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HISTOPATHOLOGICAL CHANGES INDUCED BY STAPHYLOCOCCAL ENTEROTOXIN PRODUCED IN YOGHURT

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ABSTRACT

In this study, six Staphylococcus aureus strains isolated from contaminated yoghurt were evaluated for enterotoxigenicity. Two of the strains were enterotoxigenic and caused fluid accumulation in rabbit ileal loops. Fluid aspirated from the loops was bloody and histopathological changes in sections collected from rabbit ileum, inoculated with crude enterotoxin, were characterized by circulatory disturbances, degenerative/ necrotic and inflammatory changes, including hyperaemia, fibrinous exudation and necrosis of villi epithelial cells. These findings showed that although SE are typically associated with vomiting and diarrhoea, which often abate within 24 hours, there was potential for more serious disturbances such as inflammation, tissue damage and toxic shock. Moreover, the production of potent SE by strains isolated from commonly consumed products such as yoghurt emphasizes the need for complete elimination of staphylococcal contaminants from foods in order to protect consumers.

Keywords: *Staphylococcus aureus,* Enterotoxins, Hyperaemia, Histopathological changes, Necrosis of villi

INTRODUCTION

Staphylococcal foodborne intoxication has been reported to be one of the most common bacterial foodborne outbreaks in many countries (Balaban and Rasooly, 2000; Adwan *et al*., 2005). A survey of sixteen European countries implicated *Staphylococcus aureus* in 1 – 13% of all reported foodborne disease outbreaks and in the United States there were 42 documented outbreaks due to *S. aureus* between 1993 and 1997 (Mead *et* al., 1999; ECHCPD, 2003). The entrotoxicosis typically occurs 30 min to 8 h after ingestion of heat-stable staphylococcal enterotoxins (SEs) synthesized in food by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly *S. aureus* (Holeckova *et al*., 2002; Le Loir *et al*., 2003).

The staphylococcal enterotoxins are a group of pyrogenic, heat-stable exotoxins with molecular weights of 25 to 29 KDa (Holeckova *et* al., 2002; ECHCPD, 2003; van Gessel *et al*., 2004). Currently, there are about 14 serotypes of SE described, which are named sequentially by letter (SEA – SEO), in order of discovery (Jarraud *et al*., 2001; ECHCPD, 2003; Le Loir *et al*., 2003). There are no established patterns of SEs production in foods. However, studies have shown a predominance of SEB and SEC producing strains in raw milk, particularly from mastitic cows (ECHCPD, 2003; Kuroishi *et al*., 2003; van Gessel *et al*., 2004; Adwan *et al*., 2005). *S. aureus* are ubiquitous in nature, existing in dust, sewage, water, environmental surfaces, humans and animals body surfaces and may contaminate foods through raw materials or handling during the preparation or manufacturing process. Not all strains produce enterotoxin. Growth and enterotoxin production are subject to a number of genetic and environmental factors. However, once the enterotoxins are produced, they are normally resistant to both heat and digestive enzymes (ECHCPD, 2003; Le Loir *et al*., 2003).

Exposure to SE has been shown to cause a range of clinical abnormalities from gastrointestinal upset to lethal toxic shock syndrome (TSS) (Ellis *et* al., 2003; van Gessel *et al*., 2004).

In this study, six strains of *S. aureus* isolated from contaminated yoghurt products were evaluated for their enterotoxin-producing ability by the ligated rabbit ileal loop assay and histopathological evaluation conducted on the rabbit intestines.

MATERIALS AND METHODS

Isolation and Maintenance of Bacterial Strains: Six *S.* aureus isolates were recovered from contaminated yoghurt samples during a study evaluating the microbiological quality of various yoghurt products. Colonies were selected following morphological and biochemical characterization of colonies resulting from nutrient agar plates inoculated with various yoghurt samples. The *S. aureus* strains were routinely maintained in Nutrient broth and stocks were maintained on nutrient agar slants at 4^{o}C.

Culture of *S. aureus* for Enterotoxin Production: Cultures for enterotoxin production were initially prepared using nutrient broth. Ten milliliter aliquots of sterile nutrient broth, in sterile Bijou bottles, were inoculated each with approximately 10^5 cells per ml and incubated at 37^{o}C for 48 h.

Subsequently, the *S. aureus* strains were cultured in 10 ml of yoghurt, pasteurized by heating to 80°C for 30 min and cultures were incubated as with nutrient broth cultures.

Preparation of Enterotoxin: Following 48 h incubation of the nutrient broth and yoghurt cultures, cell free culture supernatants were collected by centrifugation at 5000 rpm, followed by filtration through 0.22 μm Millex syringe filters. The cell free filtrates were then used as crude toxin preparation.

Assay for Enterotoxin Activity: Assay for enterotoxin was by ligated rabbit ileal loop method (Ike *et al.*, 2005). Briefly, six to nine week old rabbits were starved for 24 h with water supplied *ad libitum*. Each rabbit was anaesthesized with 2 ml of ketamin injection and secured in dorsal recumbency. Following a midline incision, starting from the rectal end, the ileum was divided into segments of 5 cm in length with string ligatures. The crude toxin preparation (0.5 ml) was injected into different segments. Uninoculated broth and sterile saline were injected into some segments to serve as negative controls. The incisions were then sutured and the animals allowed to recover from anaesthesia. After 7 h, the animals were sacrificed and the segments examined for fluid accumulation (dilatation).

For positive loops, the volume of fluid recovered by aspiration was used to determine the dilatation index (DI) estimated as the ratio of volume of fluid to length of ileal segment. A DI $\geq$ 0.2 was taken as positive. The test was done in triplicate animals.

Histopathology: Sections of both normal and enterotoxin-inoculated rabbit ileum were fixed by immersing the cut pieces in 10 % formol saline for 24 – 48 h. Following fixation, the tissues were dehydrated in a series of ascending ethanol concentrations (70 %, 80 %, 90 %, 95 %, and absolute), and then embedded in paraffin. Sections were thereafter cut with a microtome at 5-6 μm. Cut tissues were stained with haematoxylin and eosin and examined microscopically. Photomicrographs were taken.

RESULTS AND DISCUSSION

Of six *S. aureus* isolates tested, two isolates were found to be enterotoxigenic. Cell-free culture supernatants (crude toxin preparations) of the *S. aureus* strains caused fluid accumulation when injected into rabbit ileal segments, indicating enterotoxin activity. Dilatation index (DI) values ranged from 0.2 to 0.57. Moreover, there was a dark-reddish colouration of the positive ileal loops (Figure 1) and the aspirated fluid from such segments appeared bloody. Histopathologic changes in sections collected from the rabbit ileum were characterized by circulatory disturbances, degenerative/necrotic, and inflammatory changes. Sections of the intestine collected from untreated (control) rabbits showed mucosae (including glands) and submucosae with

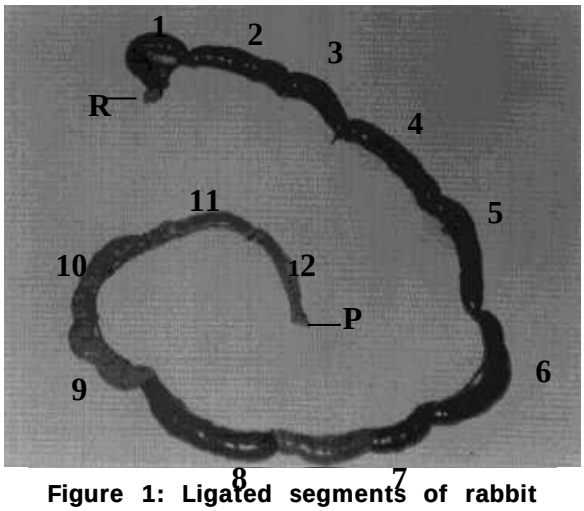

Figure 1: Ligated segments of rabbit ileal loop after injection with crude preparations of staphylococcal enterotoxin (SE) produced under different growth conditions. 1 – 8, SE produced at pH 5; 9 and 10, SE produced at pH 4 – there was change to a brownish colouration but no fluid accumulation; 11, segment inoculated with sterile saline and 12, uninoculated control. R and P, represent the rectal and pyloric ends respectively.

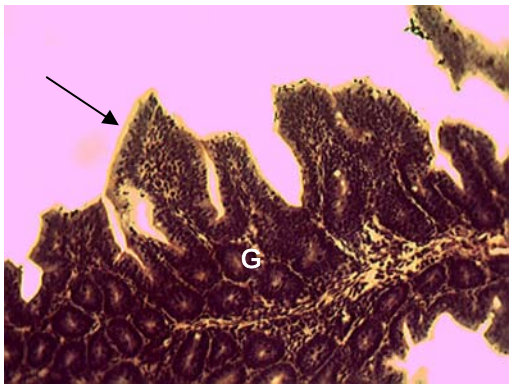

Figure 2: Section of control rabbit ileum showing normal villus (arrow) and intestinal gland (G). H and E stain, x 200.

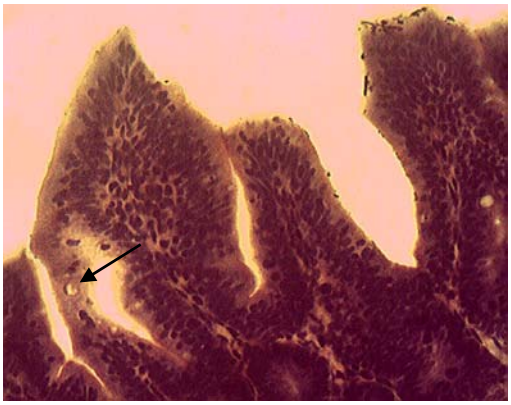

Figure 3: Section of control rabbit ileum showing columnar epithelium lining cells of villi and Goblet cell (arrow). H and E stain, x 400.

normal histomorphology (Figures 2 and 3), while sections from rabbit ileum inoculated with crude toxin preparations showed moderate to severe hyperaemia, with moderate fibrinous exudation onto the intestinal mucosae (Figure 4). In addition, there was distortion of villi epithelium at the tips and necrosis of epithelial lining cells. The intestinal glands were generally normal (Figure 5).

These findings are similar to those reported by other investigators in which various SEs induced varying degrees of inflammatory and degenerative changes. Kuroishi *et al*. (2003), elucidated mechanisms by which SEC induced inflammatory changes in bovine mammary glands. The SEC-inoculated mammary glands exhibited interstitial inflammation, with epithelial cell degeneration and migration of polymorphonuclear neutrophils.

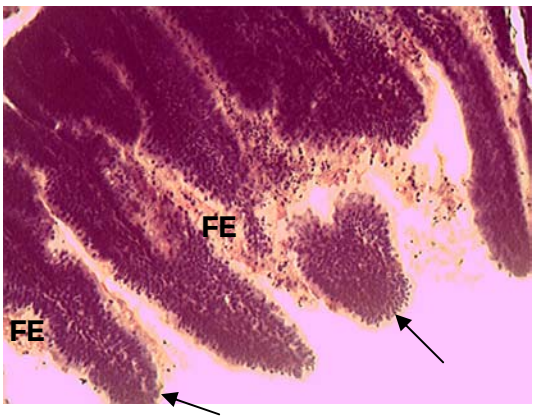

Figure 4: Section of rabbit ileum inoculated with crude staphylococcal enterotoxin, showing fibrinous exudate (FE) on mucosae with degeneration and necrosis of villi epithelial cells (arrow) epithelial cells. H and E stain, x 200.

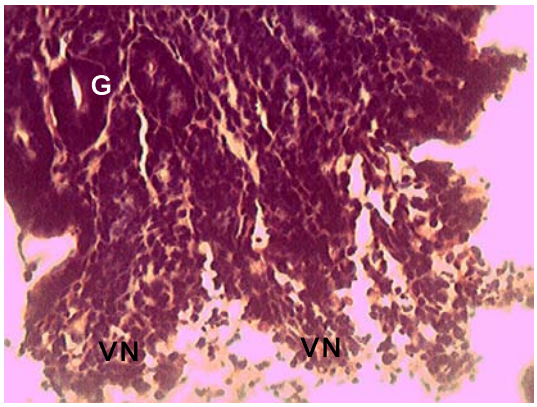

Figure 5: Section of rabbit ileum inoculated with crude staphylococcal enterotoxin, showing necrosis of villi (VN) and normal intestinal gland (G). H and E stain, x 400.

Other studies have shown that exposure to SE can cause a range of clinical abnormalities from gastrointestinal upset to lethal toxic shock syndrome (TSS) and once introduced into host tissues; these toxins have the ability to elicit pathology in different systems (Jett *et al.,* 1990; Greenfield *et al*., 2002). Van Gessel *et al*. (2004) working with piglet models demonstrated lethal SEB intoxication. Clinical signs observed in that study included pyrexia, vomiting and diarrhoea within 4 h, followed by terminal hypotension and shock by 96 h. There has even been reported involvement of SEA in a fatal case of endocarditis (Ellis *et al*., 2003).

The SEs have been reported to act as superantigens (Yokomizo *et* al., 1995; Johnson *et* al., 1996; Ferens *et al*., 1998; Kuroishi *et al*., 2003; van Gessel *et al*., 2004) and have the capacity to cause T-cell proliferation with massive inflammatory cytokine release. Much of the lethal effects of SEs have been attributed to this superantigenic activity (Miethke *et al*., 1992; Jardetzky *et al*., 1994; Johnson *et al*., 1996). However, the more common symptoms of intoxication due to SEs remain those of pyrexia, vomiting and diarrhoea, which often abate within 24 h (Jett *et* al., 1990; van Gessel *et al*., 2004). Consequently, there may be a tendency by the general population to underestimate the health risks posed by formation of SEs in food. However, the findings in this study, show that vomiting and diarrhoea may be only the least of possible health problems that could result from ingestion of preformed SEs with food, particularly in more sensitive individuals such as the very young and very old.

Although our study describes histological changes in a rabbit model, there is documented evidence that the clinical syndromes in some animal models simulate human enterotoxicosis (van Gessel *et* al., 2004). There is, therefore, a need to reassess the importance of preventing the growth of the coagulase-positive staphylococci in foods and their production of enterotoxins.

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IMPACT OF LAMDA CYHALOTHRIN PYRETHROID INSECTICIDE ON THE UPTAKE OF CATIONS AND ANIONS BY THE GILLS OF FRESHWATER CATFISH HYBRID JUVENILE

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ABSTRACT

The impact of acute exposure of karate (Lambda cyhalothrin pyrethroid) insecticide was evaluated in a 4 – day exposure period at 20, 40, 60 and 80 ppm to Heterobranchus bidosalis(+) X Clarias gariepinus(♂) fingerlings showed the 96-hlc 50 as 25.11 ppm. The threshold value was 25. 11 ppm. The gills of the exposed fish analyzed showed a significant decrease in all major cations and anions ($C1^-$, Ca^{2+}, Na^+, K^+, Mg^{2+}) at P < 0.05). There was no inhibition of uptake of the cations and anions ($C1^-$, Ca^{2+} Na^+, K^+, Mg^{2+}). Their uptake increased rapidly during the 24 hr period and dropped at 48 hr and 72 hr and gradually increased at the end of 96 hr showing that it was time dependent. During the exposure period the fish stood in upright position with their snouts above the water surface gasping for air. Other behavioral characteristics of the exposed fish were peeling of the skin, initial increase in opercula movement, curvature of the body, loss of balance, erratic swimming and quietness. Based the outcome of this research and under similar experimental condition it is the recommendation of this research that this pyrethroid will affect the uptake of the major cations and anions. It further advises environmental officers, crop farmers and insecticides habitual users to be cautious on the use of this insecticide because of the resultant consequences of the misuse.

Keywords: Karate, Uptake, Cations, Anions, Gills, Catfish, Toxicity

INTRODUCTION

Aquatic organisms are bathed in solutions of trace metals at dissolved concentrations ranging from nanograms per litre in the open ocean, through level approximating micrograms per litre in coastal seas, to even higher concentrations approaching or exceeding milligrams per litre in estuaries and acid rich streams and salt lakes (Bryan and Gibbs, 1983). However the uptake of many trace metals from solution into aquatic organisms is facilitated by the use of gills, which is an appendage of fish body primarily concerned with the exchange of gases (Hoar 1975). Fish gills that can serve this purpose comprise a large part of fish body that contacts the external environment and they play an important role in the gas and ion exchange between the organism and environment.

They are important organs for the uptake of heavy metal compounds in fishes. Thus the gills are the very first site where metal-induced lesions may occur which may result in impaired gas and ion exchange (Witeska *et al.,* 2006). Subsequently, metal ions within the blood may affect the blood cells. (David and Philips, 1993).

The uptake of dissolved metals may take place all over the body surface of small and/or soft bodied organisms, as well as at particular site of high permeability such as the gills. Metal uptake from solution will also take place in the alimentary tract, when any of the medium is swallowed during "drinking" or the ingestion of food. For example, hypoosmoregulating crustaceans in littoral or salt lake environment will drink the medium to replace the water lost by osmosis, and all also excrete excess salts actively through the gills (Mantel and Farmer, 1983). Marine teleost fish are also hypo-osmotic regulator and drink seawater routinely, subsequently disposing of excess salts via gills and feaces.

Natural pyrethroids have proved of great value for use indoors for public hygiene, medicine and animal health. Uses include the control of lice and fleas in the homes and public buildings and the control of houseflies, mosquitoes and other insects that spread diseases of animals and humans importance (Hassall, 1990). Pyrethroids are ideal for home uses because they are of low toxicity to man and other warm-blooded animals. Moreover they are readily destroyed by heat during cooking or by digestive juices should trace get into food, onto fingers of children or onto the feet of domestic animals. Their outdoor use is severely restricted reason being that they are rapidly decomposed by light (Ruzo, 1982).

Although safe to higher animals, pyrethroids, both natural and synthetic are toxic to fish (Hassall, 1990). Pyrethroids are remarkable effective insecticides because of their ability to disrupt the insect nervous system at concentration that result in no mammalian toxicity.

Reports based on the work of Laufer *et al.* (1985) using the giant axon of the cockroach showed that pyrethroids interfered with the uptake and trans-membrane movement of sodium ions. Authors like Doherty *et al.* (1986) and Brooks and Clark (1987) observed that nonomolar concentration of pyrethroids reduce calcium ion uptake by 50 percent in housefly larvae. Hassall (1990) studied the inhibitory effect of Type 1 and type II pyrethroids on Y–aminobutyric acid (GABA) – dependent chloride ion influx into rat brain micro-sacs. Their findings showed that pyrethroids possess potent neurotoxic activity on several types of ion channels, such as chloride ion channels that are activated by GABA, voltage sensitive sodium channels and calcium channels. Furthermore, they reported that pyrethroids affects membrane pumps that involved Ca^{2+} dependent ATPase and Ca^{2+}/Mg^{2+} dependent ATPase.

