit Analysis. Cambridge University Press, New York, 337.

Gül A, Yilmaz M and Isilak Z (2009). Acute Toxicity of Zinc Sulphate ($ZnSO_4.H20$) (*Poecilia reticulata* P., 1859), G.U. Journal of Science, 22(2): 59-65.

Karakoc M and Dincer S (2003). Effect of Temperature on Zinc Accumulation in the Gill, Liver and Kidney of Oreochromis niloticus. Bulletin of Environmental Contamination and Toxicology, 71: 1077-1083.

Khunyakari RP, Vrushali T, Sharma RN and Tare V (2001). Effects of some Trace Heavy Metals on Poecilia reticulate, Journal of Environmental Biology, 22 (2): 141- 144.

Malik DS, Sastry KV, Hamilton DP (1998). Effects of zinc toxicity on biochemical composition of muscle and liver of murrel (Channa punctatus), *Environment International,* 24 (4): 433-438.

OECD (Organization for Economic Co-operation and Development) (1993). OECD Guidelines for testing of chemicals, OECD, Paris.

Pierson KB (1981). Effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation of the guppy, Poecilia reticulata, Canadian Journal of Fisheries and Aquatic Sciences, 38 (1): 23-31.

Shukla V, Dhankhar M, Prakash J and Sastry KV 2007. Bioaccumulation of Zn, Cu and Cd in Channa punctatus. Journal of Environmental Biology, 28 (2): 130-159.

Uviovo EJ and Beatty DD (1979). Effects of chronic exposure to zinc on reproduction in the guppy (Poecilia reticulata). Bulletin of Environmental Contamination and Toxicology, 23 (4/5): 650-657.

Williams ND and Holdway DA (2000). The Effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (Melanotaenia fluviatilis), Environmental Toxicology, 15 (3): 165-173.

Herrera AA, Amparado EA and Santos MD (1995). Laboratory studies on the effect of heavy metals (Zn and Cu) and on organophosphate (Gusation) on Chanos chanos, Third-National-Symposium in Marine-Scince-of-the-Philippine-Associationof- Marine-Scince, Pams Philippines.

Finlayson BJ and Verrue KM (1982). Toxicities of copper, zinc and cadmium mixture to juvenile chinook salmon, Trans. The American Fisheries Society, 111: 645- 650.

Khangarot BS, Durve VS and Rajbanish VK (1981). Toxicity of interaction zinc-nickel, copper-nickel and zinc-nickel-copper to a freshwater teleost, Lebistes reticulatus (Peters). Acta Hydrochimica Hydrobiologica, 9 (5): 495-503.

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EFFECT OF SOAKED AND DRIED FALSE YAM (*Icacina Oliviformis*) SEED MEAL ON CARCASS AND SENSORY CHARACTERISTICS OF BROILER CHICKEN

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ABSTRACT: *This study was conducted to determine the effect of soaked and dried false yam seed meal (SFYSM) on the carcass and sensory characteristics of broiler chicken. A total of 48 chickens (12 birds in each treatment) were randomly selected from 120 birds fed diets containing 0% (T1, control), 5% (T2), 7.5% (T3) and 10% (T4) SFYSM. The birds were weighed and slaughtered after a 24 hours feed withdrawal. Carcass and visceral weights were taken, after which the thigh and breast muscles were bagged separately for laboratory and sensory analyses respectively. The breast muscles were thawed, grilled in an oven to a core temperature of 70°C for sensory analysis, whiles the thigh muscles were used for moisture, fat, crude protein and lipid per-oxidation analyses. The results indicated that the use of SFYSM had no significant effect on carcass and sensory characteristics of the birds. In addition, there was no significant difference in moisture and lipid per-oxidation of the products. However, the crude protein contents of the carcasses significantly (P<0.05) increased with an increasing SFYSM inclusion rates. Feeding of SFYSM to broiler birds up to 10% inclusion on weight basis has no effect on carcass, sensory and storability of the carcasses.*

Keywords: *False yam seeds, carcass, sensory quality, broiler chicken*

ORIGINAL ARTICLE

INTRODUCTION

Animal production is characterized by high input costs mainly resulting from feeding and housing. This cost in terms of feeding ruminants is generally lower than for poultry, which duel mainly on grains and legumes; important staple for humans (Ensminger, 1983). Bell and Weaver (2002) reported that 85% of the world's chicken energy is derived from maize. This brings about a competition between animals and humans for the staple, making its supply limited and expensive when available (Kekeocha, 1984). Consequently, the cost of poultry and poultry products escalates and is unaffordable to the average household.

The poultry industry supplements the protein and income of a typical Ghanaian family, as these have relatively shorter maturity period compared with ruminants (Sonaiya and Swan, 2004; Obeng-Asamoah, 1989). This short maturity period makes chicken the world's largest supplier of meat for consumption (Gopalakrishnan and Moley, 1985). In addition, the meat is of high quality, containing an average protein content of 20% and high levels of unsaturated fatty acid as well as a good source of vitamins (Niacin) compared with ruminants (Van Eekeren et al., 1990 and Oluyemi and Robert, 1979).

In order to combat the problem of high feed costs and make poultry and poultry products affordable to the average consumer, scientists are exploring the potentials of non-conventional feed ingredients which are cheaper than grains and legumes, but have potentials for use as animal feed. One of these materials is false yam seeds (*Icacina oliviformis).*

In Ghana, false yam has been identified as a new feed ingredient with potentials to substitute maize in poultry rations (Michael, 1993). The plant however, is believed to contain a bitter toxic principle called gum resin, which has a potential of adversely affecting the carcass yield and eating qualities of poultry (Okine et al., 2009). Current research is

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employing soaking of the seeds, as a means of reducing the concentration of the gum resin in the seeds. According to Teye et al. (2006) the carcass quality of livestock is affected by the type of feed given and the inclusion levels of the various feed ingredients.

This research was therefore aimed at determining the effect of soaked and dried false yam seed meal on carcass and sensory characteristics of broiler chicken.

MATERIALS AND METHODS

The experiment was conducted at the Meat Processing Unit and Laboratories of the University for Development Studies, Tamale.

Experimental birds

A total of forty-eight (48) broilers (Cobb-500) of eight weeks old with a sex ratio of 1:1 were selected from 120 birds. Twelve birds were selected randomly from four groups of birds raised on rations in which maize was substituted with soaked false yam seed meal (SFYSM) at levels of 0% (T1), 5% (T2), 7.5% (T3) and 10% (T4) inclusions.

Slaughtering of birds

Each bird was weighed (live weight) with an electronic scale (Sartorius, CP 245S) after a 24-hour feed withdrawal, and tagged to differentiate them. The birds were then stuck with a sharp knife to cut the jugular veins and allowed to bleed for approximately 60 seconds, after which they were scalded in warm water (60°C). The feathers were plucked manually and head and shanks removed. An incision was then made around the vent to remove the viscera. The hot carcass weight was then taken.

Carcass yield

The viscera were separated into intestines, gizzard, liver and spleen. The dressed carcass was chilled for 24 hours and cold weight taken. Primal cuttings were made from the chilled carcass and weighed. The breast and thigh muscles were bagged, stored and used for sensory and laboratory analyses respectively.

Sensory analysis

A total of fifteen (15) panelists, comprising staff and students were randomly selected and trained according to the British Standard Institution (BSI, 1993) guidelines to evaluate the products. Sensory evaluation was carried out on the 1, 7 and 14 days of freezer storage. The breast muscles were thawed and grilled to a core temperature of 70°C in an electric oven (Turbofan, Blue seal, UK). The products were sliced into uniform sizes (about 2cm) and wrapped with coded aluminum foils and presented to the panelists. Each panelist was provided with water and pieces of bread to serve as neutralizers between the products.

A five-point category scale was used to evaluate the sensory characteristics of the products as follows:

Color: very light (1), light (2), intermediate (3), Dark (4), very Dark (5)
Off-odor: very weak (1), weak (2), intermediate (3), strong (4), very strong (5)
Juiciness: very juicy (1), juicy (2), intermediate (3), dry (4), very dry (5)
Tenderness: very tender (1), tender (2), intermediate (3), tough (4), very tough (5)
Chicken flavor: very weak (1), weak (2), moderate (3), strong (4), very strong (5)
Flavor-liking: like very much (1), like (2), intermediate (3), dislike (4), dislike very much (5)

Proximate and Peroxide values (POV) of the meats

Thigh muscles were used to determine the nutritional composition and lipid per-oxidation according to the methods of the AOAC (1999).

Statistical Analysis

Data obtained were analyzed using the General Linear Model (GLM) of Analysis of Variance (ANOVA) of the Minitab Statistical Package, version 15 (MINITAB, 2007).

RESULTS AND DISCUSSION

Carcass characteristics of birds

The weights of the carcass and primal cuttings of the birds are presented in Table 1. The results showed no significant difference in carcass characteristics of the birds. The dressing percentage ranged between 75.64 and 77.10 percent. This result is not different from the findings of Taylor and Field (1998), who reported a dressing percentage of

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78% in commercial broilers. The weights of the drumstick, thigh, wings and breast muscles did not vary between treatments (Table 1).

Anti-nutritive factors in feedstuffs are poorly digestible, inhibit protein digestion, depress growth and are capable of increasing the incidence of skeletal disorders in birds (Saif, 2003). According to Cooke and Maduagwu (1985), soaking enables the movement of soluble cyanide into solution in cassava roots. Coffie (2011) reported a reduction in weights of carcass parameters, when raw false ram seed meal was used in broiler rations. It therefore implies that most of the soluble toxins in the false yam seeds were removed by the soaking method of processing.

Table 1 - Effect of SFYSM on carcass characteristics and primal cuttings of broiler chicken

Parameter	T1	T2	T3	T4	SED	Sig.
Dressing %	77.00	77.10	75.97	75.64	6.81	ns
Cold weight	1737	1792	1661	1642	185.45	ns
Drum stick	231	249	229.3	232.8	30.44	ns
Wings	196.2	203.0	187.0	185.3	19.93	ns
Thigh	495.2	534.3	479.5	472.0	56.89	ns
Breast	713.3	739.2	645.0	659.0	93.23	ns
Shank	45.32	47.91	43.91	42.82	9.30	ns
Head	52.81	57.23	54.99	55.62	6.14	ns
Whole intestine	83.53	82.63	83.88	82.05	5.62	ns
Whole gizzard	51.88	57.31	55.75	56.23	7.42	ns
Empty gizzard	37.79	40.25	38.28	40.93	5.16	ns
Spleen	1.89	2.74	2.16	1.90	0.53	ns
Liver	30.31	33.52	3.53	31.94	5.21	ns

Sig= significance; ns= no significance; SED= standard error of difference

Sensory characteristics of the chicken

The meat was offered to the panelists for sensory evaluation, and the results are presented in Table 2. The SFYSM had no significant effect ($P > 0.05$) on the eating qualities of the experimental chicken (Table 2).

Sensory characteristics of meat are very important factors consumers consider when buying meat and meat products. Color is a major indicator of quality of meat, as the appearance influences consumer acceptance (Van Oeckel et al., 1999; Bell and Weaver, 2002). Odor and flavor are other important parameters consumers consider when buying meat (Omojola and Adesehinwa, 2007). When these qualities appear different from those of the traditional chicken, the products are likely to be rejected by the consumers.

Since the SFYSM-fed chicken had similar sensory characteristics as those fed the traditional chicken diet, means consumers would not detect differences in the sensory parameters, hence readily accept such meats.