Pyrethroids are extremely toxic to aquatic animals due to impaired metabolism, resulting in LC_{50} values 10 -1000 times higher for fish than for mammals. Fish toxicity is greater in the presence of α-cyano moiety and is inversely related to temperature (Hassall, 1990). Field exposure studies have shown that pyrethroids are not as toxic to fish populations in their native environment as in bioassay studies conducted in the laboratory. This observation is explained in part by the hydrophobic nature of pyrethriods, which results in high levels of nonspecific binding to apolar humic substances and organic particulate matter, thereby decreasing the effective concentration of bioavailable pyrethroids (Hassall, 1990).

"Heteroclarias" hybrid juveniles were chosen for this study because of their economic viability and strong genetic potential for aquaculture programmes. They are known for their breed easy technology. They are ubiquitous in Africa and are now very popular among fish farmers and have become African most cherished and adaptable aquaculture candidate. They constitute one of the main fish families of economic value as food fish. Several authorities have advocated using them for restocking programmes but there is strong opposition to that because they might pollute the genetic strains of the catfish family (Fagbenro, 1982).

The Karate (Lambda cyhalothrin pyrethroid) insecticide was purchased from "Clemagro" Agrochemical Nigeria Limited, Umuahia, Abia State, Nigeria. This insecticide cyhalothrin (pyrethroid) was introduced by ICI Australia and ICI Agrochemicals (now Zeneca Agrochemicals). The common name is cyhalothrin (BSI, draft E – ISO BAN); cyhalothrin (f) draft F – ISO). The IUPAC name (RS) - - cyano –3 – phenoxybenzyl (Z) – (IRS, 3 RS, 3RS) – (2 – chloro = 3, 3, 3 – frifluropropenyl) – 2, 2 – dimethylcy clopropanecar boxylate. Roth: (RS) - - cyano – 3 – phenoxy benzyl (Z) – (IRS) – cis – 3 – (2 – chloro – 3, 3, 3, - trifluoro = propenyl) – 2, 2 – dimethyl – cyclopropane carboxylate (Tomlin 1997).

The objective of this study was to examine the impact of acute exposure of karate (Lambda cyhalothrin pyrethroid) on the uptake of some major cations and anions (Na^+, CI^-, K^+, Mg^{2+}, Ca^{2+}) in the gills of the freshwater catfish hybrid (*Heterobranchus bidosalis* X *Clarias gariepinus*) Juvenile.

MATERIALS AND METHODS

Catfish Hybrid Procurement and Management: Freshwater catfish (hybrid of *Heterobranchus bidorsalis* (male) X *Clarias gariepinus* (female) were obtained from African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, River State Nigeria. The hybrid juveniles averaging 9.26 ± 0.05 cm (Total length) and 6.23 ± 0.02g body weight were used for this study. The fish were held in the laboratory in large water baths of 160 L capacity at 24.3 – 26.0^0C and acclimated for two weeks prior to experiment. The fish were fed 5% body weight with Pfizer pelleted diet during acclimation. A daily photoperiod of 16: 8h light: dark was maintained during acclimation and experimental phases.

LC_{50} Screening Test: LC_{50} screening tests (Range finding tests) were carried out in one litre conical flasks containing 30 juvenile catfish. Concentrations of pyrethroid insecticide which caused 50% fish death within 30 minutes were omitted from the test. Acute concentrations of insecticide used were 80, 60, 40, 20, and 0.00 ppm (control). Ten catfish hybrid juvenile were exposed to acute concentrations of 20, 40, 60, 80 ppm of insecticide while the 0.00 ppm served as the control. The experimental set up consisted of 15- circular plastic water tanks (30L capacity) filled to 20-litre mark with well-aerated dechlorinated tap water. Insecticide concentrations were obtained by diluting stock solution prepared weekly. The already prepared concentrations were introduced into the first four tanks serially, the fifth tank served as the control (devoid of the toxicant Karate); while the remaining ten tanks served as replicates. The test media were changed every 24 h, replacing the old media with fresh solution.

Methods for acute toxicity tests as described by Sprague (1973) were employed for this investigation. The fish were not fed 24h prior to and during the exposure period which lasted for 96h. The 96–h Lc_{50} confidence limit was calculated as a summary of the percentage mortality data using formula as described by Lichfied and Wilcoxon (1949) thus: $S = (LC_{84}/LC_{50}) + (LC_{50}/LC_{16})$, where LC_{84} is the probit 84, LC_{50} is probit 50 and LC_{16} is probit 16 from graph.

Experiment 1: Mortality, Opercular Ventilation Rate and Behavioural Studies: Mortality was recorded every 24 h, though the tanks were inspected every 3h for dead fish which were immediately removed. The opercular ventilation rates per minute, were read at the start of and every 24h thereafter. Behavioural responses of the exposed fish were observed on a 3 – hourly basis.

Experiment 2: Ions and Water Chemistry: In the second experiment four fish was removed from each tank containing different concentrations of toxicant

including the control which was devoid of the toxicant with the aid of scalpel.

The gills were dissected out and analysed to determine the effects of the toxicant (Karate) on the uptake of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} by the gills. This was done daily and it lasted for 4 days. The cations and anions were determined by Atomic Absorption spectrophotometer model (AA – 670, Shimadu, Kyoto) with background compensation capability for dissolved oxygen, free carbondioxide, total hardness, alkalinity and pH. These parameters were monitored every 24h using methods described by APHA (1989).

Data Analysis: Results obtained from these investigations were analysed using the analysis of variance (ANOVA) methods to test for level of significance at the 0.05 probability level.

RESULTS

The mean of water quality parameters obtained during the period of exposure of the *H. bidosalis* X *C. gariepinus* hybrid to the different concentrations of pyrethroid insecticide showed that the temperature was 24.3 $\pm$ 0.6^{0}C, pH, 7 ± 0.04, Dissolved Oxygen, 5.79 ± 0.5 mg/l; Carbon (IV) Oxide 1.17 ± 0.1 mg/L and Total Alkalinity was 8.04 $\pm$ 0.2 mg/l. The water quality parameters in the treatment tanks did not very significantly ($P < 0.05$) from those of the control tanks (Table 1).

Behavioural patterns observed during the exposure period include; the fish stood upright with their snouts above the water surface gasping for air, curvature of body, peeling of the skin, initial increase in opercula movement, loss of balance, erratic swimming and quietness. The fish finally died.

Mortality increased with increase in pesticide concentrations. Thus mortality was highest in 80 ppm and lowest in 20 ppm. There was no mortality in the control experiment (Table 2). The 96-h LC_{50} was 25.12 ppm while the threshold value was 25.11 ppm.

The uptakes of the major cations and anions by the gills during the exposure have been presented in (Table 3). There was a significant difference between the uptake of the cations and anions among the treatment tanks ($P < o.05$). Uptake of Mg^{2+} and Ca^{2+} were significantly different at ($P < 0.05$), while the uptake of K^+, Na^+ an CI^- were not significantly different at ($P < 0.05$).

There was no total inhibition of uptake of the cations and anions (Mg^{2+}, Ca^{2+}, Na^+, K^+, and CI^-) but their uptake increased rapidly during the 24 h period and then dropped at 48 h and 72 h, and gradually increased at the end of the 96 h indicating that its uptake was time dependent (Table 3).

DISCUSSION

In the present study, there was no significant difference in the physicochemical parameters of the treatment medium (water). The water quality parameters of the treatment tanks during the exposure did not vary significantly from those of the control tanks. All were within suggested tolerance levels (Mackereth, 1963). The value of 96-h LC_{50} of 25.12 ppm reported in this experiment was within the range earlier reported by Oti (1999) for several tropical freshwater catfish species exposed to insecticides. Amadi (2004) and Nmerole (2004) reported a 96-h LC_{50} of 25.00, and 26.08 pp for fish *Heterobranchus bidorsalis and Clarias gariepinus* exposed to Dichlorvos insecticide and round up herbicide respectively. Several other workers have reported 96-h LC_{50} for several species of the African catfishes exposed to different pesticides in contrast to what was observed in this experiment. Aguigwo (2002) reported 96-h LC_{50} of 4.17 mg1-^{1} for *Clarias gariepinus* exposed to cymbush pesticide. Rahman et al (2002) reported 96-h LC_{50} of 6.55, 3.09 and 2.72 ppm for *A. testudineus*, *C. punctatus* and *B. gonionotuse* exposed to Diazinon. Perschbacher and Sarkar (1989) reported 2-h LC_{100} values of 15 ppm for snakehead exposed to rotenone. We however noted that the differences, LC_{50} values of in the present study from those of cited workers may be attributed to difference in fish species, age, size, sex, strain, fish condition and experimental techniques adopted (Brown, 1990).

During the exposure period, the test fish showed abnormal pathological sign of peeling of the skin and several behavioural changes such as increased opercula and respiratory activities, curvature of the body, erratic swimming, loss of balance, strong spasm, paralysis and finally death. These behavioural movements are in agreement with earlier reports of Avoaja and Oti (1997), Oti (1999), Witeska *et al.* (2006) and Rahman *et al.* (2002). These behavioural responses were indications of stress and nervous disorder.

The initial increase in opercular activities of fish exposed to pyrethroid insecticide as reported in this findings suggests that fish exposed to karate insecticide tended to exhibit avoidance syndrome during the first few hours of exposure. However, as the exposure period was prolonged the fish became fatigued hence subsequent drop in opercula activity. Indices of opercula activities have earlier been reported by Saroj and Gupta (1987) as a strong indicator of stress when fish were exposed to toxicants. Basha *et al.* (1984) have shown initial increases in the respiratory rate of fish exposed to malathion, this was soon followed by a decreased in respiratory rate. The combined effect of fatigue and toxicity of the karate to the exposed fish often led to death.

According to Gill *et al.* (1988) respiratory distress in *P. conchonius* exposed to dimethoate was due to degeneration of secondary gill lamellae, edematous separation of respiratory epithelium and degenerated chloride cells in the interlamellar crypts of gill tissue. It may be assumed that as a result of reduced efficiency of damaged gills to respiratory activity other metabolically active; tissues like liver and muscle receive less oxygen leading to severe tissue hypoxia. Development of such internal hypoxic conditions may be responsible for the overall stressful conditions of exposed fish observed in the present study.

Table 1: Water quality parameters during exposure to Karate

Parameter	Toxicant Concentration (ppm)				
	20	**40**	**60**	**80**	**Control**
Temperature (°C)	24.3± 0.8	24.2 ±0.	23.8 ±0.4	24.3 ± 0.01	24.9 ± 0.5
pH	10 ± 0.2	9.5 ± 0.07	7.03 ± 0.21	7.02 ± 0.3	7.15 ± 0.4
Dissolved Oxygen (Do) (mg/l)	6.20 ± 0.4	5.88 ± 0.004	5.3 ± 0.6	5.10 ± 0.5	6.43 ± 0.2
Total alkalinity (mgl)	8.2 ± 0.2	8.0 ±0.4	8.0 ± 0.6	5.0 ± 0.1	11 ± 0.2

Table 2: Mortality rates of experimental *Heterobranchus bidosalis X Clarias gariepinus* exposed to different concentration of Karate

Conc. (ppm)	Log. Conc. (ppm)	Mortality (hrs) 24	48	72	96	Total mortality	Survival %	Mortality	Probit kill
0	0	0	0	0	0	0	10	0	0
20	1.3010	0	1	1	2	4	6	40	4.75
40	1.6021	0	1	1	2	5	5	50	5.00
60	1.7782	1	2	2	3	8	2	80	5.85
80	1.9031	1	2	3	3	9	1	90	6.30

Table 3: Cations and anions uptake by gills of exposed fish

Conc. ppm	Exposure period 24h			48 h			72 h			96 h		
	Na+	**CL^-**	**K**	**Na^+**	**CL^-**	**K^+**	**Na^+**	**CL^+**	**K^+**	**Na^+**	**CL**	**K^+**
0	92.0	0.14	75.0	94	0.15	96	97	0.14	75	105	0.15	88
20	70.5	0.13	66.0	86	0.14	57	86	0.15	68	86.5	0.15	72.5
40	81.5	0.15	62.5	94	0.15	77	95	0.15	66	96.5	0.15	77
60	74.5	0.15	60.0	83	0.15	76.5	77.5	0.14	65	83.5	0.14	70.5
80	89.5	0.15	63.0	90	0.15	72.5	76.5	0.14	64	77.5	0.15	67.5
	Mg^{2+}	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**	
0	1945	4408.8		243.1	451		291.7	802		246	762	
20	972.5	4408.8		143.6	512.05		121.6	561		505.5	559.5	
40	972.5	5210.4		145.9	401		145.9	381		173.45	441	
60	1094.05	5210.4		133.75	721.5		461.9	41.5		27.1	514	
80	850.95	6613.2		121.6	501		158.05	541		139	520.5	

There was no total inhibition in the uptake of the cations and anions (Na^+, $C1^-$, K^+, Mg^{2+} studied but there was slight reduction in the uptake. Similar observation was recorded by Brooks and Clark (1987) when they exposed the rat brain to nanomolar concentrations of pyrethroid with an alpha - cyano groups. They observed the calcium ion uptake was reduced by 50 percent. Laufer et al. (1985) reported similar findings in the giant axon of the cockroach exposed to pyrethroid. They observed that pyrethroids interfere with the uptake of sodium and chloride ions as well as transmembrane movement of these ions. It has been suggested that pyrethroids appear to affect membrane pumps that involve Ca^{2+} dependent ATPase and Ca^{2+}/ Mg^{2+} dependent ATPase (Hassall 1990).

The uptake of many trace elements concentrations in the tissues of aquatic organisms at their permeable surfaces is generally considered to be a passive process not requiring the expenditure of energy (Simkiss and Taylor, 1989). This situation contrasts markedly with that pertaining to the major ions of alkali metals (e.g. sodium, potassium, calcium), which are taken up through active transport pumps. The key to this difference lies in different chemistries of the two groups of metals (Nieboer and Richarson, 1980). The uptake of dissolved metal may take place all over the body surface of small and/or soft bodied organism, as well as particular sites of high permeability such as the gills (David and Philips, 1993).

Uptake of potassium, sodium and chloride ions did not vary significantly between the concentrations at ($P < 0.05$) while the uptake of magnesium and calcium ions varied significantly at ($P < 0.05$). The relationship between metal uptake and loss dictates the particular metal accumulation strategy of on organism (David and Philips, 1993).

Whether the usual uptake of this ions from solution is by passive facilitated diffusion or by incorporation into active transport pumps the rate of uptake of an element will be directly proportional to the external concentration of dissolved metal over typical environmental range of uptake of most circumstances, however, the rate of uptake of trace elements respond proportionally to increases in external dissolved concentrations (Rainbow and White, 1990), Nugegoda and Rainbow (1989) have shown that changes in salinity and osmolatity had different effects in the uptake of zinc by *Palaemen elegans*. Campbell and Jones (1990) have shown the physiological response of prawn to the uptake of these ions. In contrast to the case of Heteroclarias" hybrid" catfish it appears that progressive physiological reduction in the uptake of cations and anions in the initial hour 24 and 72 hours of exposure observed in this research may have been the result of external concentration of pyrethroid bound linkages which exerts strong osmotic pressure with concomitant reduction in the available ions of the sample.

The gradual rise in metal ions during the 96 hours period indicated a recovery phase in the experimental animals. At the end of the recovery period the cation and anion values was near to control value. Similar trends of event have been documented by Begum (2004) for *Clarias batrachus* exposed to carnofuran insecticide.

Similarly the significance different at ($P<0.05$) in the uptake of potassium and chloride ions may be as a result of acid – base balance and osmotic regulation in the test fish. Sodium and potassium are associated with chloride in acid/base balance and osmo-regulation (McDonald *et al.,* 1995)

The increase in the uptake of these cations and anions at the 24 hour and decrease at 48 hour and 72 hour, and finally gradual increase at the end of 96 hours may be linked to initial phase of acclimation and or adjustment process to acute concentrations of xenobiotic compound. The high metal uptake of ions in the control fish may be because the control tank was devoid of the toxicant. The above result also show that the uptake of these cations and anions (Na^+, CI^-, Ca^{2+}, Mg^{2+}, K^+) is time dependent.

The uptake of these cations and anions varied significantly between the treatment tanks and the control groups ($P > 0.05$).

It may be concluded that in general that acute exposure of *"Heteroclarias"* to karate (Lambda cyhalothrin) pyrethroid insecticide under similar environmental conditions and experimental techniques will affect the uptake of the major cations, and anions, although it may not totally inhibit, the uptake of these metal ions. As a follow up to this, it is advisable for environmental officers, crop farmers and insecticide habitual users to be cautious on the use and application of this insecticide as its misuse may affect the hydrodynamics and stability of ambient water quality standard and the aquatic life therein.

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EXOGENOUS TESTOSTERONE STIMULATES GLUCONEOGENESIS IN HYPOPROTEINEMIC ALBINO RAT

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ABSTRACT

Changes in plasma glucose and protein concentrations in two experimental groups of albino rats, weighing 250 - 300g, were evaluated after 7 days of acclimatization to laboratory conditions and another 14 days of feeding the rats with low protein diets. Frank hypoproteinmia was evident by the low plasma protein levels and some clear physical manifestations, such as hair loss, change in skin colour and edema. Edema was caused by lowered plasma protein concentrations. Daily intraperitoneal (i.p.) injections of 0.2 ml of testosterone for a period of 7 days produced a statistically significant increase in plasma glucose concentrations (P < 0.01) when compared with the saline-treated controls. There was a statistically significant decrease (P <0.05) in total protein concentrations in testosterone injected hypoproteinemic rats when compared with the control rats. These findings suggest that testosterone, in addition to its anabolic function of protein build up in muscles, may also be involved in gluconeogenesis, the formation of plasma glucose from non-carbohydrate substrates. Apparently, the hypoproteinemic rats require enough glucose to survive since glucose is the only source of energy for the mammalian brain. The mechanism of action of steroid hormones on target organ cells, and the role of testosterone as a performance enhancing drug are discussed.

Keywords: Exogenous testosterone, Protein, Glucose, Gluconeogenesis, Hypoproteinemic rat

INTRODUCTION

Protein-energy malnutrition, also referred to as kwashiorkor, is a disease condition that results from hypoproteinemia (Heaton, 1986). Hypoproteinemia means a drop in blood protein. This condition often leads to edema since a drop in blood protein, chiefly serum albumin, means a decrease in colloid osmotic pressure (COP) which, in turn, means an increase in filtration pressure (FP). This explains the edema of malnutrition (Pihs, 1963; Zaloga and Roberts, 1994).