Proximate composition of the meat

The moisture, crude protein and fat contents of the carcasses are presented in Table 3. The moisture content of the chicken was not affected by the diets (Table 3). The crude protein contents of the meats were however, significantly increased ($P<0.05$) in birds fed the SFYSM diet, compared with the control birds. The crude protein contents of the carcasses increased with increasing SFYSM inclusion (Table 3).

Higher crude protein in diets is advantageous to the consumer. This is because proteins are required in higher levels in growing children and also for productive functions such as pregnancy and lactation, because of increased output of proteins in the products of conception and in milk (Pond et al., 1995). Therefore, with higher crude protein levels in the chicken, a small quantity would be required by consumers to meet their nutrient requirement, hence reduce expenditure on meat, as well as satisfy health concerns associated with excessive meat consumption.

The fat content in the chicken ranged between 5.31 and 10.08% of the fresh chicken with T2 being the least and significantly different from the rest of the treatments. These however, fall within the range of 6.9 - 12% reported by Panda (1995) in chicken. Consumption of high levels of saturated fats has been associated with high incidence of coronary heart diseases in humans (Mike and Floyd, 1999). Several health organizations including the World Health Organization (WHO, 1990) therefore recommend a reduced dietary fat intake. Since the use of SFYSM did not cause a significant increase in the fat content of the meat, it is an indication that the consumption of such meats will not pose health threats to consumers.

Peroxide value of products

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Lipid per-oxidation in the products was determined on the 15th day of storage to determine the effect SFYSM on lipid per-oxidation in the products. The results are presented in Figure 1. The peroxide values of the products ranged between 1.45 and 1.78 mill equivalent of oxygen per kg product. These values are significantly lower than 25millequivalent of active O_2/kg, which is considered as the limit of acceptability in fatty foods (Narasimhan et al., 1986).

Table 2 - Sensory characteristics of broilers fed SFYSM

Storage period (days)	Parameter	T 1	T 2	T 3	T 4	SED	Sig.
1	Color	2.07	1.80	1.53	1.80	0.51	ns
	Off-odor	2.47	2.53	2.40	2.40	0.79	ns
	Juiciness	3.13	3.07	2.93	3.00	0.63	ns
	Tenderness	3.33	3.13	2.87	2.73	0.69	ns
	Chicken flavor intensity	2.60	2.73	2.67	2.73	0.55	ns
	Flavor liking	2.47	2.40	2.07	2.33	0.53	ns
7	Color	2.53	2.33	2.40	2.73	0.61	ns
	Off-odor	2.40	2.53	2.53	2.53	0.93	ns
	Juiciness	2.60	2.87	2.57	3.13	0.58	ns
	Tenderness	2.80	2.47	2.67	2.60	0.62	ns
	Chicken flavor intensity	2.47	2.87	2.53	3.00	0.84	ns
	Flavor liking	2.40	2.07	2.33	2.33	0.68	ns
14	Color	2.33	2.33	2.07	2.13	0.56	ns
	Off-odor	2.40	2.53	2.53	2.67	0.90	ns
	Juiciness	2.53	2.40	2.13	2.07	0.51	ns
	Tenderness	3.07	2.87	2.60	2.67	0.44	ns
	Chicken flavor intensity	2.93	3.07	2.80	2.80	0.70	ns
	Flavor liking	2.27	2.20	2.13	2.27	0.63	ns

SED= standard error of difference; ns= not significant; sig= significance

Table 3 - Proximate composition of the meat

Parameter	T1	T2	T3	T4	SED	Sig.
Moisture	75.36	72.72	74.51	74.84	6.66	ns
Crude protein	16.08_b	17.33_{ab}	17.94_{ab}	18.27_a	0.44	*
Fat (ether extract)	8.02_{ab}	5.31_b	10.08_a	8.63_a	0.67	**

SED= standard error of difference; sig.= significance; ns= not significant; *= significant (P<0.05), **= significant (P<0.01)

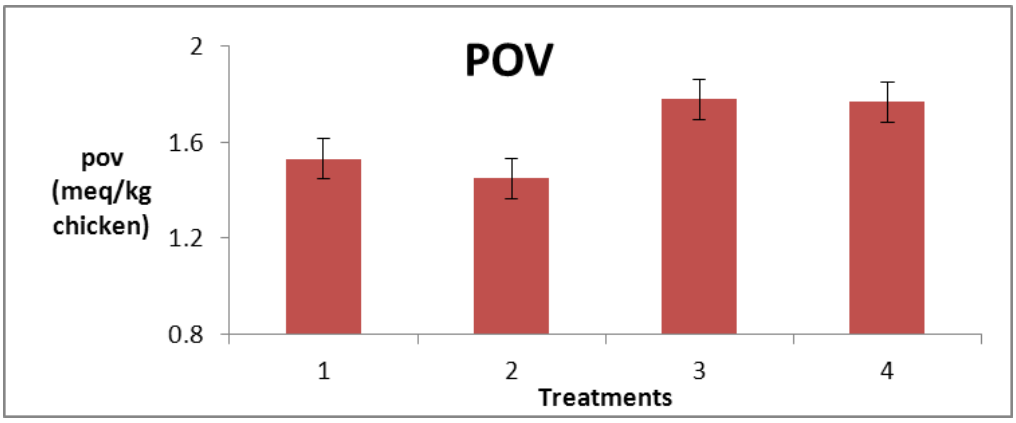

Fig. 1 - Peroxide value of experimental chicken, POV= peroxide value

According to Warriss (2010), lipid per-oxidation progresses at faster rates in fats rich in unsaturated fatty acids, than those high in saturated fatty acids. The unsaturated and polyunsaturated fatty acids present in these, react with oxygen to form fatty acid hydro peroxides. Hydro peroxides are unstable, and breakdown into various compounds which can produce off-flavours; leading to a rather stale, rancid flavor in food products (Kerler and Grosch, 1996).