The major source of blood glucose during prolonged periods of fasting comes from protein (Mayer and Thomas, 1967). For survival of the brain, plasma glucose concentration must be maintained. Glycogen stores, particularly in the liver, act as the first line of defense. They are quickly broken down to glucose by a process known as hepatic glycogenolysis (Meyer and Thomas, 1967) and released into the bloodstream. They can supply the body's needs for several hours, but under prolonged conditions of starvation protein and fat are mobilized. Utilization of substrates, such as amino acids and glycerol, provides a glucose-sparing action, and the molecules may also be employed directly in glucose formation, a process known as gluconeogenesis (Meyer and Thomas, 1967)

In human beings, kwashiorkor kills more people than all other malnutrition diseases put together (Fakunle, 1986). Five million children in Africa, Latin America, and the Far East, die every year from kwashiorkor. It is a very common disease in tropical countries where the population lives on staple food such as plantains, cassava, or maize, which provides enough energy as carbohydrates but not enough protein for a growing child (Fakunle, 1986). Added factors are poverty, ignorance and taboos against giving milk, eggs, fish or meat to children (Heaton, 1986). In mammals, treatment for kwashiorkor or hypoproteinemia has often centered mainly on protein and amino acids supplementations (Heaton, 1986).

Androgens are known to inhibit the pituitary gland, stimulate protein anabolism or buildup in muscles, and cause the retention of potassium and phosphate (Olsen, 1979; Maclusky and Naftolin, 1981; Sairam *et al.,* 1981). Testosterone is the chief androgen, and the present investigation was designed to assign a possible role for exogenous testosterone in the treatment and maintenance of hypoproteinemic albino rats.

MATERIALS AND METHODS

Experimental Animals: 15 adult male albino rats, weighing 250 – 300 g, were collected from the department's animal house and acclimatized in the laboratory for 7 days. During acclimatization, all the animals were fed and watered *ad libitum* with diet formulated from mixture of maize 50g, fishmeal 20g, and groundnut cake 40g. The proximate composition of the balanced diet is presented in Table 1.

Hypoproteinization of Rats: 10 of the acclimatized rats were placed on a diet of carbohydrates consisting of a mixture of maize, plantain, cassava, and rice for 14 days. This feeding regime (Table 1) rendered the rats hypoproteinemic as compared with the rats maintained on the balanced diet. The hypoproteinemic rats were divided into 2 groups, B and C, and housed in separate rat cages. The

proximate composition of the high carbohydrate diet is presented in Table 1.

Group A rats served as controls and were given daily intraperitoneal (i.p.) injections of physiological saline (0.6 % saline) of 2.5 ml/kg body weight. Group B rats were given daily i.p. injections of saline, while group C rats were injected with 0.2 mg/kg body weight of testosterone for a period of 7 days.

At the end of the experiment, animals were properly anaesthetized with chloroform prior to being sacrificed. Blood was collected by cardiac puncture into heparinized test tubes. The test tubes were centrifuged for 15 minutes at 2000 revolutions per minute (rpm). The plasma rich supernatant was decanted into clean test tubes for protein and glucose determination and the packed blood cells residue were discarded. This was repeated three times and the mean values recorded.

Table 1: Gross and Proximate compositions of balanced and high carbohydrate diets

Food items	A	B
Maize	50g	20g
Plantain	-	20g
Rice	-	20g
Fishmeal	20g	-
Groundnut cake	40g	-
Composition		
Moisture	19.9%	24.4%
Ash	1.6%	0.7%
Lipid	25.8%	11.6%
Protein	12.1%	4.6%
Carbohydrates	40.7%	44.8%

A - Balanced diet, B - High carbohydrate diet

Analytical Procedure: The proximate compositions of both the balanced diet and the hypoproteinemic diet were analyzed by methods described by Windham (1996)

Physical Changes in Experimental Albino Rats: The five rats, which served as controls were fed *ad libitum* with balanced diet. The 10 rats, which served as experimentals were fed with high carbohydrate diet throughout the period of experimentation. Physical changes between the control and hypoproteinemic rats were observed and recorded daily.

Weighing of Liver and Kidneys: After drawing the blood from the control and experimental rats, livers and kidneys were removed, cleaned with blotting paper and weighed on a Mettler balance. The weights of the livers and kidneys of each group were added together and the mean calculated by dividing the total weight of each group by the number of rats in that group, which is 5 rats.

Quantitative Determination of Total Plasma Protein and Glucose Levels: The glucose concentrations of plasma were determined by the glucose oxidase method (Folin and Malmrose, 1929; modified by Free, 1963). The experiment was replicated three times.

Quantitative Determination of Total Plasma Protein Concentration: The total protein concentrations of plasma were determined by the biuret method (Tietz, 1995, 1999). The experiment was repeated three times and the mean value taken.

Statistical Analysis: All results were expressed as means ± standard deviation (SD). Statistical comparisons between the two groups of hypoproteinemic albino rats were performed using the Student's two - tailed test for unpaired data. The analysis of variance (ANOVA) for statistical difference among the different groups of rats was performed by means of Steel and Torrie (1980).

RESULTS

Physical Manifestations of Hypoproteinemia: Physical manifestations of hypoproteinemia were evident from this investigation. Normal albino rats had weight gains, no hair losses, and no changes in colour. In contrast, the hypoproteinemic rats developed edematous appearances, hair losses and changes in skin colour. They remained miserable or listless as compared with the normal rats.

Mean Weights of Livers and kidneys: There was no significant change in the mean weights of the livers of Group A rats (7.2 ± 0.33 mg) and the Groups B and C rats (7.1 ± 0.30 mg). Similarly, there was no significant change in the mean weights of the kidneys of Group A rats (1.2 ± 0.12 mg) and Groups B and C rats (1.1 ± 0.12 mg)

Mean Plasma Glucose Concentration of the Groups of Rats: The mean plasma glucose concentrations of the three groups of rats (A, B, and C) are presented in Table 2. There was a statistically significant increase ($P < 0.01$) between the plasma glucose concentrations in Group A rats (84.75 ± 1.35 mg%), Group B rats (104.69 ± 3.46 mg%) and the Group C rats (128.50 ± 1.89 mg%).

Table 2 Changes in Plasma glucose and total protein concentrations of the groups of rats (mg/100 ml)

	Plasma glucose ± SD mg%	Total protein ± SD mg%
A	84.75 ± 1.35	7.45 ± 0.13
B	104.65 ± 3.46	6.41 ± 0.19
C	128.50 ± 1.89	5.90 ± 0.20

A = Normal rats treated with saline, B = Hypoproteinemic rats injected with saline, C = Hypoproteinemic rats injected with testosterone

Mean Plasma Total Protein Concentration of the Groups of Rats: The mean plasma total protein concentration of the three groups of rats (A, B and C) is presented in Table 2. There was a statistically significant decrease ($P < 0.05$) between the plasma total protein concentrations in Group A rats (7.45 ±

0.13 mg %), Group B rats (6.41 ± 0.19 mg %) and group C rats (5.90 ± 0.20 mg %).

DISCUSSION

Hypoproteinemia leads to a disease condition known as kwashiorkor. In mammals, treatment for kwashiorkor has often centered on amino acid supplementations. In humans, patients suffering from protein-energy deficiency, or kwashiorkor, manifest signs of edema (Heaton, 1986).

Androgens, like other steroid hormones, are known to act by stimulating protein synthesis within their target tissues (Chan and O'Malley, 1976; Vesely, 1980; Brooks, 1981). The major functions of testosterone include stimulation of the development of male secondary sexual characteristics, and protein anabolism (buildup) in muscles (Edelman and Marvar, 1980).

In the present investigation, some Albino rats were experimentally rendered hypoproteinemic by feeding them with high carbohydrate diets for a period of two weeks. Frank hypoproteinemia was established by lowered plasma protein concentrations in addition to physical manifestations. In normal rats there was increase in mean body weight, no hair losses, and no changes in skin colour. In contrast, the hypoproteinemic rats developed edematous appearances, hair losses, and changes in skin colour. They remained miserable or listless as compared with the normal rats. The results showed no significant changes in the mean weights of the livers and kidneys between the saline- treated rats and the testosterone-injected rats.

The mean plasma glucose concentration of the testosterone-treated rats was statistically higher ($p<0.01$) than the saline-treated hypoproteinemic controls. There was a statistically significant decrease ($P<0.05$) in plasma total protein concentration between the saline-treated control rats and the testosterone-injected experimental rats (Figs. 1, 2 and Table 2).

These findings suggest that testosterone significantly lowered the plasma total protein and elevated the plasma glucose levels. In fact, the rise in blood glucose concentration is concomitant and proportional to the fall in blood total protein concentration when compared with the control rats. It can be conclusively surmised that the androgen, testosterone, in addition to its anabolic function of protein buildup in muscles, may also be involved in the formation of plasma glucose from non-carbohydrate substrates, such as proteins, amino acids and lipids, a process referred to as gluconeogenesis (Perssin and Bell, 1992). Apparently, the hypoproteinemic rats require enough glucose to survive since glucose is the only source of energy for the brain of mammals, including human beings (Guillemin, 1978).

Recent report (Ndukuba, 2004) showed that exogenous thyroxine stimulated gluconeogenesis in normal albino rats. The present finding is interesting since, like thyroid hormones, steroid hormones employ the mobile receptor mechanism and act on the genome to induce messenger ribonucleic acid (mRNA) synthesis and subsequent protein synthesis (Jacobs and Cuatrecasas, 1977; Iynedjian, 1993). Testosterone has been classified as a performance enhancing drug by World Athletic Federations. Thus, athletes who test positive to testosterone during routine checks are banned for life from competitive sporting events.

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A NEW POLYSACCHARIDE, *Detarium microcarpium* FROM TRADITIONAL NIGERIAN PLANT FOOD: ITS PHYSIOLOGICAL EFFECTS ON RATS

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ABSTRACT

Detarium microcarpium is a leguminous plant food used traditionally among the Ibos in the South Eastern part of Nigeria as a thickening agent in vegetable soups. Detarium is largely uncharacterised and under exploited. There is a dearth of information in the literature on this plant food. The aim of the study is to process, analyze and characterise detarium flour; screen detarium using rats to investigate it's physiological effect on the general metabolism of rats, compare detarium to guar gum (GG) as a positive control, to determine the effects of the two foods on the plasma cholesterol level of rats. The result of the analysis showed that powdered detarium has a mean particle size of 464µm. The SNSP content per 100 g food sample was 59.8 g. The viscosity of 1% aqueous dispersion of the powdered detarium food sample obtained using the U tube capillary viscometer was 4000 – 24000 cp. The main SNSP fraction of detarium was identified to be a high molecular weight xyloglucan. In the rat study, the experimental diet contained detarium or guar gum, as positive control, at a level providing 80g soluble NSP/kg diet. Food intake, faecal output, weight gain, digestibility, food efficiency ratio and plasma cholesterol (after overnight fasting) were measured. The result showed that the cholesterol levels of rats fed detarium and guar gum diets were significantly lower than the control (P < 0.05) using the analysis of variance. Detarium and guar gum covariates such as weight gain, food intake and faecal output. The results obtained indicate that detarium may possess properties as guar gum which maybe useful in the management of diabetes and disorders of lipid metabolism in humans.

Keywords: *Detarium,* Guar Gum, Soluble Non starch polysaccharides, General rat metabolism

INTRODUCTION

Water-soluble non starch polysaccharides (SNSP) such as guar gum, a glactomannan from locust bean, have received widespread attention as dietary agents that modulate gastrointestinal function as well as lipid and carbohydrate metabolism. Many studies have demonstrated that this polysaccharide when incorporated into starchy foods and glucose drinks, attenuate the postprandial rise in blood glucose and insulin concentrations in healthy and diabetic subjects (Jenkins *et al.,* 1976; Ellis *et al.,* 1981, 1991; Morgan *et al.,* 1990; Fairchild *et al.,* 1996). Animal studies have shown that the postprandial effects of SNSP depend mainly on their capacity to increase the viscosity of the digesta in the upper part of the gastrointestinal tract (Cherbut *et al*., 1990; Ellis *et al*., 1995, 1996; Johansen *et al.*, 1996). In vitro and animal experiments indicate that an increase in intraluminal viscosity of digesta is a major factor in inhibiting the rate of digestion and absorption of available carbohydrate and improved glycemic index (Blackburn *et al.,* 1984; Mann, 1985, 1995; Edwards *et al*., 1988; Ellis *et al.,* 1995).

Soluble NSP has also been shown to modify plasma lipids by reducing the availability of bile acids which interferes with the absorption of fat (Kelly and Tsai, 1978; Gee *et al.,* 1983). The binding of bile acid and cholesterol is the basic mechanism for the hypocholesterol effect of dietary fibre (Kay and Truswell, 1977). This has been shown in animals (Judd and Truswell, 1976; Judd, 1980; Judd and Truswell, 1985) and in humans (Judd and Truswell, 1981; 1982; Anderson and Chen, 1983; Anderson *et al.,* 1984).

In the Eastern part of Nigeria numerous plant food preparations are used as food condiments. Flour produced from these plant foods are traditionally used to thicken vegetable soups and liquid foods. The thickening property is due to the presence of starch and/or SNSP. On the basis of their ability to thicken soups and liquid foods, it was suggested that these plant materials may have properties similar to guar gum and could be useful sources of water-soluble dietary fibre (SNSP) in glucose control and lipid metabolism in humans. Some of the plant foods are legumes; others are derived from different parts of the plant. It was therefore necessary to undertake a detailed investigation of the physicochemical and nutritional properties of one of these plants foods. The food selected in this study is *Detarium senegalensis*, leguminous plant. The local name for *Detarium* is 'Offor'. *Detarium* is unexploited and largely uncharacterized, commonly used as condiment in the Eastern part of Nigeria. There is a dearth of information in the literature regarding this food.

The present investigation was designed to process, analyze and characterise and determine the polysaccharide composition of detarium flour using a

range of chemical and physical techniques; screen *Detarium* using rats to establish it's physiological effect on the general metabolism of rats by comparing it to Guar gum, as a positive control, to determine it's effect on the plasma cholesterol level of rats.

MATERIALS AND METHODS

Preparation and Processing of Plant Food Extracts: *Detarium senegalensis* Gmelin is a leguminous plant belonging to the subdivision Caesalpinoideae (Balogun and Fetuga, 1986) and is considered to be synonymous with *Detarium microcarpium* (FAO, 1988). Each pod produced by the plant contains one seed, which is usually rounded, oval or flattened and about 40 mm in diameter (FAO, 1988). The legumes grow predominantly in West Africa, Chad and Sudan. The seed samples were purchased in Nsukka and transported to the UK for processing into flour.

The processing method involved boiling the seed for 45 – 60 minutes until the deep brown-purple seed coats (testae) were peeled off easily when touched. The testae were then removed and the white cotyledon was soaked in water for 60 min. The cotyledons were washed three times with cold tap water and discarded after each washing. The cotyledon was then soaked in water overnight to wash away some of the gummy exudates. The washed cotyledons were sun-dried for 24h and ground into fine powder that passes through a 1 mm screen using a coffee grinder (Moulinex blender/mill). The powdered material was air-dried at room temperature for 24h until the powder did not form lumps when touched. The detarium powder (Table 1) was yellowish-white in colour and possessed strong characteristic odour (Onyechi, 1995).

Chemical and Physical Methods of Analysis of the Plant Food Extract: The test food was analysed using standard methods (Kirk and Sawyer, 1991) for moisture (104^{o}C for 16h); ash (Total minerals; 525 ^{o}C for 12h); fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (micro-Kjeldahl method; N x 5.7). The starch content of the flour was determined by an enzymatic method (Englyst *et al.,* 1992a.).

The Englyst method (Englyst *et al.,* 1992b) was used to determine total NSP and the water-insoluble fraction of the NSP; the water-soluble fraction of the NSP was determined as the difference. The procedure involves acid hydrolysis of the NSP followed by gas chromatography of the alditol acetate derivatives of the neutral sugars. The test food sample was boiled with 80% ethanol for 1 hour under reflux. The residue obtained by filtration was washed with 95% ethanol and air dried at room temperature. The dried residue was extracted with 7 ml of distilled water then followed by centrifugation. The supernatant was collected, pH adjusted and centrifuged. The SNSP in the supernatant was precipitated by addition of absolute ethanol. The precipitate was collected by filtration and stored at 4 ^{o}C

The particle size distributions of the test foods were determined by a standard laboratory mechanical sieve analysis method (Lauer, 1966); water binding, by the method described by Quin and Paton (1978) and the viscosity of 1% aqueous dispersion of the test foods obtained by the U tube capillary viscometer.

Rats: Six litters, each containing four male Sprague Dawley rats weighing between 86 g and 141 g were used. The rats were supplied by A. Juck and Sons, London. Each litter of rats was placed in a cage. The rats were fed stock diet (CRM Labsure, Christopher Hill, London), for the first two days and then placed on ground stock diet for a further five days to acclimatise them to eating a ground diet.

Formulation of the Control Diet: The batch size of diet prepared was 5 kg. The calculated quantities of casein (New Zealand Milk Products UK Ltd), vitamin mix and mineral mix (King's College, London mix), sucrose (Booker Fitch Food Services), solka floc (Jordensen and Wettre Limited) and corn starch (Cerestar, Manchester HHIPA), were each weighed and transferred to Hobart mixer and blended for 15 minutes. Sufficient corn oil was heated in beaker to approximately 80 ^{o}C and calculated amount of cholesterol (BDH Chemicals Limited) was weighed and stirred into the corn oil and mixed well to dissolve. This mixture of cholesterol and corn was added to the dry ingredients and blending continued for another 30 min until well distributed. The mixture was passed through a 1/8 inch mesh size. Homogenization of the total mixture was ensured by mixing for a further 30 minutes in the Hobart mixer. The diet was stored at -20^{0}C in self- sealed freezer bags. The proximate composition of the diet is shown on Table 1.

Table 1: Composition of the control diet, positive control diet, containing guar gum and the test diet containing detarium

Ingredients (g/kg).	CD	GGD	DTD
Casein	150	150	129
Fat (corn oil)	100	100	90
Vitamin mix	40	40	40
Sucrose	100	100	100
Cholesterol	10	10	10
Solka floc	50	50	50
Guar gum (M90)	-	100	-
Detarium	-	-	180
Starch	550	430	381

Note: The starch, casein and corn oil content of each was corrected so that all diets had equivalent nutritional content. Each test diet contained approximately 80g/kg total NSP. The vitamin and mineral mix were King's College London mix.

Formulation of the Test Diets: The test diets contained 80g NSP/kg. Corn starch was added to substitute the test flour, in order to supply 80 g NSP/kg diet. The NSP content of the test flour was obtained from the analysis of the foods (Onyechi,

1995). The protein and fat contents were also adjusted as required to accommodate the protein and fat already in the foods. The positive control diet GGD, contained medium grade guar gum, (M90) to provide 80g NSP/kg diet (Meyhall Chemical Company Ltd, Switzerland). The proximate composition of the diet is shown on Table 1.

Feeding of the Rats: On arrival the rats were fed stock diet for 2 days and placed on ground stock diet for a further 5 days to acclimatise them to eating a ground diet. After one week of acclimatization the rats were assigned into groups so that one rat from each litter went into each experimental group. The groups were therefore assumed to be genetically similar and fed for 14 days. The rats were individually housed in stainless steel cages with suspended trays containing filter paper linings for collection of spill and faeces. The rats were weighed daily for the first two days and then on alternate days. Weight was determined by difference from week to week.

Food intake was recorded by providing each rat with an individual weighed pot of food, weighed on alternate days before topping up the food supply and reweighed. At the end of each week of the two experimental periods, the spillage was collected by sifting the faeces from the spilt food.

The rats were pair fed. Each rat was provided with individual weighed pot of food. The daily food intake for the poorest feeders was calculated. This amount plus additional 2-3g of food was fed to each rat. On subsequent days each litter mate across the groups was given an amount of food equal to the previous day's intake of the poorest feeder. If all the food provided was eaten by the rats in a litter-group, the food available for intake was increased by 3-5g on subsequent days.

The faeces samples were collected separately from each animal and stored in self-sealed bags at -20^{o}C until analysis. Dry weight (DW) of spilt food was determined by drying spilt food together with the cage lining paper and food adhering to it, in an oven at 105^{o}C for 48 hours. The dry weight of the paper was subtracted from the total to give dry weight of spillage. Dry weight of the remaining food in the pot was similarly determined after drying for 48 hours at 105^{o}C. Food intake was calculated.

Food intake, faecal output, weight gain, energy digestibility and plasma cholesterol were the parameters that were assessed in the rats.

Bleeding of the Rats and Plasma Cholesterol Analysis: At the end of 14 days of experimental feeding, the rats fed the experimental diets were anaesthetized and bled from the heart using a heparinized needle and syringe to prevent the blood from clotting. The blood was collected in a centrifuge tube and centrifuged at 2,500 rpm for 15 minutes. The plasma was separated from the cells and stored in LP tubes at a temperature of -20^{o}C until analysis. The fasting plasma cholesterol levels were determined by enzymatic method (Roschkur, 1975; Sidel *et al*., 1981) using Boehringer-Mannheim kit method.

Statistical Analysis: The differences between the effects of the types of diets on the rats were analysed by analysis of variance, ANOVA (Statistical analysis system package, SAS Institute, 1995) The level of significance was fixed at $p<0.05$. The results were further investigated using Tukey's method and Least Square Means. Tukey's method compared the means of groups by modified t-test which takes into account the multiplicity of comparison of adjusted means which would have resulted if all experimental units were identical with regard to covariates. Thus the statistical analysis model examined not only the effect of diet on rat plasma cholesterol level but also the effect of a number of additional covariates such as weight gain, food intake, faecal output and diet digestibility.

RESULTS

Chemical and Physical Characteristics of Plant Food Extract: The analysis of detarium flour was based on 100g flour of the food sample (dry weight). The results indicated that detarium contained 5.9g fat, 12.1g protein, 0.4g starch and 1.9g ash (Table 2). The total NSP determined by the Englyst method (Englyst *et al.,* 1992), was 63.8g/100g of which 59.8 g/100g was the SNSP fraction and 4.0g/100g was the water insoluble NSP (Table 3). The sugar composition of the SNSP fraction indicated a high proportion of glucose, xylose and galactose (Table 4). The mean particle size of the detarium flour was 464µm and the viscosity of a 1% aqueous dispersion of the flour was between 4,000 and 40,000 cps ().

Table 2: Constituents of Detarium food samples g/100g

Parameter (g/100g)	Detarium
Moisture	6.4
Fat	5.9
Protein	12.1
Ash	1.9
Total CHO	73.8
Available CHO	18.3
Dietary fibre (by difference)	55.5
NSP	59.7
Kcal (by bombing)	369.0
Available energy	179.0
Starch	0.4

Onyechi 1995.

Table 3: Soluble, insoluble and total NSP content (g/100g) of wet and dry detarium flour

Food sample [Detarium]	Wet powder (g/100g)	Dried powder (g/100g)
Soluble NSP	55.9	55.8
Insoluble NSP	3.7	4.0
Total NSP	59.7	63.8

Onyechi, 1995.

The food intakes across the groups of rats were similar. There was no significant difference in weight gain of the groups of rats fed the Control, Guar and Detarium diets. However, rats on the control diet had higher weight gain.

The mean faecal output of the groups of rats fed control (25.9g); guar (25.3g) and detarium diets (27.7g) were similar with detarium having the highest faecal output. The mean plasma cholesterol levels of groups of rats fed the control diet (3.38 mmol/L) was significantly higher than the plasma cholesterol levels of the groups of rats fed the two test diets detarium diet (2.25 mmol/L); guar gum diet (2.14 mmol/L) at $p<0.05$. The percentage reduction was 34% for detarium as shown in Table 5.

Table 4: Non-cellulosic polysaccharide composition (g/100g) of Detarium flour

Detarium sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Uac
Soluble NSP	t	t	2.3	18.0	0.3	10.0	26.7	2.5
Insoluble NSP	t	t	0.5	0.1	0.1	0.3	t	0.3
Total NSP	t	t	2.8	18.5	0.4	10.3	2.6	2.8

Rha= rhamnose; Fuc= fructose; Ara= arabinose; Xyl= xylose; Man= mannose; Gal = Galactose; Glu= glucose; Uac = uronic acid (Onyechi, 1995)

Table 5: Mean plasma cholesterol levels (mmol/L); food intake (g/14d); weight gain (g/14d); food efficiency ratio (g wt gain/g food eaten); faecal weight (g/dw/14d) and diet digestibility for rats CD, GGD and DT treatments

Parameters	CD	GGD	DTD
Plasma cholesterol	3.38 ± 0.19^{abc}	2.14 ± 0.21^{a}	2.25 ± 0.14^{c}
Food intake(g)	169 ± 4.0	144 ± 9.0	157 ± 8.0
Weight gain (g)	42.8 ± 3.30^{a}	30.2 ± 2.80^{b}	37.2 ± 3.20^{c}
Food efficiency ratio	0.25 ± 0.02	0.20 ± 0.02	0.24 ± 0.01
Faecal output (g)	25.9 ± 0.05	25.3 ± 1.50	27.7 ± 1.60
Digestibility (%)	84.7 ± 0.30	81.9 ± 0.70	82.1 ± 1.30

Values in the same row with same superscript are significantly different $p<0.05$. Note: CD= Control diet; GGD= Guar gum diet; DTD=Detarium microcarpium diet (Onyechi, 1995)

ANOVA was also used to examine the effect of all the variables interacting with the type of diets to determine their effect on plasma cholesterol levels. Only the type of diet had a significant effect on cholesterol level. This is probably because the group of rats fed guar diet ate slightly less of the diet (144 g) than the rats fed on the control diet (169 g) and detarium (157 g). However, the effect of the food intake was less significant than the effect of the type of diet in affecting the plasma cholesterol level of the rats. ANOVA followed by the LSM test did not reveal any significant effect by such variables as food intake, weight gain, FER, faecal output and digestibility. The mean plasma cholesterol levels obtained after adjustment for effects of different co-variants is highlighted in Table 6.

Table 6: Mean plasma cholesterol levels (mmol/L) of rats unadjusted and adjusted for co-variates fed control and positive control diet (Guar gum diet) and test diet containing detarium

Diet	A	B	C
Control diet	3.18	2.70	2.68
Guar gum diet	2.14	2.05	1.78
Detarium diet	2.25	1.99	1.89

Note: A - Unadjusted means; B - Cholesterol levels adjusted for faecal output and weight gain; C - Cholesterol levels adjusted for weight gain and digestibility.

It appeared that rats fed guar and detarium diets had similar covariate levels. The LSM test verified that the cholesterol lowering effect was due to the diet. Also when the cholesterol means were adjusted for covariates, the two diets still had a similar effect.

DISCUSSION

The chemical analysis of detarium showed that it is high in SNSP. On a dry weight basis the SNSP content was 55.8g/100g. *Detarium* was shown to have a significant amount of SNSP and very viscous. The high SNSP fraction of detarium seed extract and the viscous nature could have positive physiological effects. The chemical analysis has shown that the main monosaccharide component of the extracted SNSP fraction of detarium flour consists mainly of xyloglucan. This is structurally similar to the main polymer found in tamarind gum, a seed extract of the plant *Tamarindus indica L*. (Reid, 1985) which is also known to have beneficial effects on lipid metabolism in rats (Yamatoya *et al.,* 1996).

An important determinant of the biological activity of SNSP, which has been found in significant amount in detarium and shown in guar gum studies, is their capacity to generate viscosity in the lumen of the stomach and small intestine. This has been shown to be of primary importance in reducing the rate (and possibly the extent) of digestion and absorption of available carbohydrate (Ellis *et al.,* 1996). An increase in the viscosity of stomach content can impair gastric function, such as sieving and mixing, one consequence of which is an increase in the size of large-sized food particles entering the small intestine (Meyer and Doty, 1998). Furthermore, an increase in digesta viscosity is thought to reduce the rate of emptying from the stomach (Ellis *et al.,* 1996). Increased viscosity in the gut lumen, inhibits the propulsive and mixing effect of intestinal contractions (Blackburn *et al.,* 1984). Under these conditions, SNSP, which are contained in detarium, just as guar gum, may likely impair the rate of digestion of starch as a result of less disruption of food particles, reduced mixing of food with intestinal secretions and decreased transport of hydrolysed products of starch to the mucosal surface. Recent evidence also suggests that such polymers in addition to increasing digesta viscosity, may act as a physical barrier to amylase-starch interaction in the lumen of the small intestine (Brennan *et al*., 1996).

The proximate analysis showed that detarium contains 55.8g/100g of NSP (Onyechi, 1995). Results of the rheological study (Onyechi, 1995) indicated that a dilute solution of detarium when fully hydrated has properties similar to medium grade guar gum (M90) with similar intrinsic viscosity

of 8.9 +/- 0.2 dl/g and 8.7 dl/g respectively. Thus, the NSP extracted from detarium like guar gum appears to be a high molecular weight polymer which when hydrated gives viscous solution. The two diets had similar effects on the rats food intake, faecal output, digestibility, weight gain and food efficiency ratios and resulted in a similar decrease in the level of plasma cholesterol thus apparently confirming the rheological study.

The higher fat excretion of rats fed detarium, seem to indicate an effect of the fibre in reducing fat absorption rather than solely the lower food intake. The effectiveness of dietary fibre in reducing cholesterol levels probably lies in its ability to reduce availability of fatty acids and cholesterol for absorption in the upper intestine. This maybe seen as increased faecal fat; increased cholesterol and its bacterial metabolites, and reduced bile acid re-absorption, which affects cholesterol esterification (Dreher, 1987).

It can therefore be suggested that the mode of action by which detarium reduced plasma cholesterol levels in rats is similar to that of guar gum. The mechanism by which guar gum reduces glucose, insulin and cholesterol levels is not completely understood but several hypotheses have emerged regarding these cholesterol level lowering effects. These hypotheses include alteration in bile acid absorption and metabolism; decreased activity of the digestive enzymes; effect of short chain fatty acids (SCFA); changes in hormone concentrations and reduction in fat absorption (Cummings, 1981; Anderson, 1986 and Anderson *et al*., 1990). Dietary fibre like detarium, may affect lipid absorption by such mechanisms as altered gastric function, decreased availability of bile acids interference with effective micelle formation, altered digestive enzyme activity (Anderson *et al.,* 1990). NSP bind bile acids and decrease their availability for optimal fat digestion absorption. This may be indicated in high faecal fat content of detarium fed rats. *Detarium* contains some carbohydrate (CHO), however, analysis shows that the starch content is very low 0.4g/100g dry weight (Onyechi, 1995). It is therefore likely that the effects of the SNSP in the detarium outweigh the possible effects of CHO digestion and SCFA production in the large gut.

In conclusion, the result of this study suggested that detarium may have beneficial physiological effects and there is need to study it's effect on glucose and insulin profiles in humans.

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VARIATION IN RELATIVE PALATABILITY OF DIFFERENT FORAGES FED TO RABBITS

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ABSTRACT

Twenty four 10-week old crosses of (New Zealand White X Chinchila) rabbits was used to determine relative palatability differences in leaves of Calopogonium mucunoides (Calopo), Elaeis guineensi (Oil palm), Musa sapientum (Banana) and Andropogon gayanus (Gamba). Centrosema pubescens (Centro) was included as control. Significant differences (P < 0.01) in relative palatability index (RPI) were detected among the different forages offered. Based on their RPI, rabbits preferred in descending order of magnitude Centrosema pubescens, Calopogonium mucunoides and Elaeis guineensi (RPI > 95 %) to Musa sapientum (RPI > 70 %) and Andropogon gayanus (RPI > 40 %). The preference of Oil palm leaves to Banana leaves according to the RPI ranking in this study is an interesting observation from the study.

Keywords: Forage plants, Relative palatability, Rabbit

INTRODUCTION

Rabbits have potential as meat-producing animals in the tropics, particularly on subsistence-type farms. Characteristics such as small body size (thus low daily feed requirements), short generation interval, high reproductive potential, rapid growth rate and the ability to utilize forages and fibrous agricultural by-products are attributes in favour of rabbit production (Cheeke, 1986; Cheeke *et al.,* 1987). Rabbit is also a good source of meat, which is of high quality with low cholesterol and therefore suitable for special diets (Owen, 1981). It has an advantage over poultry and pigs because it can convert locally available plant products and by-products such as *Leuceana leucocephala* (Raharjo and Cheeke, 1985) and by-product feeds (Raharjo *et al.,* 1986) into animal protein for human consumption.

It is heart warming however, to note that the ability of rabbits to subsist on forages derived from leguminous browse plants and multi-purpose trees (MPTs) has brought tremendous relief to rabbit producers. In spite of these apparent advantages, rabbit production has not yet achieved its optimum potential in the tropics.

In selecting herbage for rabbits the most important factors for consideration are: Availability of the browse, the crude protein level, the digestibility factors and toxic constituents.

The major classes of browse plants are grasses and legumes. Based on the crude protein, crude fibre and digestibility, legumes are more superior to grasses but their utilisation is seriously limited by their levels of toxic constituents. The grasses on the other hand will dry up during the dry season and thus unavailable. Within the rabbitary unit, Ebonyi State University, perennial plants such as banana and oil palm trees abound in addition to herbaceous legumes and grasses. However, during the late dry season, most of the herbaceous legumes and grasses dry up leaving the oil palm and banana leaves.

This trend has necessitated the need for a study to determine the relative palatability of *Andropogon gayanus* (gamba grass), *Musa sapientum* (banana leaves), *Calopogonium mucunoides* (calopo), and *Elaeis guineensis* (Oil palm leaves), using *Centrosema pubescens* (centro) as control fed to rabbit.

MATERIALS AND METHODS

Study Area: The study was carried out at the Rabbitary Unit, Teaching and Research Farm, Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki. The station is located between latitude 06o 21N and longitude 08o 51E. The annual rainfall ranges from 1500 to 1800 mm with a temperature range from 21^{o} to 30^{o} C (Ofomata, 1975).

Rabbit: Twenty four ten-week old crosses of (New Zealand White X Chinchila) rabbits were introduced into the pen for five hours each day. The rabbits had been accustomed to feeding on Centrosema pubescens since weaning.

Palatability: A palatabilty study using the cafeteria method was conducted. Fresh leaves from 8-weeks early dry season regrowth of Andropogon gayanus, Calopogonium mucunoides, and leaves from matured Musa sapientum, and Elaeis guineensi were harvested and fed to rabbits. Centrosema pubescens was included as control. Leaves were harvested during the morning (08.00 - 11.00) of each collection day. Equal amounts were offered separetely in plastic feeding troughs randomly placed around the

perimeter of an 8 x 6 m roofed pen. Weights of residual material were recorded and consumption of each forage calculated. The procedure was repeated for 10 days following a five-day adjustment period. Feeding throughs were re-randomised each day. A daily relative palatability index (RPI) was calculated for each forage by dividing all consumption values by that of the highest value, and multiplying the result by 100.

Statistical Analysis: The experimental design was a split-plot with two pen replications. Analysis of Variance (ANOVA) was used to analyse the data according to the procedure outlined by Steel and Torrie (1980). Detection of differences among treatment means were carried out using the least significant difference (LSD) procedure.

RESULTS

The chemical composition of the forages has been shown in Table 1. The crude protein contents of Centro, Calopo and Elaeis were 20.8, 18.4 and 12.5 % respectively while the Banana leaves and Gamba had Crude protein contents of 12.2 and 11.8 % respectively. The organic matter as well as the crude fibre contents of the forages were also shown (Table 1).

Table 1: Proximate composition of forages (% DM basis)

	Centro	Calopo	Oil palm	Banana leaves	Gamba grass
Ash	7.0	9.8	7.0	13.0	6.0
Organic matter	93.0	90.2	93.0	87.0	94.0
Crude fibre	30.7	21.6	29.8	23.1	29.3
Ether extract	2.9	3.1	6.5	5.6	2.5
Crude protein	20.8	18.4	12.5	12.2	11.8
Nitrogen free extract	38.4	47.1	44.2	46.1	50.4

The relative palatability index (RPI) and preference ranking (PR) of the forages are shown in Table 2. Analysis of RPI data showed no significant ($P > 0.05$) forages by collection day interaction. However, significant ($P < 0.01$) differences were detected among the different forages offered when data were accordingly analysed across days. Rabbits preferred in descending order of magnitude Centrosema pubescens, Calopogonium mucunoides and Elaeis guineensi (RPI > 95 %) to Banana leaves (RPI > 70 %) and Andropogon gayanus (RPI > 40 %).