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The values were not significantly different among the treatments, and acceptability was also not adversely affected; an indication that the use of SFYSM in poultry rations has no detrimental effect on product storability.

CONCLUSIONS

The use of soaked and dried false yam seed meal to substitute maize up to 10% in broiler rations has no effect on carcass and sensory characteristics of the meat. Meanwhile, the crude protein contents of the carcasses increased. In addition, there was no significant difference in lipid per-oxidation of the carcasses, indicating that feeding SFYSM is not detrimental to product storability. Soaked false yam seed meal can be used in poultry rations up to 10% inclusion rate on weight basis.

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REFERENCES

AOAC International (1999). In P. Cunniff (Ed.), Official methods of analysis of AOAC international (16th ed.). Gaithersburg, MD, USA: AOAC International.

Bell DD and Weaver WD (2002). Jr. (Eds), Commercial chicken meat and egg production, 5th edition, Kluwer Academic publishers. Pp 7, 8, 47 and 873-910.

British Standard Institution (1993). Assessors for sensory Analysis. Guide to Selection, Training and Monitoring of Selected Assessors. BS 17667. British Standard Institute, London, United Kingdom.

Coffie M (2011). Effect of raw false yam seed meal on carcass and eating qualities of broiler chicken. Unpublished BSc. Dissertation, University for Development Studies, Tamale, Ghana. 38pp

Cooke RD and Maduagwu EN (1985). The effect of simple processing on the cyanide content of cassava chips. Journal of food Technology 13: 299-306.

Ensminger ME (1983).The Stockman's Handbook. The Interstate printers & publishers, Inc Pp 215.

Gopalakrishnan CA and Moley LG (1996). Livestock and Poultry Enterprise for Rural Development, Vikas publishing House, PVT Ltd. Pp 886 and 974.

Kekeocha CC (1984). Poultry production handbook. Pfizer Corporation in association with Macmillan publishers Ltd. Pp 4(5): 92-94.

Kerler J and Grosch W (1996). Odorants contributing to warmed-over flavour (WOF) of refrigerated cooked beef. Journal of Food Science 61: 1271–1274.

Michael JF (1993). A close look at an underexploited food plant, iii. Ecology a production. Economic Botany, 42(2): 163-170.

Mike E and Floyd K M (1999). Non-ruminant nutrition and meat quality. Reciprocal meat conference proceedings, Vol. 52. Pp 15 and 19.

Minitab (2007). Minitab Statistical Software, release 15 for Windows 95/98/2000 and Windows NT. Minitab Inc, USA.

Narasimhan S, Raghuver KG, Arumngham C, Bhat KK and Sen DP (1986). Oxidative rancidity of groundnut oil evaluation by sensory and chemical indices and their correlation. Journal of Food Science and Technology 23: 273–277.

Obeng-Asamoah J E (1989). The Secrets of Profitable Poultry Production. Macmillan Nigeria Publishers Ltd. Pp. 67-74.

Okine C A, Teye GA and Dei H K (2009). Effects of Processed Mucuna pruriens beans on carcass characteristics of broiler chicken. In Proceedings of the Ghana Association of Animal Production (GSAP), 16th Biennial Conference. Pp 145-147.

Oluyemi JA and Robert FA (1979). Poultry production in the tropics. Macmillan publishers Ltd., Hong Kong. Pp 121-163.

Omojola A B and Adesehinwa AOK (2007). Performance and Carcass Characteristics of Broiler Chicken Fed Diets Supplemented with Graded levels of Roxazyme G®. International Journal of Poultry Science 6 (5): 335-339.

Panda PC (1995). Textbook on egg and poultry production technology. Vikas publishers house PVT. Ltd., New Dehli. Pp 139-141, 146, 157 and 173.

Pond WG, Church DC and Pond KR (1995). Basic animal nutrition and feeding. 4th edition, John Willey and Sons Pp 119-138

Saif Y M (2003). Diseases of poultry.11th edition. A Blackwell publishing company. Pp 1138.

To cite this paper: Teye, G.A., Adua, E.A. and Teye, M. 2011. Effect of soaked and dried false yam (*Icacina oliviformis*) seed meal on carcass and sensory characteristics of broiler chicken. *Online J. Anim. Feed Res.*, 1(4):144-149.
Journal homepage: http://www.ojafr.ir

Sonaiya EB and Swan S B (2004). Small scale poultry production, FAO Animal production and Health. Rome. Pp 134.
Taylor RE and Field TG (1998). Scientific animak production 6th edition. Printice Hall, Upper Saddle River, New Jersey. Pp. 113, 115, 269 and 273.
Teye GA, Munkaila L and Dei HK (2006). Carcass Characteristics of the male and female broiler chicken. Agricultural and Food Journal of GhanaVol.5 December 2006.
Van Eekeren N, Mass A and Saatkamp H W (1990). Small scale poultry production in the tropics. Agrodok No. 4. Agromisa, Netherlands. Pp. 2, 24 and 42.
Van Oeckel M J, Warrmant N and Bookque L V (1999). Measurement and prediction of poultry colour. Meat Science 52: 347-354.
Warriss PD (2010). Meat Science, An Introductory Text (2nd Edition). CAB International, Wallingford Oxfordshire, OX10 8DE, UK. 234 pages
World Health Organization (WHO) Study Group (1990). 'Diet, Nutrition and the Prevention of ChronicDiseases' WHO Technical Report Ser. 797.