Table 2: Relative palatability index (RPI), and preference ranking (PR) of the forages

Forages	Relative palatabilty index	Preference ranking
Centrosema pubescens	100	1
Calopogonium mucunoides	98.5	2
Elaeis guineensi	97.6	3
Musa sapientum	73.2	4
Andropogon gayanus	49.2	5

The results of forage comsumption by rabbits during the ten days cafeteria feeding is shown on Figure 1. The consumption of centro, calopo and oil palm leaves were significantly ($P < 0.01$) higher than those of banana leaves and gamba grass. Banana leaves was consumed more ($P < 0.01$) than gamba grass. There were no significant ($P > 0.05$) differences in the consumption of centro, calopo and oil palm leaves

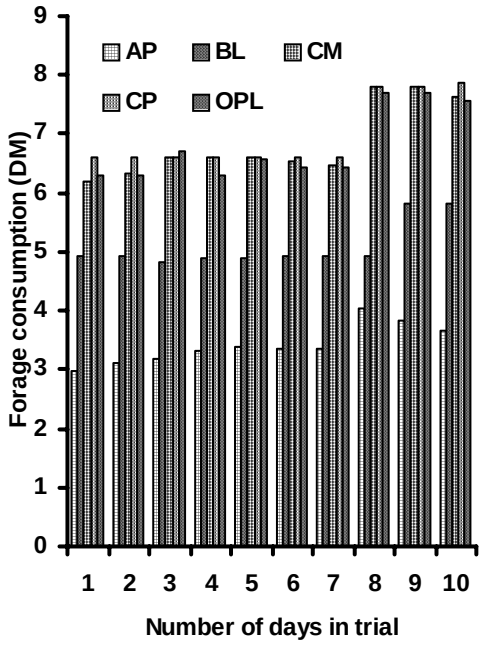

Figure 1: Forage consumption of rabbits during cafeteria feeding. Legend: AP = *Adropogon gayanus*; BL = Banana leaves; CM = *Calopogonium mucunoides*; CP = *Centrosema pubescens*; OPL = Oil palm leaves

DISCUSSION

The exact reasons for the differences in relative palatability of rabbits fed different forages is not known, and could not be explained by this study. This is because palatability is a complex phenomenon determined by dietary type and environmental variables (Marten, 1978; Molyneux and Ralph, 1992). However, it could be argued that rabbits preferred familiar and novel foods that complemented the flavours and macronutrient contents of their basal diet (Cheeke, 1986).

The relative palatability index (RPI > 95 %) observed in centro, calopo and oil palm leaves was corroborated by the high crude protein contents of 20.8, 18.4 and 12.5 % observed in this group. Differences in anti-nutritional factors (ANF) and astringent tastes in forage legumes may be partly accountable for the difference in observed RPI. A key concept in palatability explanation is aversion, the decrease in preference for food just eaten as a result of sensory input (taste, odor, texture, i.e. food's

flavour) and postingestive effects (of nutrients and toxins on chemo-, osmo-, and mechano-receptors) unique to each food (Provenza, 1995).

The crude fibre levels in the forages were in the range of 21.6 30.7 % (Table 1). According to Champe and Maurice (1983), rabbits require more than 9 % crude fibre in feed for normal growth.

The preference of oil palm leaves to banana leaves according to the RPI ranking in this study is an interesting observation. Banana plants are around the farm and its leaves usually given to rabbits as against oil palm leaves that is equally available. This observation is in agreement with the reports of Provenza (1996) who noted that aversion also occur even when nutritious food are eaten too frequently or in excess.

The decision to feed Banana leaves was made by owners of rabbits and it may interest them to try out oil palm leaves now. Further research is needed to establish anti-nutritional factors in forages using preference ranking.

Conclusion: Data from this study showed that there is a great potential for improvement in rabbit production. Our study indicated differences in relative palatability among selected forages. Identification of other palatable forages such as Musa sapientum and Oil palm leaves during dry season could stimulate interest in rabbit production.

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MACROINVERTEBRATE FAUNA OF A NIGERIAN FRESHWATER ECOSYSTEM

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ABSTRACT

A survey of macro invertebrate fauna of Anambra River was carried out for 22 months at Otuocha, Ogurugu and Nsugbe. The macro invertebrates were sampled using kick sampling techniques and scoop nets. Sampled specimens were identified to generic level. During the study a total of 21 genera of macroinvertebrates belonging to 13 families were identified. The fauna was composed of Gyrinus sp. (29.2%), Macrobranchium sp. (19.6%), Ranatra sp. (13.2%) and Agabus sp. (3.5%). The margalef's index of fauna richness showed that Otuocha station had the highest species richness (12.70), followed by Nsugbe (7.01), and Ogurugu (6.80) stations. The least fauna diversity of 0.21 was registered at Nsugbe as against 3.15 at Otuocha and 0.86 at Ogurugu. The Mc Naughton community dominance index was more pronounced at Nsugbe (53.1) than at Otuocha (49.69) and Ogurugu (47.04). Jackson's fauna similarly index showed that the fauna at Otuocha and Ogurugu were more closely related (0.64) than the fauna at Nsugbe.

Keywords: Macroinvertebrates, Anambra River, Nigeria

INTRODUCTION

The lotic and lentic inland water, as well as brackish and marine waters in the tropics are habitats for a variety of macroinvertebrate fauna. Work on macroinvertebrate fauna in the tropics has shown that the quantitative collection of key species from natural aquatic habitats or that modified by man can provide a means of estimating various ecological parameters, such as richness or evenness in diversity (Holloway and Barlow, 1983).

Pickavance (1991) reported that mayflies (Ephemeroptera) and Gyrius (Coleoptera) are predominant members of the fauna of polluted stream. The presence of large detrital material has profound effect on the macroinvertebrate community, especially in those deeper parts of the lake where the homogeneous sediments support only a limited diversity of species. In such cases animals which are normally only found in the littoral may extend into the benthic zone (Me Lachlan, 1974). This may be due either to the availability of food or the provision of a suitable substratum or a combination of these factors.

Recent faunistic, quantitative works include those leading to the provision of keys for the identification of tropical fresh water fauna (Miles and Graham, 1970), checklist of macroinvertebrates of Ikpoba rivers, (Ogbeibu and Oribhabor, 2001), and an x-ray of macroinvertebrate fauna of flood plain (fadama) of the Anambra River (Eyo and Ekwonye, 1995).

The primary aim of this paper is to provide quantitative information on aquatic macroinvertebrate fauna in the Anambra river to supplement the only existing information (Eyo and Ekwonye, 1995).

MATERIALS AND METHODS

Study Area: The study area was the Anambra River (Figure 1) covering about 14014 km^2 (Awachie, 1976). The Anambra river is about 207.4 km length, it rises from the Ankpa hills (caa. 305 - 610 m above sea level), flows in southerly direction through a narrow trough that gradually broaden as it courses down. It crosses the Kogi/Anambra State boundary a bit north of Ogurugu, then meanders through Ogurugu to Otuocha and Nsugbe. From there it flows down to its confluence with the Niger at Onitsha. The basin lies between latitude $6^0 10^1$ and $7^0 20^1$, longitude $6^0 35^1$ and $7^0 40^1$, east of the River Niger into which the Anambra river empties. There are two

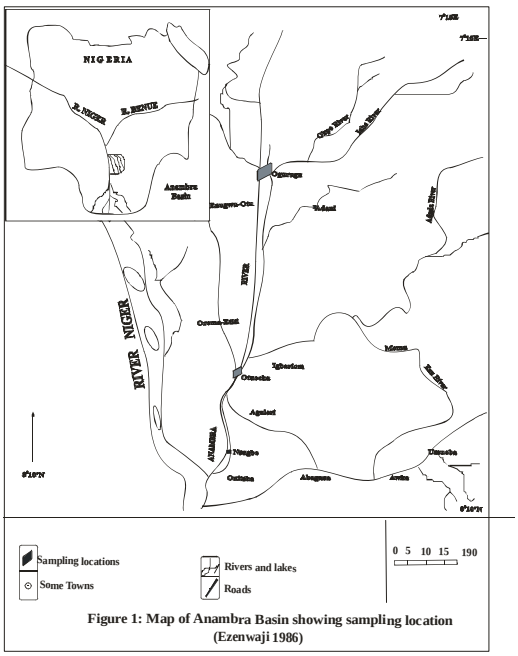

Figure 1: Map of Anambra Basin showing sampling location (Ezenwaji 1986)

main seasons the dry season October – March and the rainy season (April – September) approximately corresponding to the dry and flood phase, respectively of the hydrological regime. The vegetation is derived guinea savannah. The riparian vegetation, ecology and productivity of the river basin have been extensively studies (Awachie and Ezenwaji, 1981). The area it drains is one of the agriculturally rich area of this country. The macroinvertebrates were sampled from Anambra river at Ogurugu, Otuocha and Nsugbe. Samples were collected using scoop net and by "kick sampling techniques" (Ogbeibu and Oribhabor, 2001).

Macroinvertebrate Sampling: The kick sampling technique was used in collecting macroinvertebrates from the bank root biotope of each station. A hand scoop net (154 um mesh size) was used in sampling $0.3m^2$ of the substratum at four different points to form one composite sample per station.

The sampling period was from January 1998 – October 1999. Collected samples were preserved in formalin. Sampled specimens were identified to the generic level. Faunal abundance and biomass were computed thus; numbers per $0.3m^2$ x total weight (mg) (Ogbeibu and Oribhabor, 2001).

Identification of the macroinvertebrate fauna to generic level was done by using the keys of Miles (1970), Need ham and Needham (1962) and Mellamby (1963).

Margalef's index of taxa richness was used in computing taxa richness (Margalef, 1974) while the Shannon-Wiener (H) and Evenness (E) indices (Shannon and Wiener, 1963) were used to evaluate species diversity.

RESULTS

Composition, abundance and biomass of macroinvertebrate in Anambra river: A total of 21 genera belonging to 13 families of macroinvertebrates were identified (Table 1). The fauna was composed of *Hydrophilus sp.*, *Agabus sp.*, *Gyrinus sp.*, *Macrobranchium sp., Nepa sp., Hirudo sp.* and *Velia sp.* all of which constituted 87.02% abundance and 88.19% biomass. *Gyrinus sp.* were the most abundant group, accounting for 29.77% abundance and 6.35% biomass. *Caridina sp.* had 13.93% abundance and 17.79% biomass.

Agabus sp. 8.47% abundance and 3.67% biomass, occupied an intermediate rank. *Hydrophilus sp.*, 7.05% abundance and of 3.50% dominated the *Hydrophlidae, Ranatra sp.*, 13.93% abundance and 18.2% biomass, *Velia sp.,* 5.19% abundance and 14.75% biomass dominated the nepidae and velidae respectively.

Mesovelia sp., 0.16% abundance and 0.34% biomass dominated the *Mesovelidae sp.*, *Rhabdolaimus sp.*, 0.60% abundance and 2.13% biomass dominated the nematodes and was among the least abundant group in the river system.

Taxa richness and diversity indices of macroinvertebrate in the Anambra river: The macro invertebrate richness as computed by Margalef's index showed that *Hydrophilus sp.*, had 31.9% taxa richness at Otuocha and ranked highest followed by 3.3% in Ogurugu and 0.4% in Nsugbe (Table 2). *Gyrinus sp.*, accounted for 14.8% taxa richness at Otuocha, 10.1% at Ogurugu and 5.0% at Nsugbe.

The *Bulinus sp.*, *Libyodrilus sp.*, and *Rhabdolaimus* sp., were least available in the three sampled stations. *Hydrophilus* sp., accounted for 35.6% taxa richness followed by *Gyrinus sp.* 29.8% taxa richness and Caridina 13.9% taxa richness. *Rhabdolaim* sp. had 0.1% taxa richness, *Bulinus sp.* 0.1% taxa richness and *Baetis sp.* 0.2% were among the least occurring macroinvertebrates.

The macroinvertebrate diversity deduced from Shannon-Weaner's diversity index was highest at Otuocha (9.93), closely followed by Nsugbe (8.01) and lowest in Ogurugu (6.29). Diversity Equitability (Evenness) Index (E) of 0.32 was recorded at Otuocha, 0.03 at Nsugbe and 0.01 at Ogurugu. The value for Otuocha was significantly different from Nsugbe and Ogurugu ($P < 0.05$).

The macroinvertebrate community similarly index indicated a pronounced variation in the similarity index among macroinvertebrates of Ogurugu, Otuocha and Nsugbe stations (Table 2). The Mc Naughton community dominance index was more pronounced at Nsugbe than at Otuocha and Ogurugu respectively. Seasonal variations in the relative abundance of the 21 genera of macroinvertebrates are shown in Table 3. *Gyrinus sp., Hydrophilus sp., Agabus sp.* and *Ranatra sp.* were more abundant in the dry season months of December and January than in the months of wet season (Table 3). *Rhabdolamus sp., Donacia sp.* and *Libyodrilus sp.,* showed a temporal trend that was the reserve of that *Gyrinus sp.* and *Agabus sp.* They were generally more prominent in the rainy season than in the dry seasons. The mean abundance and biomass of major macro invertebrate among the three stations of the river system are shown in Table 4. The *Gyrinus sp.* ranked highest in Otuocha and was closely followed by *Macrobranchium sp.* and *Ranatra sp.* In Nsugbe *Gyrinus sp.* was most abundant and was followed by *Ranatra* and *Macrobranchium sp.* respectively. In Ogurugu the least abundant species were *Velia* and *Nepa species*.

In all, *Gyrinus sp.* ranked highest followed by *Macrobranchium sp* and *Ranatra sp*. The *Nepa sp* and *Velia sp*. were the least occurring of all the major macro invertebrates collected during the study (Table 4).

DISCUSSION

Water quality and food availability are important factors governing abundance and distribution of macro invertebrate fauna in aquatic environment (Bishop, 1993, Dance and Hynes, 1970).

Table 1: The composition, percentage number, Abundance and Biomass of total macroinvertebrate fauna of Anambra river system

Family	Genera	Number (%)	Density (%)	Biomass (%)
Hydrophilidae	*Hydrophilus*	129(6.94)	430.0(7.05)	30.10(3.50)
Dytiscidae	*Hydrobius*	27(1.45)	90.0(1.47)	5.40(0.63)
	Agabus	155(8.34)	516.69(8.47)	31.53(3.67)
	Hybius	19(1.02)	63.33(1.04)	3.99(0.46)
	Dytiscus	30(1.61)	100.00(1.64)	16.00(1.86)
Gynnidae	*Gyrinus*	545(29.32)	1816.67(29.77)	54.50(6.35)
Chrysomelidae	*Donacia*	29(1.56)	69.67(1.58)	26.10(3.04)
Nepidae	*Ranatra*	247(13.29)	823.33(13.49)	156.43(18.21)
	Nepa	72(3.88)	240.0(3.93)	48.0(5.59)
Velidae	*Velia*	95(5.11)	316.67(5.19)	126.67(14.75)
	Mesovelia	31(1.67)	10.0(0.16)	2.9(0.34)
	Lymphula	11(0.59)	36.67(0.60)	5.87(0.68)
Chronomidae	*Chronomus*	3(0.16)	10.0(0.16)	0.3(0.03)
Baetidae	*Baetis*	5(0.27)	16.67(0.27)	6.50(0.76)
Palaemonidea	*Macrobranchium*	110(5.92)	366.67(6.01)	8.07(0.94)
	Caridina	255(13.72)	850.00(13.93)	170.0(19.79)
Lymnalidae	*Lymnaea*	8(0.43)	26.67(0.44)	8.00(0.93)
	Bulinus	3(0.16)	10.0(0.16)	17.0(0.20)
Libyodridae	*Libyodrilus*	9(0.48)	30.0(0.49)	6.3(0.73)
Hirudidae	*Hirudo*	65(3.5)	216.67(3.55)	132.17(15.39)
Rhabdolaimidae	*Rhabdolaimus*	11(0.59)	36.69(0.60)	18.33(2.13)
Total		**1859(100.00)**	**6103.36(100)**	**858.86(100.00)**

Table 2: The percentage number, Taxa richness, diversity indices and faunal similarities of total macro invertebrates at Ogurugu, Otuocha and Nsugbe stations of Anambra river system January 1995 – October 1999

Family	Taxa	Ogurugu		Otuocha		Nsugbe		Total		
		N (%)	Taxa richness (%)	N (%)	Taxa richness (%)	N (%)	Taxa richness (%)	N	%	%TR
Hydrophilidae	*Hydrophilus*	61(3.28)	18.4(3.3)	59(3.17)	17.7(31.9)	9(0.48)	2.4(0.4)	129	6.94	35.6
	Hydrobius	6(0.32)	1.5(0.3)	21(1.13)	6.1(1.1)	0	0	27	1.45	1.4
Dytiscidae	*Agabus*	63(3.39)	19.0(3.4)	81(4.36)	24.5(4.4)	11(0.59)	3.1(0.6)	155	8.34	8.5
	Hybius	10(0.54)	2.8(0.5)	9(0.48)	2.4(0.4)	0	0	19	1.02	0.9
	Dytiscus	16(0.86)	4.6(0.8)	11(0.59)	3.1(0.6)	3(0.16)	0.6(0.1)	30	1.61	1.3
Gynnidae	*Gyrinus*	184(9.90)	56.0(10.1)	270(14.52)	82.3(14.8)	91(4.90)	27.5(5.0)	545	29.32	29.9
Chrysomelidae	*Donacia*	19(1.02)	5.5(1.0)	10(0.54)	2.8(0.5)	0	0	29	1.56	1.5
Nepidae	*Ranatra*	60(3.23)	18.0(3.2)	141(7.58)	42.8(7.7)	46(2.47)	13.8(2.5)	247	13.29	13.4
	Nepa	30(1.61)	8.9(1.6)	30(1.61)	8.9(1.6)	12(0.65)	3.4(0.6)	72	3.87	3.8
Velidae	*Velia*	5(0.27)	1.2(0.2)	56(3.01)	16.8(3.0)	34(1.83)	10.1(1.8)	95	5.11	6.4
Mesovelidae	*Mesovelia*	0	0	31(1.67)	9.2(1.7)	0	0	31	1.67	1.7
	Lymphula	5(0.27)	1.2(0.2)	6(0.32)	1.5(0.3)	0	0	11	0.59	0.5
Chronomidae	*Chronomus*	4(0.22)	0.9(0.2)	7(0.38)	1.8(0.3)	0	0	11	0.59	0.5
Baetidae	*Baetis*		0	5(0.27)	1.2(0.2)	0	0	5	0.29	0.2
Palaemonidae	*Macrobrauchium*	110(5.92)	33.3(6.0)	0	0	0	0	110	5.92	6

	Caridina	0	0	215(11.57)	65.5(11.8)	40(2.15)	11.9(2.1)	255	13.72	13.9
Lymnalidae	*Lymnaea*	0	0	5(0.27)	1.2(0.2)	3(0.16)	0.6(0.1)	8	0.43	0.3
	Bulinus	0	0	3(0.16)	0.6(0.1)	0	0	3	0.16	0.1
Lumbriculidae	*Lumbriculus*	0	0	3(0.16)	0.6(0.1)	6(0.32)	1.5(0.3)	9	0.48	0.4
Hirudidae	*Hirudo*	52(2.80)	15.6(2.8)	11(0.59)	3.1(0.6)	2(0.11)	0.3(0.1)	65	3.5	3.5
Rhabdolaimidae	*Rhabdolaimus*	0	0	2(0.11)	0.3(0.1)	1(0.05)	0	3	0.16	0.1

Table 3: Seasonal mean abundance and biomass of the major macroinvertebrates of Anambra river system

Taxonomic group	Dry season		Wet season	
	Abundance (Nm^{-2})	Biomass (Mgm^{-2})	Abundance (Nm^{-2})	Biomass (Mgm^{-2})
Hydrophilus sp	214	15	65	4.7
Agabus sp	250	4.21	63	1.68
Gyrinus sp	891	25.31	184	4.8
Macrobranchium sp	180	41.2	60	15.3
Ranatra sp	477	90.6	75	13.1
Caridina sp	403	88.8	76	16.06
Nepa	140	28.2	41	8.4

Table 4: Mean taxonomic abundance and biomass of main macro invertebrates per station, January 1998 - October, 1999 of Anambra river system

Taxonomic Group	Ogurugu		Otuocha		Nsugbe		Mean Value	
	Abundance (Nm^{-2})	Biomass (Mgm^{-2})	Abundance (Nm^{-2})	Biomass (Mgm^{-2})	Abundance (Nm^{-2})	Biomass (Mgm^{-2})	Abundance (Nm^{-2})	Biomass (Mgm^{-2})
Hydrophilus sp	203.33	14.23	196.67	13.77	30	2.1	143.33	10.03
Agabus sp	196.67	13.77	270	16.47	36.67	2.24	167.78	10.83
Gyrinus sp	613.33	18.4	900	27	303.33	9.1	605.33	18.17
Ranatra sp	200	38	470	42.3	153.33	29.13	274.44	22.38
Macrobranchium sp	366.67	154	716.67	301	133.33	56	405.56	170.33
Velia sp	16.67	5.57	186.67	74.67	40	8	81.11	29.78
Hirudo sp	173.33	105.73	36.67	2.01	113.33	45.33	107.78	65.11
Nepa sp	100	20	100	20	6.67	4.07	68.89	14.69

In Anambra river system there was pronounced diversity of species among the macroinvertebrates sampled.