To cite this paper: Teye, G.A., Adua, E.A. and Teye, M. 2011. Effect of soaked and dried false yam (*Icacina oliviformis*) seed meal on carcass and sensory characteristics of broiler chicken. *Online J. Anim. Feed Res.*, 1(4):144-149.
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HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF STARTER BROILERS FED NEEM (*Azadirachta indica*) LEAF MEAL

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ABSTRACT: *A 28-day feeding trial was conducted to evaluate the effects of dietary inclusion of Neem (Azadirachta indica) leaf meal on the haematological and serum biochemical indices of starter broilers. The Neem leaves used in the experiment were manually harvested, air-dried and milled to become Neem leaf meal. The Neem leaf meal was included in broiler starter diets at 0, 2.5, 5.0, 7.5 and 10% levels, respectively. One hundred and fifty (150) Anak broiler starter chicks raised on a commercial starter mash for one week were used. They were divided into 5 groups of 30 birds each and randomly assigned to the 5 experimental diets in a completely randomized design (CRD). Each group was sub-divided into 3 replicates of 10 birds each and each replicate housed in a pen fitted with necessary brooding facilities. Feed and water were given to them ad libitum for 4 weeks. Proximate analysis of the Neem leaf meal displayed same characteristics as leaf meals from other tropical browse plants - high crude fibre (15.56%) and moderate crude protein content (18.10%). At the end of the feeding trial, blood was collected from the birds, 4 per treatment and analysed for haematological and serum biochemical indices. Haemoglobin (Hb) and packed cell volume (PCV) of the birds were significantly reduced (P<0.05) but not below the level considered normal for birds. No traces of monocytes, eosinophils and basophils were observed. Blood sugar was significantly raised (P<0.05) by the leaf meal but cholesterol was significantly (P<0.05) decreased. Alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) decreased with increase in leaf meal (P<0.05). Serum electrolytes: calcium, sodium, potassium, chloride and bicarbonate tended to show that Neem leaf meal up to 10% dietary inclusion level could still maintain the integrity of the kidney in boosting cation/anion exchange. The haematological and serum biochemical parameters obtained from this study suggested that dietary Neem leaf meal has no deleterious effects on some physiological indices of starter broilers.*

Keywords: *Neem leaf meal, starter broilers, haematological, serum, biochemical indices.*

ORIGINAL ARTICLE

INTRODUCTION

The growth of human and livestock population which has created increased demand for food and feed in the developing countries suggests that alternative feed resources must be identified and evaluated (Nworgu et al., 2007). In evaluating such unconventional feed resources, it is important to also check the effects of such feed resources on the health status of the livestock. Esonu et al. (2001) stated that haematological constituents reflect the physiological responsiveness of the animals and the influence of diet on haematological traits is very strong (Church et al., 1984; Babatunde et al., 1987). Restricted low energy feed intake results in elevated MCHC. Haemoglobin and packed cell volume are very sensitive to the levels of protein intake as the values increase with increase in dietary protein concentration (Edoziem and Switzer, 1977). It has also been observed that serum urea, total protein and creatinine contents depend on both the quality and quantity of protein supplied in the diet (Iyayi and Tewe, 1998).

The use of leaf meals of plants as feed ingredients as alternative to conventional feed resources is a novel area of research in animal nutrition. Leaf meals of some tropical legumes and browse plants, rich in nutrients like vitamins, minerals and carotenoids have been reported (Vohra et al., 1972; Udedibie, 1987; Udedibie and Opara, 1996; Esonu et al., 2002).

One of the tropical plants that have attracted attention of animal nutritionists in recent time is the neem tree (*A. indica*). Various parts of the tree have medicinal value (Chakraborty et al., 1989) and recent studies by Esonu et al.

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(2006) have shown that its leaf meal could be of some value in the diet of laying hens both as feed ingredient and egg yolk pigmenter. There is the need to also evaluate its effects on haematological and serum biochemical constituents of poultry.

The studies herein reported were therefore designed to examine the haematological and serum biochemical indices of starter broilers as affected by dietary Neem leaf meal.

MATERIALS AND METHODS

Study sites

This study was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology and Animal Science Laboratory of the Federal University of Technology, Owerri, Imo State, Nigeria. Imo State lies between latitude 4˚4' and 6˚3' N and longitude 6˚15' and 8˚15' E. Owerri is about 100m above sea level. The climatic data of Owerri as summarized in Ministry of Lands and Survey Atlas (1984) of Imo State is as follows: mean annual rainfall, 2500 mm; temperature range, 26.5 – 27.5˚C and humidity range of 70 – 80 %. Dry season duration (i.e. months with less than 65mm rainfall) is 3months. The annual evapo-transpiration is 1450 mm and the soil type is essentially sandy loam with average pH of 5.5.

Source and processing of Neem leaves

Fresh green Neem leaves used for the experiment were harvested within the University environment. Each batch of collection was air-dried. They were considered adequately dried when they became crispy to the touch. They were then milled, using a hammer mill with 2 mm sieve, to produce Neem leaf meal (NLM). Samples of the leaf meal were subjected to proximate analysis according to AOAC (1995).

Experimental Diets

Five white maize-based experimental broiler starter diets (23% CP) were made, incorporating the leaf meal at 5 levels of 0.00, 2.50, 5.00, 7.50 and 10% designated as T_0, $T_{2.50}$, $T_{5.0}$, $T_{7.5}$ and T_{10}, respectively. The ingredient composition of the experimental diets is shown in Table 1. The diets were balanced for crude protein and caloric content as per the requirements of this class of birds in the tropics (Sansbury, 1980).