Coleopteran, for instance, exhibited a reasonable diversity of aquatic fauna. They were found in all the bank root biotopes where collections were made. Majority of the Coleopteran were collected during the dry season period (October – April) when the river level was drastically reduced and the insects concentrated in small ponds that were formed along the river course during residing flood. The high concentration accounted for more specimens caught, per unit effort during the sample periods.

The large number of aquatic beetles (Coleopteran) sampled, especially from Otuocha 461 (24.79%) and Ogurugu 359 (19.11%) (Table 2) stations of the river may be attributed to their adaptation to the environment, availability of food, reproduction and time of collection (Mbah and Vajime, 1989).

Belostomid water-bugs (Hemiptera) found in Anambra river were of four types. The *Velia sp.* 95(5.11%) were common and more frequent than the *Mesovelia sp* 31(1.6%). The *Velia sp* were morphologically adapted to the aquatic environment by the possession of a short, retractile respiratory siphon. The female glues her eggs to the back of the male, which carries it until they hatch thus ensuring high percentage of hatchability and subsequent survival.

Specimens of water sticks insect, *Ranatra sp.* 247(13.29%) and *Nepa sp*. 72(3.87%) were found below the water surface clinging to aquatic vegetation with long respiratory siphon thrust upward to obtain oxygen at the water-air interface. This morphological adaptation contributed to their survival in the habit (Umeham, 1989).

The sampled aquatic fauna from the River basin suggest coleopteran and hemiptera dominance.

Baetis (Ephemeroptera) usually form a major part of the fauna of normal streams (Mba and Vajime, 1989). They were only collected in Otuocha station, the (Baetis) absence in Ogurugu and Nsugbe could be ascribed to the fact that the majority of the tree shrubs which provided ideal habitat for these fauna have decayed; thus eliminating the natural habitat.

Generally, the chironomids were periphytic. The absence of chironomid in Nsugbe station and 4(0.22%) at Ogurugu may be due to the ability of this fauna to colonize all kinds of aquatic environment especially those which are badly polluted (Burton, 1987). This is possible because the chironomid species possess haemoglobin used to extract oxygen from water in areas where the concentrations of oxygen is very low. *Hirudo* respond to organic enrichment macrophytes by increase in abundance. This clearly manifested in Ogurugu station that registered 52(8.3%) of *Hirudo.*

Their significantly higher density in Ogurugu than in Otuocha and Nsugbe stations could be attributed probably to organic enrichment leading to growth of macrophytes.

Macrobranchium is a common taxon in the Anambra river. These macro invertebrates were available in Ogurugu, Otuocha and Nsugbe stations especially during the dry season, October – April. The abundance of this fauna could be attributed to favourable organic enrichment of the river.

Fewer numbers of macro invertebrates were collected from Ogurugu and Nsugbe stations than Otuocha. This could be attributed to the lotic and relatively lack of macrophytes in Ogurugu and Nsugbe stations.

The significant interaction ($P<0.05$) between *Hydrophilus sp.*, *Agabus sp.*, *Ranatara sp* and *Gyrinus sp.* of the river and dissolved salts, oxygen concentration, water current, temperature indicated the importance of these parameters in the River system for growth and survival of the aforementioned fauna. The significant relationship signified that the variables concerned did t favour abundance and distribution of the fauna. The picture that emerges when species richness is compared in descending order is depicted below; Coleoptera, Hemiphtera, Decapoda, Oligochaeta, Diptera and Ephemeroptera.

Hynes (1970) and Macan (1974), reported that presence or absence of aquatic fauna is associated with other factors such as predators, behaviour, food, concentration of dissolved salts, hydrogen ion concentrations, oxygen concentration, water current, water level and water temperature.

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LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF THE ELEPHANT FISH, *Mormyrus rume* (Valenciennes, 1846) IN RIVER OSE, SOUTHWESTERN NIGERIA

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ABSTRACT

A total of 791 elephant fish, Mormyrus rume specimens of various sizes were sampled from River Ose, southwestern Nigeria. Length-weight relationship and condition factor of the M. rume specimens were studied. Their standard lengths ranged from 15.0 to 45.0 cm. Mean standard length for males, females and combined sex were 27.86 cm, 30.08 cm and 28.97 cm, respectively. The body weight ranged from 75.5 to 610.0 g. Mean body weight for males, females and combined sex were 167.57 g, 237.38 g and 202.48 g, respectively. Length-weight relationship for males, females and combined sex were 1.699, 2.134 and 1.990, respectively. The fish exhibited allometric growth in the river. The predictive equation was log W = -0.636 + 1.99 log L. The mean condition factor varied between seasons. The mean condition for males, females and combined sex were 0.787, 0.859 and 0.823, respectively. The condition factor decreased with increase in individual sizes.

Key words: Length-weight relationship, Condition factor, *Mormyrus rume*, River Ose, Nigeria

INTRODUCTION

Mormyrus rume Valenciennes, 1846 (Pisces: Mormyridae) are found in fresh waters of tropical Africa (Meek, 1916; Greenwood *et al*., 1966; Fawole, 2002). They occur in fast moving waters with demersal habits. Members of the family have rudimentary electric organs situated on each side of the terminal portion of the tail and they possess large brains (Holden and Reed, 1972).

Length-weight relationships of fishes are important in fisheries biology because they allow the estimation of the average weight of the fish of a given length group by establishing a mathematical relation between the two (Beyer, 1987). Like any other morphometric characters, the Length-weight relationship can be used as a character for the differentiation of taxonomic units and the relationship changes with various developmental events in life such as metamorphosis, growth and the onset of maturity (Thomas *et al*., 2003). Besides this, the length-weight relationship can also be used in setting yield equations for estimating the number of fish landed and comparing the population in space and time (Beverton and Holt, 1957). Furthermore, the empirical relationship between the length and weight of the fish, enhances the knowledge of the natural history of commercially important fish species, thus making the conservation possible.

The condition factor (K) (Le Cren, 1951) is a quantitative parameter of the well-being state of the fish and reflects recent feeding conditions. This factor varies according to influences of physiologic factors, fluctuating according to different stages of the development. Anderson and Neumann (1996) refer to length-weight data of population as basic parameters for monitoring study of fisheries, since it provides important information concerning the structure and function of the populations.

The objective of this study was to determine the length-weight relationship and condition factor among seasons, sexes and sizes of *M. rume* populations in river Ose, southwestern Nigeria.

MATERIALS AND METHODS

Study Area: River Ose is a major perennial river in the southwestern part of Nigeria. The river took its source from Apata hills and flows through the Savanna, the rainforest down to the mangrove forest and discharges into the Atlantic Ocean through a series of creeks and lagoons. The river lies between longitudes 5°20'E to 6°10'E and latitude 6°20'N to 8°00'N. It flows approximately 300 km from its source before breaking into series of creeks and lagoons (Figure 1). Traditional fishing has been known in this river and has been sustaining a thriving fishery in that part of the country (Fagbenro *et al*., 1991). The fish fauna is very rich and varied. Fishes of commercial importance found in the river are *Oreochromis niloticus, Sarotherodon galilaeus, Tilapia zillii, Parachanna obscura, Heterobranchus longifilis, Clarias gariepinus, C. anguilaris, Labeo coubie, Hepsetus odoe, Malapterurus electricus, Mormyrus rume, M. hesselquisti, M. macrophthalmus* and *Mormyrops* spp. The fishermen operate from non-powered dug-out canoes and use a variety of gears which include gill nets of various mesh sizes, long lines (with baited and unbaited hooks), traps (trigger type and non-return valve type) and cast nets.

Fish landing reaches the peak twice in November - January (during the dry season when river level is low) and in May - July (when river level rises due to frequency in rainfall). In August - October, landing is minimal because of flood.

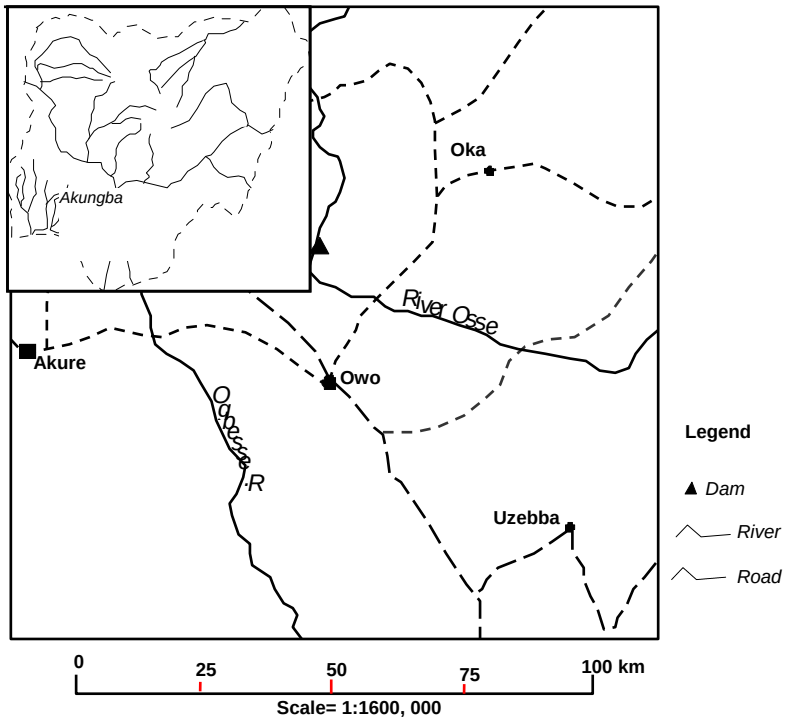

Figure 1: Map of Ose River Basin showing Nigeria Inset

Fish Sampling: *M. rume* specimens were collected once weekly, directly from fresh landings of fishermen from river Ose over 24 months. The specimens, totaling 791, were selected randomly to include various size ranges. The specimens were sorted into three size groups namely, small (<20.00 cm), medium (20.01-34.99 cm) and large (>35.00 cm). Records of total length (cm), standard length (cm) and body weight (g) measurements of individual fish were made in the field before preserving them in ice. The specimens were dissected, sexes were noted, gonads and digestive systems were removed and kept in cross-reference plastic bottles for laboratory investigations.

Data Analysis: Length-weight relationship was calculated using Le Cren (1951) equation $W = aL^b$. The data were transformed into logarithms to determine the growth pattern thus: Log W = Log a x b Log L, where W = body weight of fish (g), L = standard length of fish (cm), a = constant, b = exponent. The condition of the fish was expressed by Fulton's condition factor (K), calculated using the formula: $K = 100W/ L^3$.

RESULTS

Length-weight Relationship: The total lengths of 791 specimens of *M. rume* examined in this study ranged from 17.0 to 50.0 cm while the standard lengths ranged from 15.0 to 45.0 cm. The weights ranged from 74.50 to 610.0 g. Figure 2 illustrate the length-weight relationship of the species. Table 1 presents the length-weight regression analysis of the species. Relationship between standard length and body weight of all the specimens were estimated as: Log W = -0.636 + 1.99 Log L, r = 0.865. The b values for males, females and both sexes were shown in Table 1. The females had better b values of 2.134 indicating the possibility of better growth patterns than males (1.699) and combined sex (1.990).

Condition Factor (K): The condition factor (K) value was calculated for *M. rume* and examined in relation to sex, size and season; and ranged from 0.41 to 1.21, 0.44 to 2.21 and 0.41 to 2.21 for males, females and combined sexes, respectively. Mean K values were 0.784, 0.849 and 0.817 for males, females and combined sexes, respectively. Figure 3 illustrates the mean condition factor for the three size groups of combined sexes while Table 2 presents the mean seasonal condition factor (K) for the combined sexes.

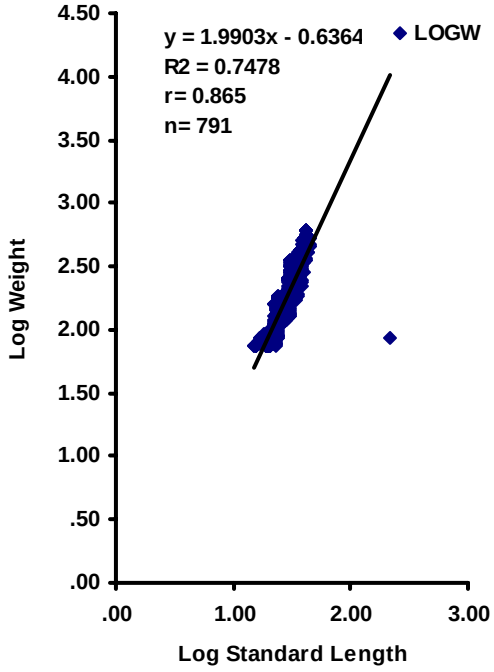

Figure 2: Length-weight relationship of *M. rume* from River Ose (October 2004 - September 2006).

Table 1: Length-weight regression analysis of *M. rume* in River Ose

Sex	No. of fish examined (n)	Log a	Log b	Correlation coefficient
Male	352	-.266	1.699	0.822
Female	439	-0.812	2.134	0.910
Combined sexes	791	-0.636	1.990	0.865

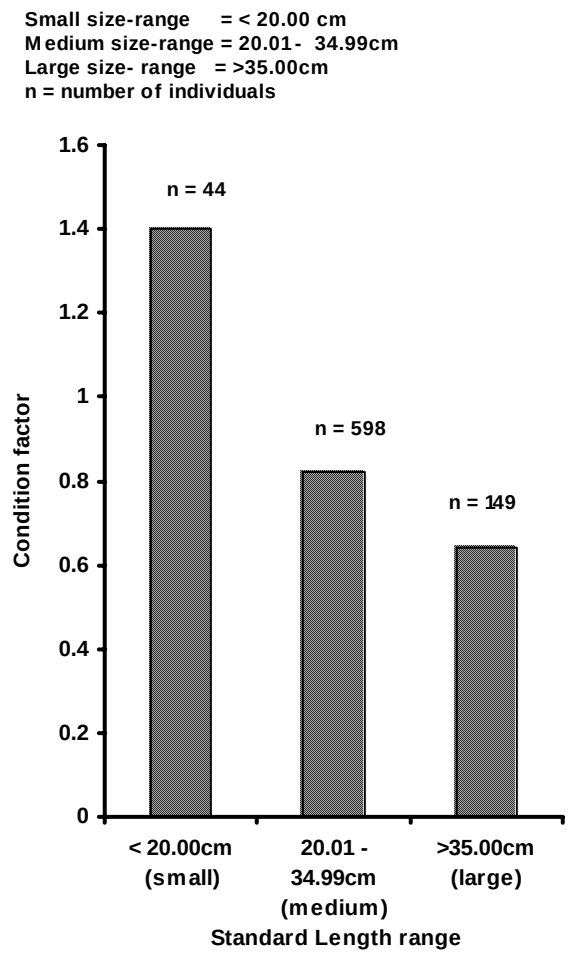

Figure 3: Means of condition factor for *M. rume* by size-range in River Ose (October 2004 - September 2006).

Table 2: Seasonal mean condition factor of *M. rume* from River Ose

Season	No. of specimens examined	Mean condition factor (K)
Dry (October 2004-March 2005)	190	0.793 ± 0.028
Wet (April-October 2005)	247	0.916 ± 0.097
Dry (November 2005-March 2006)	151	0.782 ± 0.040
Wet (April-September 2006)	203	0.908 ± 0.073

DISCUSSION

Fish weight is considered to be a function of length (Weatherley and Gill, 1987; Zafar *et al.,* 2003). For an ideal fish, which maintains dimensional equality, the isometric value of b would be 3.0. This has occasionally been observed (Allen, 1938; Thomas *et al*., 2003). A value significantly larger or smaller than 3.0 indicates allometric growth (Tesch, 1978). A value less than 3.0 shows that the fish becomes lighter (-ve allometric) or greater than 3.0 indicates that the fish become heavier (+ve allometric) for a particular length as it increases in size (Wootton, 1998; Zafar *et al*., 2003). From Figure 2, it was evident that the weight of *M. rume* increases as the length increases. The b values for males, females and combined sexes (Table 1) show a negative allometric growth with the general assumption that the specific gravity of the fish remained constant (Tesch, 1978). Le Cren (1951) and Fagbenro *et al*. (1991) stated that obedience to the cube law (isometric growth, b = 3) was rare in a majority of fishes and this was true for *M. rume* in this study in which there was a deviation from the cube law. This value was close to the values reported for some freshwater fish species (Thomas *et al*., 2003).

Correlation coefficients were very high and highly significant (Table 1) an indication that changes in standard length and weight of this Elephant fish species were proportional in one direction. Nikosky (1963) reported that the larger the condition factors the better the condition of the fish. In this study the female *M. rume* were in a better condition than the males. This agrees with the results of Oben *et al*. (1999) on *M. rume* in Lekki lagoon. There was a general decrease in condition factor with increasing length of the specimens (Figure 3). This means that increase in length did not bring about proportional increase in weight. Mgbenka and Eyo (1992) and Fawole (2002) attributed the differences in condition factor to the deposition of materials for gonad formation, which led to increase in weight and actual spawning which led to reduction in fish weight respectively.

Mean monthly indices of condition, K values (Table 2) shows that there was an improvement in fish condition between April and September (wet season). This observation agrees with the findings of Welcomme (1979) and Fagbenro *et al*. (1991) for many freshwater fishes in Africa. Lagler *et al*. (1977) attributed such differences in seasonal values of condition to availability and abundance of food supply, timing and duration of breeding cycle; physiological stress caused by changes in water quality properties within the habitat; sexual differences age; changes in seasons; and gonad maturity stages in fish. The seasonal variations in fish condition observed in this study may be attributed to the availability of natural food in the habitat consequent upon flooding during the rainy season which resulted in the inundation of previously dry ground thereby altering a number of water quality properties which ensured growth and production of natural food.

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AFZELIA AFRICANA, A NOVEL NON STARCH POLYSACCHARIDE, RAISED FASTING PLASMA CHOLESTEROL AND TRIGLYCERIDE LEVELS OF RAT

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ABSTRACT

The effects of vegetable flour prepared from indigenous plant Afzelia africana, a legume, on the fasting plasma cholesterol and triglyceride levels of rats were investigated. Chemical analysis indicated that Afzelia flour contained significant amount of non-starch polysaccharides (NSP). The flour of Afzelia was incorporated into semi-synthetic diet to provide 10g of dietary fibre which is 300g/kg Afzelia flour. This replaced some of the casein, oil and starch in the control diet .The test and control diets were fed to young Sprague-Dawley rats for 14 days ad libitum. Food intake, weight gain, crude digestibility, faecal fat excretion, fasting plasma cholesterol and triglyceride were evaluated. The result showed a statistically significant difference (p > 0.05) between the control diet and the test diet in food intake, weight gain and energy digestibility. Afzelia fed rats had a significant higher fasting plasma cholesterol and triglyceride levels than rats fed the control diet.

Keywords: *Afzelia africana*, Plasma cholesterol, Triglyceride levels, rat

INTRODUCTION

The ability of certain sources of dietary fibre to lower serum cholesterol has been demonstrated in human clinical studies. Water soluble dietary fibre sources (SNSP) tend to be most effective in lowering total cholesterol and LDL cholesterol. Jenkins *et al.* (1975) first reported the hypocholesterol effect of guar gum, a water soluble NSP. Similar reports have also shown reduction in total and low density lipoprotein (LDL) cholesterol in normal subjects (Khan *et al.,* 1981; Smith and Holm, 1982; Penagini *et al.,* 1986).

Different fibres have various effects on rat metabolism. The early studies of Wells (1961) and Judd and Truswell (1985) demonstrated variable effects of supplementing rat diets with different forms of dietary fibre. The authors showed that SNSP such as pectin, guar gum, locust bean gum and carrageen in the diet decreased cholesterol levels, while insoluble dietary fibre does not usually demonstrate hypocholesterolemic effects.

The suggested mechanism involved in the lowering of plasma cholesterol are reduction in bile acid re-absorption, alteration of the metabolism and ratio of bile acid absorbed by changing the intestinal secretion and the hepatic production of lipoproteins or modification of the peripheral disposal of lipoproteins (Chen and Anderson, 1979, 1986). Viscosity is an important factor in the lipid lowering effects of gel-forming polysaccharide such as guar gum (Kay and Truswell, 1977). Furthermore, dietary fibre tends to swell within the aqueous medium of the intestine thus increasing intestinal filling (Eastwood, 1973) which may reduce food intake in rats.

Afzelia africana (AA) is an underexploited and under utilised leguminous crop seed indigenous to the Ibos in the South Eastern part of Nigeria. The fruits of *Afzelia* are very hard and woody, nearly black and busting violently to discharge the seeds (Hutchinson and Dalziel, 1931). This legume is cheap, and is traditionally processed in homes and used on a daily basis for thickening vegetable soups. *Afzelia* is locally known as *'akparata'.* On the basis of these observations, the authors believed that such plant foods could be a useful source of water-soluble dietary fibre and was selected for the study. There is dart of information in the literature on this food and it is largely uncharacterized. The present investigation was designed to determine the effects of *Afzelia* on the plasma cholesterol and triglyceride level of rats. The flour of *Afzelia* was incorporated into semi–purified rat diet to find out if it will lower the plasma cholesterol and triglyceride concentration in rats. Chemical analysis showed that *Afzelia* is rich in non-starch polysaccharides (Onyechi, 1995)

MATERIALS AND METHODS

Preparation and Processing of Plant Food Extracts: The seed samples used in the present study were purchased at the local market in Nsukka, Enugu State and transported to the United Kingdom for processing into flour. *Afzelia* was processed using the traditionally processing method. This involves sorting the seeds to remove spoilt ones. The seeds were then roasted for 10 - 20 minutes in wide aluminium stainless steel pan. The roasted seeds were cracked with the use of wooden pestle to remove the skin. The roasted endosperm was cracked in smaller pieces and ground into fine powder in a coffee grinder (Moulinex blender/mill) and air dried at room temperature. The processing yields a fine yellowish powder with strong aromatic odour (Onyechi, 1995).

Chemical and Physical Methods of Analysis of the Plant Food: Afzelia flour was analysed using standard methods (Kirk and Sawyer, 1991) for moisture (104 ^{o}C for 16h), ash (total minerals; 525 ^{o}C for 12h), fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (micro-Kjeldahl method; N x 5.7). The starch content of the flour was determined by an enzymatic method (Englyst *et al.,* 1992a.). The Englyst method (Englyst *et al.,* 1992b) was used to determine total NSP and the water-insoluble fraction of the NSP; the water-soluble fraction of the NSP was determined as the difference. The procedure involves acid hydrolysis of the NSP followed by gas chromatography of the alditol acetate derivatives of the neutral sugars. The test food sample was boiled with 80 % ethanol for 1 hour under reflux. The residue obtained by filtration was washed with 95 % ethanol and air dried at room temperature. The dried residue was extracted with 7 volume of distilled water then followed by centrifugation. The supernatant was collected, pH adjusted and centrifuged. The SNSP in the supernatant was precipitated by addition of absolute ethanol. The precipitate was collected by filtration and stored at 4 ^{o}C. The particle size distributions of the test foods were determined by a standard laboratory mechanical sieve analysis method (Lauer, 1966); the viscosity of 1% aqueous dispersion of the test foods was obtained by the U tube capillary viscometer.

Rats: Ten litters each containing two male Sprague-Dawley rats, supplied by A. Jack and sons, London, were used for the experiment. On arrival, each rat weighed between 71 - 87g. Each litter of rats was placed in a cage and fed a stock diet (CRM Labsure, Christopher Hill, London)

Formulation of the Control Diet: The batch size of diet prepared was 5 kg. The calculated quantities of casein (New Zealand Milk Products UK Ltd), vitamin mix and mineral mix (King's College, London mix), sucrose (Booker Fitch Food Services), solka floc (Jordensen and Wettre Limited) and corn starch (Cerestar, Manchester HHIPA), were each weighed and transferred to Hobart mixer and blended for 15 minutes. Sufficient corn oil was heated in beaker to approximately 80 ^{o}C and calculated amount of cholesterol (BDH Chemicals Limited) was weighed and stirred into the corn oil and mixed well to dissolve. This mixture of cholesterol and corn was added to the dry ingredients and blending continued for another 30 min until well distributed. The mixture was passed through a 1/8 inch mesh size. Homogenization of the total mixture was ensured by mixing for a further 30 min in the Hobart mixer. The diet was stored at -20^{0}C in self- sealed freezer bags. Composition of the diets is shown in Table 1.

Formulation of the Test Diet: The test diet was prepared to contain afzelia flour and was formulated to provide approximately 10g total dietary fibre per 100g diet while maintaining similar protein and fat levels. This was determined from the result of preliminary analysis (Onyechi, 1995). The amount of casein and corn oil was adjusted to maintain similar protein and fat contents with the control diet. The test food replaced some of the protein, fat and starch in the diet as shown in Table 1. Enough corn starch was used to make up a batch size of 5 kg diet. Proximate composition of the diets is shown in Table 1.

Table 1: Quantity and proximate composition of the Control and test diet containing Afzelia flour

Ingredients	**Quantity of ingredient (g/1000g) diet**	
	CD	**AAD**
Casein	150	99
Fat (corn oil)	100	100
Vitamins	20	20
Minerals	40	40
Sucrose	100	100
Cholesterol	10	10
Solka floc	50	50
Food sample	-	300
Corn starch	530	281
Proximate Composition		
Moisture %	6.11	5.61
Fat %	10.00	10.4
Protein %	15	15
Ash %	4.8	5.0
Available CHO	58.0	42.7
Fibre difference %	6.1	21.4
Total CHO	64.1	64.0
Kcal by bombing	406.0	409.0

Note: CD = Control diet, AAD = Afzelia Africana diet

Feeding of the Rats: On arrival the rats were fed stock diet for 2 days and placed on ground stock diet for a further 5 days to acclimatise them to eating a ground diet. After one week the rats were assigned into two groups with mean weight of 115 – 116 g, so that one rat from a litter went into each experimental group. The groups were therefore assumed to be genetically similar and fed for 14 days *ad libitum.* The rats were individually housed in stainless steel cages with suspended trays containing filter paper linings for collection of spill and faeces. The rats were weighed daily for the first two days and then on alternate days. Weight was determined by difference from week to week. Food intake, faecal out put, weight gain, energy digestibility, plasma cholesterol and triglyceride were the parameters assessed in the rats.

Food intake was recorded by providing each rat with an individual weighed pot of food. These food pots and food were weighed on alternate days before topping up the food supply and reweighing. At the end of each week of the two experimental periods, the spillage was collected by sifting the faeces from the spilt food. The faeces samples were collected separately from each animal and stored in self-sealed bags at -20 ^{o}C until analysis. Dry weight (DW) of spilt was determined by drying this in an oven at 105 ^{o}C for 48 hours, together with the cage lining paper and food adhering to it. The dry weight of the paper was subtracted from the total to give dry weight of spillage. Dry weight of the remaining food

in the pot was similarly determined after drying for 48 hours at 105 ^{o}C. Food intake was calculated.

Bleeding of the Rats and Plasma Cholesterol and Triglyceride Analysis: At the end of 14 days of experimental feeding, the rats fed the diets were anaesthetized and bled from the heart using a heparinized needle and syringe to prevent the blood from clotting. The blood was collected in a centrifuge tube and centrifuged at 2,500 rpm for 15 minutes. The plasma was separated from the cells and stored in LP tubes at a temperature of -20 ^{o}C until analysis. The fasting plasma cholesterol and triglyceride levels were determined by enzymatic method (Roschkau *et al.,* 1975; Sidel *et al.*, 1981) and (Tiffany *et al.,* 1974) respectively using Boehringer-Mannheim kit method.

Statistical Analysis: The data collected were analysed using descriptive statistics for their central tendencies and analysis of variance with Fischer least significant level at 0.05 probability.

RESULTS

Chemical and Physical Analysis of *Afzelia:* Chemical analysis showed that A*fzelia* flour contained 3.3g/100g of fat; 17.7g of protein; 1.4g starch; 3.3g ash. The total NSP was 35.8g/100g; 29.3g was the value for the water soluble NSP; 6.5g was the insoluble NSP content per 100g afzelia flour. The sugar composition of the SNSP fraction showed that afzelia contained a proportion of xylose, glucose and galactose. The mean particle size of afzelia flour was 272 µm and the viscosity of a 1% aqueous dispersion was 1,000-15,000 cps (Onyechi, 1995).

Food Intake: Food intake of rats at 1 - 7 days, 8 – 14 days and cumulative are shown in Table 2. The mean dry food intake for rats fed the control diet was significantly (P<0.05) higher than the food intake of rats fed afzelia diet.

Table 2: Dry food intake (g) of rats fed control and test diets containing *Afzelia* over 1 - 7, 8 - 14 and 14 cumulative days

Diet	Days 1-7	Days 8-14	Cumulative
Control	192 ± 25.60^{a}	138 ± 25.60^{b}	332 ± 23.42^{c}
Afzelia	72 ± 8.91^{b}	74 ± -5.79^{c}	147 ± 12.23^{d}

Column values with unsimilar superscripts are significantly different from each other

Faecal Fat Excretion: There was no significant difference in the faecal fat excretion of the rats fed the two diets at 1 - 7, 8 – 14 and 14 cumulative days (Table 3).

Table 3: Fecal fat excretion (g) of rats fed control diet and test diets containing *Afzelia* for days 1 - 7, 8 - 14 and 14 cumulative days

Diet days	Days 1-7	Days 8-14	Cumulative
Control	1.75 ± 0.08	1.85 ± 0.21	3.60 ± 0.19
Afzelia	1.149 ± 0.21	1.95 ± 0.13	3.44 ± 0.38

Weight Gain: The rats fed the control diet had more significant weight gain (P < 0.05) than the rats fed the test diet through out the feeding periods (1 – 7 and 1 – 7, 8 – 14 and cumulative days) (Table 4).

Table 4: Weight gain (g) of groups of rats fed control diet and test diet containing *Afzelia* during days 1 – 7, 8 – 14 and 14 cumulative days

Diet	Days 1 – 7	Days 8 – 14	Cumulative
Control	44 ± 3.11^{a}	67 ± -25.39^{b}	111± -539^{c}
Afzelia	17 ± -1.02^{b}	21 ± -2.57^{c}	38 ± 2.98^{d}

Column values with the unsimilar superscripts are significantly different from each other p < 0.05.

Energy Digestibility: Energy digestibility was determined at 14 days of the feeding period. There was a higher significant difference (P < 0.05) in the energy digestibility of rats fed the control diet compared to the ones fed the test diet containing afzelia diet (Table 5).

Table 5: Mean energy digestibility (%) of control and test diet containing *Afzelia* fed to rats at 1 – 7 and 8 – 14 days

Diet	Days 1 – 7	Days 8 – 14
Control	0.93 ± -0.02^{a}	0.85 ± -0.004^{b}
Afzelia	0.82 ± 0.01^{b}	0.74 ± -0.02^{c}

Value in column with unsimilar superscripts are significantly different from each other at P < 0.05

The fasting plasma cholesterol and triglyceride levels: The fasting plasma cholesterol and triglyceride levels of groups of rats fed *Afzelia* diets was significantly (P < 0.05) higher than the rats fed on the control diet (Table 6).

Table 6: Mean fasting plasma cholesterol and triglyceride levels (mmol/L) of rats fed control diet and the test diet containing Afzelia four for 14 days

Diets	Plasma cholesterol level (mmol/L)	Plasma triglyceride level (mmol/L)
Control	3.97 ± -0.17^{a}	0.80 ± -0.07^{b}
Afzelia	566. ± -0.31	2.10 ± -0.28^{c}

Column values with unsimilar superscripts are significantly different from each other p<0.05.

DISCUSSION

The result of the study showed a lower mean body weight of *Afzelia* fed rats compared to the rats fed the control diet. This is in line with Fleming and Lee, 1983; Judd and Trustwell, 1985 studies that reported consistent lower weight gain of rats fed pectin. Studies have shown that fibre swells within the aqueous medium of the intestine (Eastwood, 1973). This property of dietary fibre has a tendency to increase intestinal filling. Judd (1980) showed that gut content of rats fed 10% pectin diet for 21 days were heavier than the content of the control group despite a lower food intake of the pectin fed animals. Therefore the rats in the current study may have eaten less food due to the intestinal filling caused by

the water trapped in the GIT and the slower gastric emptying giving the rats a sense of fullness.

Previous works (Riccardi *et al.,* 1967; Chen and Anderson, 1979; Judd, 1980; Judd and Truswell, 1985) have shown cholesterol lowering effect of diets containing SNSP. The different effects of dietary fibre on blood lipids have been reported in previous studies. The addition of cellulose (insoluble NSP) to a diet containing 1% cholesterol increased serum cholesterol levels (Tsai *et al.,* 1976). However viscous soluble NSP have consistently been shown to lower plasma cholesterol levels in rats fed hypercholesterolaemic diets (Judd *et al.,* 1977; Chen and Anderson 1979; Asp 1981). The suggested mechanism by which NSP affect plasma lipid level include, reduction in total energy intake, bile acid excretion/ sequestering, changes in fat absorption, changes in endocrine secretion, resistance to diffusion and effects of short-chain fatty acids (Story and Kritchevsky, 1976). The effectiveness of dietary fibre in reducing cholesterol levels probably lies in its ability to reduce availability of fatty acids and cholesterol for absorption in the upper intestine. This maybe seen as increased faecal fat; increased cholesterol and its bacterial metabolites, and reduced bile acid re-absorption, which affects cholesterol esterification (Dreher, 1987).

The increased level of plasma cholesterol and triglyceride levels seen in rats fed *Afzelia* was contrary to expectation. The result of the chemical composition of the plant seeds showed that Afzelia flour is high in SNSP which has been shown to lower plasma lipid levels in hypercholesterolemic rats. The authors postulated that the negative effect of *Afzelia* to the plasma lipid levels of the rats could be attributed to possible toxic substances that may be contained in *Afzelia* seeds. Hutchinson and Dalziel (1931) noted that the processing method of *Afzelia* seeds include roasting and soaking for several days to rid the seeds of the poisonous substances they may contain. However, the traditional processing method which was used was different, as the seeds of *Afzelia* were not soaked after roasting. This could account for the negative effects of *Afzelia* on the rats like poor weight gain, loss of hair and poor condition at the end of the experimental period.

Furthermore, this confounding result of increased plasma lipid levels maybe due to the fact that in these rats, fat absorption may have been continuing even during the fasting period as food remained in the guts of these animals. It has been suggested that the presence of fibre in the gut generates an increased unstirred water layer in the intestine (Johnson and Gee, 1981). Consequently there is delay in fat absorption in the GIT.