Table 1 - Ingredient composition of the broiler starter experimental diets

	Dietary levels of leaf meal (%)				
Ingredients (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
White Maize	50.00	49.00	47.00	46.00	45.00
Neem Leaf meal	0.00	2.50	5.00	7.50	10.00
Soybean meal	26.00	26.00	26.00	26.00	26.00
Wheat offal	10.00	8.50	8.00	6.50	5.00
Palm kernel cake	5.00	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Blood meal	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Common Salt	0.25	0.25	0.25	0.25	0.25
Vitamin/Trace min. premix	0.25	0.25	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis (% of Dm)					
Crude protein	21.97	21.96	21.95	21.92	21.94
Crude fibre	4.41	4.70	5.00	5.28	5.57
Ash	4.02	4.06	4.11	4.16	4.20
Ether extract	4.34	4.32	4.33	4.31	4.30
Calcium	1.82	1.82	1.82	1.82	1.82
Phosphorus	0.98	0.98	0.97	0.96	0.95
Metabolizable Energy(kcal/kg)	2734.71	2702.91	2690.27	2683.45	2685.45

Each kg of feed contained: Vit. A, 2, 000,000 i.u; vit. D_3, 100 iu ; vit. E, 8g ; vit. K, 0.4g ; vit. B_1, 0.3g ; vit. B_2, 1.0g ; vit .B_6, 0.6g ; vit. C, 2.40g, vit. B_{12}, 40g ; Mn, 160g ; Fe, 8.0g ; Zn, 7.2g; Cu, 0.3g ; Iodine, 0.25g ; Co, 36.0mg ; Se, 16.0mg.

Experimental Birds and Design

One hundred and fifty (150) Anak broiler chicks raised on a commercial starter mash for one week were used. They were divided into 5 groups of 30 birds each and each group randomly assigned to one of the 5 experimental diets in a completely randomized design (CRD). Each group was further sub-divided into 3 replicates of 10 birds each and each replicate housed in a pen fitted with necessary brooding facilities. Feed and water were given to them *ad-libitum*. The birds were weighed at the beginning of the trial and thereafter, weekly. Daily feed intake per group was determined by weighing the feed offered and the left-over the following morning. The feeding trial lasted 4 weeks.

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Blood collection and Analysis

At the end of the feeding trial (5th week) blood samples were collected from one broiler bird per replicate, making three samples per treatment. Bleeding was done from the punctured wing vein with a 5 ml scalp vein needle set. About 2 ml of blood was collected from each bird into two sets of sterilized bottles, one containing ethylenediaminetetraacetic acid (EDTA) as the anti–coagulant for determination of haematological parameters. Haemoglobin concentration (Hb) was determined, using Sahli method and the value recorded in g/100 mls (WHO, 1980), RBC and WBC using the improved Neubauer haemocytometer as described by Dacie and Lewis (1991). PCV was determined by the Microhaematocrit method, while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the appropriate formulae.

The second set of bottles without EDTA was centrifuged in a macro centrifuge to obtain serum for biochemical analysis. Total protein was determined, using the burette method as described by Daumas (1975); urea by di-methethyl monoxide method as described by Varley et al. (1980). Creatinine was determined by Jaffe reaction method as described by Henry et al. (1974). Albumin was measured using dye–binding technique with bromocresol green as described by Doumas and Biggs (1972). Serum potassium (K^+) and sodium (Na^+) were determined by the calorimetric method, while serum cholesterol was by a modification of the Liebermann- Burchard test.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA). Where analysis of variance indicated significant treatment effects, the means were separated using Duncan's New Multiple Range Test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION:

Proximate composition of the leaf meal is presented in Table 2. The leaf meal displayed same characteristics as leaf meals from other tropical browse plants – high crude fibre and moderate crude protein content as reported for *Jacaranda mimosifolia* (Okorie, 2006) and for *Microdesmis puberula* (Esonu et al., 2002). With relatively high crude fibre content (15.56%), the metabolizable energy must be low even though its gross energy content was high (4.16 Kcal/g).

Table 2 - Proximate Composition of Neem Leaf Meal (100% DM basis)

Components	% of dm
Crude Protein	18.10
Crude Fibre	15.56
Ether Extract	2.50
Ash	5.62
Nitrogen free Extract	58.22
Gross Energy (Kcal/gm)	4.16

Haematological Indices

The haematological indices of the starter broilers fed graded levels of Neem leaf meal are presented in table 3. The Hb value for $T_{5.0}$ compared favourably ($P>0.05$) with that of control. There were no significant differences ($P>0.05$) among the Hb values for $T_{2.5}$, $T_{7.5}$ and T_{10}. The values of Hb recorded by all the groups were, however, within the normal range for chicken (Aiello and Mays, 1998; Okeudo, 2003). There was significant difference ($P<0.05$) between the ESR values for T_0 and those of $T_{7.5}$ and T_{10}. There were no significant differences ($P>0.05$) among the control and those of $T_{2.5}$ and $T_{5.0}$. The ESR was rather decreasing with increase in leaf meal inclusion. Sedimentation rates are increased in cases of acute general infection, malignant tumours and pregnancy.

Table 3 - Effects of graded levels of Neem leaf meals on the haematological parameters of starter broilers

	Dietary levels of Neem leaf meal					
Indices	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	T_{10}	SEM
Hb (g/dl)	9.60^a	8.00^b	9.17^{ab}	8.40^b	8.67^b	0.28
ESR (Mm/1hr)	6.33^a	4.67^a	4.67^a	2.00^b	3.00^b	0.75
TWBC x 10^3 (uL)	1.143^a	0.787^b	1.325^a	1.578^a	1.119^a	0.08
PCV (%)	28.33^a	23.33^b	27.33^a	25.00^b	26.00^{ab}	0.87
Heterophils (%)	4.00^b	4.67^b	5.00^b	6.00^a	4.00^b	0.45
Lymphocytes (%)	96.00^a	95.33^a	95.00^a	95.33^a	96.00^a	0.45
Monocytes (%)	0.00	0.00	0.00	0.00	0.00	0.00
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

^{ab}Means in the same row with different superscripts are significantly different ($P < 0.05$)

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It shows therefore that the birds did not suffer from any of the aforementioned. Except for $T_{2.5}$, the values of TWBC for the Neem leaf groups compared favourably with that of the control and also were within the normal range. There were significant differences ($P<0.05$) between the PCV of the control and those of $T_{2.5}$ and T_{10}. However, $T_{5.0}$ compared favourably ($P>0.05$) with the control. The lymphocytes were not significantly affected by the treatments ($P>0.05$). No traces of monocytes, eosinophils and basophils were observed in all the treatment groups as earlier reported by Akpan (2007). It therefore shows that Neem leaf meal did not produce any form of infection since these parameters only observed when there is infection (Frandson, 1974).

Serum Biochemical Indices

The serum biochemical constituents of the birds are shown in table 4. Neem leaf meal tended to elevate the blood glucose level of the birds while reducing the cholesterol level. The increase in blood sugar level as the dietary Neem leaf meal increased was quite interesting because birds generally maintain a high and relatively constant blood sugar level even in low feed intake (Liukkonen-Anttila, 2001). The decline in cholesterol level with increase in dietary Neem leaf meal is in agreement with the report of Ogbuewu et al. (2008) in a similar work with rabbits. Upadhyay (1990) also reported a decline in blood cholesterol levels of broilers and rats fed Neem leaf meal.

Dietary Neem leaf meal did not significantly ($P>0.05$) affect the calcium, sodium and potassium levels but significantly ($P<0.05$) decreased the phosphate level while significantly ($P<0.05$) and steadily, increasing the chloride level as its level increased. The non-significant increase in serum calcium is an indication that the integrity of the kidney was maintained as reported by Ogbuewu (2008). Serum total protein steadily decreased with increase in dietary Neem leaf meal although the differences were not statistically significant ($P>0.05$)

Serum albumin and globulin did not show much consistency although at 10% dietary Neem leaf meal inclusion, they became significantly ($P<0.05$) depressed. Serum albumin and globulin depend on availability of dietary protein. This means that the proteins of the treatments T_0 -$T_{7.5}$, were similarly available to the birds, confirming the earlier observation by Hoffenberg et al. (1966). Urea level declined with Neem leaf meal but creatinine was not affected by the treatments ($P>0.05$), an indication that the proteins in the diets were effectively utilized. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were depressed as the level of dietary Neem leaf meal increased, indicating no toxic effect within the liver parenchyma of the birds.

Table 4 - Effects of dietary Neem leaf meal on serum biochemical indices of starter broilers

	Dietary levels of Neem leaf meal					
Indices	**T_0**	**$T_{2.5}$**	**$T_{5.0}$**	**$T_{7.5}$**	**T_{10}**	**SEM**
Glucose (mg/dl)	160.50^{a}	190.47^{ba}	224.73^{b}	195.23^{b}	272.37^{c}	13.22
Calcium (mg/dl)	9.9^{a}	9.57^{a}	10.60^{a}	10.10^{a}	9.80^{a}	0.31
Inorg. Phosphate (mg/dl)	5.93^{a}	4.93^{bc}	5.22^{b}	4.87^{c}	4.17^{c}	0.23
Cholesterol (mg/dl)	144.83^{a}	151.87^{a}	51.87^{c}	77.80^{bc}	85.17^{b}	1.36
Protein (g/dl)	3.20^{a}	3.03^{a}	3.03^{a}	2.90^{a}	2.76^{a}	1.67
Albumin (g/dl)	1.23^{a}	1.37^{ab}	1.47^{a}	1.43^{a}	1.03^{b}	0.07
Globulin (g/dl)	1.96^{a}	1.67^{b}	1.57^{bc}	1.47^{c}	1.73^{a}	0.09
Urea (mg/dl)	30.53^{a}	29.27^{a}	28.03^{a}	27.27^{ab}	27.03^{b}	1.23
Creatinine (mg/dl)	0.90^{a}	0.90^{a}	0.09^{a}	0.09^{a}	0.09^{a}	0.05
Sodium (Mmol/L)	147.93^{a}	153.37^{ab}	158.37^{a}	156.70^{a}	148.53^{a}	1.77
Potassium (Mmol/L)	3.50^{a}	3.20^{a}	3.80^{a}	5.07^{b}	3.87^{a}	0.37
Chloride (Mmol/L)	79.07^{a}	69.60^{a}	78.20^{a}	81.03^{a}	103.10^{b}	3.49
Bicarbonate (Mmol/L)	16.67^{a}	17.17^{a}	22.03^{b}	19.03^{b}	17.17^{a}	0.74
Tot. Billirubin (mg/dl)	0.70^{a}	0.50^{b}	0.37^{b}	0.43^{b}	0.70^{a}	0.51
Conjugated billirubin (mg/dl)	0.37^{a}	0.23^{b}	0.20^{b}	0.20^{b}	0.40^{a}	0.05
ALP (iu/l)	419.77^{a}	395.93^{a}	396.50^{a}	340.77^{b}	311.33^{b}	11.13
ALT (iu/l)	23.33^{a}	19.00^{b}	15.67^{bc}	12.67^{c}	12.00^{c}	1.26
AST (iu/l)	53.33^{a}	43.00^{b}	39.00^{b}	32.00^{c}	18.00^{d}	3.64

^{abc} Means within a row with different superscripts are significantly different ($P<0.05$)

CONCLUSION

We therefore, concluded that Neem leaf meal can be included in the diets of young broiler chicks up to 10% without any deleterious effects on their haematological and serum biochemical constituents. It reduces blood cholesterol and tends to maintain the integrities of both the kidney and liver.

REFERENCES

Aiello SE and Mays M (1998). The Merck-Veterinary Manual, 8th edition. Merck and company.

Akpan MJ (2007). Effects of neem (*Azadirachta indica*) leaf extract on productive performance, egg quality, haematological indices and coccidial load of laying hens. Msc. Thesis, University of uyo, uyo- Nigeria. pp 59-60.

AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis, 7th Edition. Washington D.C.

Babatunde GM, Pond WO, Krook L, Dvan L, Walker ER and Chapman D (1987). Effect of dietary safflower oil or hydrogenerated coconut oil on growth rate and on swine blood and tissue components of pigs fed fat-free diets. Journal of Nutrition, 92: 1903.

Church JP, Young JT, Kebau CW, Kebay JC and Ken WW (1984). Relationships among dietary constituents and specific serum clinical components of subjects eating self selected diets. The American Journal of Clinical Nutrition, 40: 1338 – 1344.

Chakraborty T, Verotta L and Podder G (1989). Evaluation of *A. indica* leaf extract for lypoglycaemic activity in rats. Phytotherapeutic Research, 3: 30 – 32.

Dacie JV and Lewis SM (1991). Practical haematology 7th (ed.) ELBS with Churchill living tone, England pp 37-85.

Doumas BT (1975). Standards for total Serum Protein assay. Clinical Chemistry, 21: 1159.

Doumas BT and Biggs HG (1972). Determination of serum albumin.in standard methods of clinical chemistry. vol.7. (ed.) Cooper, G.R. Academic press New York pp: 175.

Edoziem JC and Switzer B.R (1977). Effects of dietary protein, fat and energy on blood haemoglobin and haematocrit in rat. Journal of Nutrition, 107: 1016 – 1021.

Esonu BO, Emenalom OO, Udedibie ABI, Herbert U, Ekpor CF, Okoli IC and Iheukwumere FC (2001). Performance and blood chemistry of weaner pigs fed raw *Mucana* bean (velvet) meal. Tropical Animal Production Investigation, 4: 49 – 54.

Esonu BO, Iheukwumere FC, Emenalom OO, Uchegbu MC and Etuk EB (2002). Performance, nutrient utilization and organ characteristics of broilers fed *Microdesmis puberula* leaf meal. Livestock Research for Rural Development, 14(16)146.

Esonu BO,Opara MN, Okoli IC, Obikaonu HO, Udedibie C and Iheshiulor OOM (2006). Physiological Response of Laying Birds to Neem (*Azadirachta indica*) Leaf Meal-Based Diets: Body Weight ,Organ Characteristics and Haematology. Online Journal of Health and Allied Sciences, 2:4,www.ojhas.org/issue 18/2006-2-4.htm

Frandson RD (1974). Anatomy and Physiology of Farm Animals. Lea & febiger, Philadelphia. 494p.

Hoffenberg R, Black E and Black JF (1966). Serum Metabolites. The Journal of Clinical Investigation, 45: 143-150.

Henry R, Cannon DC and Winkelman JW (1974). Clinical chesmetry principles and techic Hagerstown, M. D. Harper,A.H and Row London, pp 543.

Iyayi EA and Tewe OO (1998). Serum total protein, urea and creatinine levels as indices of quality cassava diets for pigs. Tropical Veterinary, 16: 57 – 67.

Liukkonen-Anttila J (2001). Nutritional and genetic adaptation of gallitorns birds: Implications for hand rearings and resticking. Acanic Dissertation, Faculty of Science, University of Oulu, Oulu Yilopisto, Finland. Retrieved September 17, 2007 from http://herkulesoulu.fi/isbn951425990index.html

Okeudo, N. J., Okoli, I. C. and Igwe, G. O. F. (2003). Haematological characteristics of ducks (*Cairina moschata*) of South Eastern Nigeria. Tropicultura, 21(2): 61 – 65.

Ogbuewu IP, Okoli IC and Iloeje MU (2008). Serum biochemical evaluation and organ weight characteristics of buck rabbits fed graded levels of neem (*Azadirachta indica*) leaf meal diets. Vet online international. p 3 – 10.

Nworgu FC, Ogungbenro SA and Solesi KS (2007). Performance and some Blood Chemistry indices of Broiler Chicken served fluted pumpkin (*Telferia occidentalis*) leaves Extracct supplement. American-Eurasian Journal of Agricultural & Environmental Science, 2(1) 90-98.

Okorie, K. C. (2006). Evaluation of leaf meals of *Pentaclethra macrophylla, Jacaranda mimosifolia* and *Mucuna pruriens* as feed ingredients in poultry diets. PhD Thesis, Federal University of Technology, Owerri – Nigeria. p. 67.

Sansbury D (1980). Poultry health and management, chicken, Ducks and Turkeys. Pp21, 25.

Steel RGD and Torrie JH (1980). Principles and procedures of statistics ,New York, McGrae Hill, pp 137-269

Udedibie, A.B.I. (1987). Comparative evaluation of leaf of paw paw (*C. papaya*), Jackbean (*C. ensiformis*), swordbean (*C. gladiata*) and pigean pea (*C. cajan*) as feed ingredients and yolk coluring agents in laying diets. Nigeria Journal of Animal Production, 14: 61-66.

Udedibie ABI and Opara CC (1996). Response of growing broilers and leaf meal from *Alchonea cordifolia* Anim. Food Science Technology, 71: 157-164.

Upadhyay C (1990). The medicinal properties of neem (*Azadirachta indica*) tree. In: Animal pharmacology 2nd edition. Longman England.

Varley H, Gowshock AH and Bell M (1980). Determination of Serum Urea using Biochemistry. 5th Edition William Heineman Medical Books, Ltd., London.

Vohra P, Hjenrick RB, Wilson WC and Scopes TD (1972). The Use of ipil-ipil (*Leucaena leucocephala*) in the diets of laying chickens and laying quails. The Philippine Agriculturist, 56: 104-133.

WHO. (1980). Manual of Basic Techniques for a Health Laboratory. World Health Organization, Geneva, pp. 360 – 406.