In conclusion *Afzelia* which is consumed in most households as thickening agents in the South Eastern Nigeria among the Ibos may contain undesirable substances that maybe toxic. These toxic substances may require that the seeds to be roasted and soaked for days to rid the seeds of the poison (Hutchinson and Dalziel, 1931). It is important to reconsider the traditional processing method and adopt the new method of roasting and soaking of the seeds.

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DIPTERAN FAUNA OF AN ABATTOIR AND ITS CONTIGUOUS FALLOW PLOT IN A GUINEA SAVANNA ECOSYSTEM

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ABSTRACT

The pitfall trap was used in the study of the dipteran populations of an abattoir and a contiguous fallow plot, in relation to their relative abundance and distribution. A total number of 140 adult species of Synydas and Stomorhina cribrata, and 400 dipteran larvae were captured at the abattoir using pitfall techniques, with correspondingly fewer species of similar dipterans trapped at the contiguous fallow plot. Significant difference existed in the trapping of the Diptera larvae with more trapped at the abattoir than the fallow plot using Student t-test. There was also a preponderance of calliphorid species at the abattoir when the sweep net was used, with these species implicated as being potential pests of medical and forensic importance. The presence of Sarcophaga sp. and Fannia canicularis in the sweep net collection at the abattoir was also traced to the presence of decaying fall-offs from carcass. Other possible implications of the collected dipteran species at the abattoir and its vicinity were also discussed.

Keywords: Dipteran fauna, Abattoir, Contiguous fallow plots, Guinea savanna

INTRODUCTION

Animals serve as essential sources of protein. This has led to the establishment of animal forms and ranges for mass production of animal protein. The slaughter of animals for human consumption leads to the production of a large quantity of abattoir waste, with many slaughtering being carried out on rural slaughter slabs and at unauthorized places. In Nigeria there are only a few standard abattoirs (Abubakar, 1998). Environmentally, the establishment of these abattoirs has led to the development of peculiar insect population in response to such human activities. The importance of the insect fauna and the ever-increasing need to have in-depth knowledge of them have given rise to the evolution of sampling methods and even the modification of the existing ones in order to study them in their natural and artificial habitats.

The pitfall trap and the sweep net are devices for studying insect population. Both devices are capable of providing impressive collections of data from situations where few animals will be recorded by absolute estimated (Ewuim and Nwoye, 2002). The pitfall trap has been found useful as collecting devices for studying daily rhythms of activity, seasonal incidence and the dispersion of single species in one type of vegetable. In general, pitfall traps are useful in studying spatial distribution of population and the seasonal occurrence of species (Sing and Lal, 1988).

The sweep net is perhaps the most widely used device for sampling insects from vegetation; simple and capable of collecting sparsely dispersed species (Southwood, 1978). It is however important to define a standard sweeping before commencing a sampling programme because the method and pattern of sweeping can affect capture rates (Kogan and Pitre, 1980; Gauld and Bolton, 1988).

Some instances on the use of pitfall techniques in Nigeria in studying epigaeic fauna include those of Badejo and Lasebikan (1996) on the effect of habitat disturbance on collembolan fauna; Ewuim (1996; 1997), Ewuim and Ezenwugo (1997), Ewuim *et al*. (1997) on ant fauna in some Nigeria ecosystem. Ewuim (2002, 2004) dwelt on the efficacy of killing-preserving agent in pitfall traps. Ewuim *et al*. (2004) also compared the epigaeic insect populations of a forest and a fallow farmland in the same locality in Nigeria. Instances in the use of sweep nets by Nigerian workers have been reported (Ewuim and Nwoye, (2002).

This paper focuses on the use of two devices, the pitfall trap and the sweep net, in sampling the dipterans populations of an abattoir and its contiguous plot. Data obtained were compared using student's t-test to ascertain whether significant differences occurred in the pitfall and sweep net catches of the dipterans from both habitats.

MATERIALS AND METHODS

Study Area: The study site was the Awka abattoir. Awka is located latitude 5^0 and 6^0 25′N and longitude 7^0E and 8^0 E. The town stretches for 8 km in an East-West direction along the Enugu-Onitsha Expressway and about 5 km in a North-South orientation. The dimension of Awka is 1,207,800m^2 or 12,007 hectares.

Ecologically, Awka lies in the Guinea Savanna experiencing between 1,000 mm and 1,500 mm of rain annually (Iloeje, 1981). It also experiences two seasons – the dry and the wet season with a bout of harmattan from December to January. A North-South and East-West escarpment gives Awka its topographic character. From this escarpment, flood surges down towards Amikwo in which the abattoir is located.

Groundwater saturation here leads to the formation of intermittent streams / ponds used in meat slaughtering processes (Muoghalu and Omocho, 1997).

The abattoir is located between Amikwo and Obunagu villages. The present location resulted from its relocation from Ekenwida along the Old Enugu-Onitsha Road to Eke-Awka and finally to its present location in 1980. It lies in a low stretching across the present Enugu-Onitsha Expressway into Okpuno. Some of the inherent environment problems relate to these location attributes. The dominant species of plants found at the abattoir and its adjoining habitat were *Amaranthus* sp., *Eleusine* sp. *Indica* (L.) Gaertn, *Sida acuta,* Burm f. *Solanium,* sp., *Cyperus sp., Esculentus* L*, and Amaranthus Hybridus* L*.*

Sampling Methods: The sampling instruments used were pitfall traps and the sweep nets. The pitfall traps consisted of circular plastic dishes, 9 cm in height with mouth diameter of 8.4 cm. The preservative used was 5 % formalin, poured at least two-thirds filled of each container. 5 % formalin was effective in trapping, killing and preservation of insects. Six traps were set at random at the sites on each sampling day. Each trap was buried in the soil so that the rim flushes with ground level. The traps were collected after 24 hours of sampling. Recovered traps were closed and taken to the laboratory. Dissecting microscope was used to sort out the animals into various taxonomic groups after emptying the contents of each trap into a girded Petri dish.

The sweep net used was made of thick white cotton mesh with a round mouth measuring 92 cm in perimeter. Aerial insects were collected by twenty-five random strokes. Sampling was done for one hour between 10.00 and 11.00 a.m. Sampling for ground and airborne insects were done during the months of April – July 2005 and August to October 2004.

The collected insects were placed in a jar containing cotton wool soaked in chloroform. The dipterans and their larvae were identified using "Insect of Nigeria – Check list and Bibliography" by Medler (1980). The identification of the specimens was verified in the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The voucher specimens were also kept as reference point for further studies.

RESULTS

Table 1 shows the bi-monthly pitfall catches of dipterans from both the abattoir and the contiguous plots. A total number of 607 dipterans including larvae were collected from both sites. The pitfall catches were made up of 140 adults species of *Synydas* sp. and *Stomorhina cribata,* and 400 diptera larvae. For the contiguous fallow plot, 57 adults of similar species were trapped with 10 Diptera larvae. There were no significant differences in the trapping of the dipterans except for their larvae, with more trapped from the abattoir than from the fallow plot.

The various types of flies obtained using the sweep net are also shown on Table 2. Majority of the flies collected were the calliphorids with four species viz. *Chrysomia putoria, C. albiceps, Rhinia apicalis* and *Stomorhina cribrata.* The highest collection of Diptera (28.33 %) was made for *Syndas* sp. (Empididae), followed by *Atherigona* (Muscidae) (24.70%). A genus of Diopsidae family (*Diasemopsis* sp.) recorded 7.99 % of the total collection while the least collection of 6.05 % was recorded for *Hermetia illucens* (Stratromyiidae).

There was also no statistical differences in the Student t-test carried out to determine whether significant differences exist in the fly populations obtained at both the abattoir and the contiguous plots (Table 2), since the tabulated t-value (2.365) was less than the calculated value (0.1654). From the bi-monthly sweep net catches of Diptera made between August and October, 2004, *Chryosomia* was the most abundant (with a percentage relative abundance of 52.90%), followed by *Sarcophaga* sp.

Table 1: Bi-monthly dipteral populations sampled using pitfall technique

Dipterans	April 1	May 11	May 111	June 1V	June V	July V1	Total catches	Calculated t value+
***Synydas* sp**	4	10	13	19	13	15	74	1.242
	3	8	10	8	4	10	43	
Stomorhina cribrata	12	10	10	9	13	12	66	0.498
	4	5	4	5	2	4	24	
Dipteran	7	250	78	18	18	34	400	7.105+
larvae	-	-	-	-	-	-	0	
Total	**23**	**270**	**101**	**46**	**44**	**61**	**540**	**1.578**

**Dipteran groups for abattoir occupy the first row while those for contiguous plot occupy the second row. +Significant at 5% probability level: $t_{0.05}$ =2.228*

Table 2: Types of flies at the Awka abattoir sampled using both the pitfall trap and the sweep net

Family	Species	No of Flies Collected	% of Flies Collected
Calliphoridae	*Chrysomia putoria*	21	5.07%
	Chrysoma albiceps	17	4.11%
	Rhinia apicalis	9	2.17%
	Synydas sp.	90	21.7%
Muscidae	*Atherigona* sp.	102	24.6%
Empididae	*Stomorhina cribrata*	117	28.3%
Diopsidae	*Diasemopsis* sp	33	7.97%
Stratromyiidae	*Hermetia illucens*	25	6.04%
Total		**414**	**100**

(Sarcophagidae), which had a percentage relative abundance of 21.02 %. *Fannia canicularis* (Muscidae sp.) has a percentage relative abundance of 18.84 %, with Culicidae family recording 7.24 % relative abundance.

Table 3: Total catches of insect groups obtained at the Awka abattoir in 2004 using sweep net technique

Dipterans	Bi-Monthly Sweep Net Catches [2004]						Total	Relative Abundance
	Aug		Sept		Oct			
	1	2	3	4	5	6		
Culicidae								
Caliphoridae	0	3	1	0	2	4	10	7.24
***Chysomya* sp**	18	8	9	13	19	6	73	52.90
Muscidae								
Fannia canicularis	6	2	7	3	5	3	26	18.84
Sarcohagidae								
***Sarcophaga* sp.**	3	6	5	8	4	3	29	21.02
Total	**27**	**19**	**22**	**24**	**30**	**19**	**138**	**100**

DISCUSSION

The various flies obtained from the investigation belong to five families Calliphoridae Muscidae Empididae, Diopsidae and Stratromyiidae. The increased incidence in the captures of Diptera larvae in the installed pitfall traps especially at the abattoir is not only as a result of the crawling and wandering activities of the dipteran forms but also as a result of their feeding activity on patches of rumen of slaughtered animals and other slaughter house by-products. The slaughterhouse by-products, whose availability is assured by regular slaughtering of animals (Abubakar, 1988) at the abattoir are often not properly disposed and are usually at various stages of decay at the sites. The significant difference existing in the trapping of the Diptera larvae, with majority of the larvae trapped from the abattoir is no doubt not only related to their increased relative abundance at the abattoir, but also to their locomotory activity (Table 1). This observation is in line with Chapman (2000) who noted that the relative low speed of locomotion is associated with crawling movement in Diptera larvae, which is often effected by changes in body shape rather than legs. This therefore decreased their chances of availability in the contiguous fallow plot likely to favour only population. The presence of *Synidas* sp. and *Stomorhina cribrata* in the pitfall traps are likely related to the properties of the killing – preserving agent which are known to be attractive (Luft 1968; Skuhravy, 1970; Ewuim, 2002).

The significant difference, which however did not exist when the dipteran populations of the abattoir was compared with the contiguous plot (Table 2) no doubt is indicative of the nearness of the two habitats allowing them to support similar dipteran fauna, though in variable numbers.

In addition, the existences of intermittent streams near the abattoir, and the constant availability of decaying fall-offs from carcass at the abattoir may have respectively favoured the existence of *Synidas* sp. (empedids) and *Stomorrihina cribrata* (callophorids) at the abattoir. The preponderance of calliphorid species at the abattoir when the sweep net was used may not be unrelated to the fact that at most times their species are garbage and carcass feeders, making them potential pests of medical and forensic importance (Ekanem and Usua, 2000). Boorman, (1981) also noted that despite the unpleasant habits of the calliphorids, they perform a very valuable service since their maggot dispose off the corpses of animals. There is no doubt therefore that the ever-present fall-offs from the carcass at the abattoir markedly contributed to the presence and preponderance of the calliphorids. The presence of the sarcophagid (*Sarcophaga* sp.) and the muscid. (*Fannia canicularis)* is also attributed to the presence of these decaying fall-offs from these carcass including decaying spilled blood.

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THE USE OF BANANA FLAVOUR ESSENCE, FORMALIN AND ORDINARY WATER IN PITFALL TRAPS IN THE STUDY OF THE DIEL ACTIVITIES OF INSECTS FROM A FALLOW PLOT IN AWKA, NIGERIA

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ABSTRACT

A study was carried out to access the insect fauna of a fallow plot in Awka, Nigeria, in relation to their diel activities and to report any differences in the pitfall catches as a result of differences in the fluid used. The fluid used in the three sets of six traps installed bimonthly at the sites for 12 hours in each case were 5% formalin, water with 0.01% banana flavour essence and ordinary water. Using Student t-test, statistical differences existed in the diurnal and nocturnal activities of the Sminthurididae, Poduromorpha, Diptera, Acantholepsis, Paratrechina sp. and Camponotus, at probability level P<0.05, with more nocturnal catches obtained in all cases for water with banana flavour essence. Similarly statistical differences also existed in the trapping of Poduromorpha, Entomobryomorpha, Acantholepsis and Camponotus sp., for pitfall traps containing water. For pitfall traps with 5% formalin statistical differences, existed in the trapping Sminthurididae Poduromorpha, Diptera, Acheta lefevrei, Acantholepsis sp., Hymenoptera (other than formicids), and orthopteran larvae, with more nocturnal catches recorded for the pitfall traps with banana flavour essence, possibly indicating the attractive properties of this particular flavour essence. The Analysis of Variance (ANOVA) test also showed that statistical differences existed in the diurnal and nocturnal catches of insects obtained using the three killing agents. The Fisher's Probability Least Significance Difference (F-LSD) also established statistical differences in the catches made using banana flavour essence and water and also with ordinary water and formalin, with the nocturnal catches being higher than the diurnal catches. The F-LSD also confirmed that the total nocturnal catches were significantly higher than the total diurnal catches obtained using all the three killing agents. An approximate ratio of 1:2 was also obtained in the catches in relation to diurnal and nocturnal activities respectively.

Keywords: Pitfall traps, Diel activities, Insects, Fallow plot, Killing agents, Awka

INTRODUCTION

Pitfall traps have been used in diverse situations in the study of epigaeic forms especially the arthropods. The pitfall traps have been used with or without preservatives (Ewuim, 1996) The use of preserving fluids in pitfall traps e.g. 5% formalin, 70% alcohol or picric acid solution depends on the goal of the investigator (Ewuim, 2004) Studies on surface – dwellers such as Collembola, ants and beetles using pitfall traps containing preservatives have also been reported (Ewuim, 1996; 1998; Ewuim and Ezenwugo, 1997; Ewuim, *et al.,* 1997; Badejo, *et al.,* 1997

Preservatives used in pitfall traps may be attractive or repellent on arthropods. Ewuim (2004), for example, have implicated 3-5% ethylene glycol and formaldehyde solutions as being mainly attractive while picric acid solution is neutral, but water and alcohol may be repellant. It also appears that methylated spirit seems to be neutral for ants (Ewuim and Nwuba, 2002).

Pitfall traps have also been used for investigation on seasonal incidence of adults insects, their spatial pattern of distribution, the relative member of species in different types of vegetation, and even the daily activity rhythms (Ewuim, 1998). It has been observed that the level of activity of insects is controlled by its diurnal cycle (Lewis and Ewuim, 1998), and by the prevailing climatic conditions (Southwood, 1996). Hitherto, even though some studies have been carried out in both tropical and temperate countries, there is however the need to investigate further the insect fauna in this part of the world and in relation to their diel activities.

In this paper, the diel activities of epigaeic insects in fallow plots will be studied using pitfall traps having either water with .01% banana flavour essence, 5% formalin or ordinary water. Asymmetrical relationship between nocturnal and diurnal activities in these insects will be statistically established.

MATERIALS AND METHODS

Sampling Site: The investigation was carried out in a fallow plot behind the Amaku General Hospital, Awka. Awka is the capital of Anambra State of Nigeria and situated in the lowland rain forest zone of Southern Nigeria (Charter, 1970). The town is located between latitude 5^0 and 6^0 25′N and longitude 7^0E and 8^0E and stretches for 8 km in an East-West direction along the Enugu-Onitsha expressway to

about 5 km in a North-South orientation. In the lowland rainforest zone in Nigeria, the wet season starts in late. February or March and ends in October or early November with a bout or short dry spell in August (Badejo 1995), an observation which is applicable to Awka (Ewuim, 2004). The daily average ranges of rainfall and humidity in Awka falls between 0.00 – 13.60 mm or higher and 44.50 – 96.00% which may record slightly lower or higher values (Ewuim, 2004). In Awka the daily average for mean aerial temperatures falls between 27.25 – 38.00^{0}C while that of mean soil temperature falls between 26.00 – 38.00^{0}C respectively (Ewuim, 2004) with possible higher or lower values of these physical variables depending on habitat and year. The Awka is about 12, 007 hectares in dimension. The sampling site was located about ½ km away from Enugu – Onitsha expressway and covers about 3 hectares of land. The foristic features of the fallow plot include few tree species like *Gmelina* sp. and *Elaeis guineensis* (Jacq) while the herbaceous vegetation includes *Centrosema pubescens, Chromolaena odorata* (L.) *Sida actuta* (Burm. f) and *Panicum maximum* (Jacq).

Sampling Method: In the fallow plot sampling was carried out between the months of Augusts and November 2002 using pitfall technique. The traps consisted of plastic containers 6 cm deep and 4.50 cm in diameter with each of the three sets of traps positioned 2 cm apart. Each of these three sets was also positioned in 6 different locations in a triangular manner in the plot at a distance of 4 cm in each case.

Each trap was sunken into the soil until each rim flushes with, with the ground level. Each set of traps were set at 6 am in the morning and collected at 6 pm in the evening with these set of traps replaced in the same position immediately at 6 pm the same day only to be collected at 6 am the following day. Precaution was taken not to disturb the soil markedly to avoid "digging –in-effects". Each killing agent was poured up to two-thirds the size of each container.

At the laboratory, the sorting of the contents of each container into various taxonomic groups were carried out with the aid of calibrated Petri dish, fine brush and stereomicroscope. A chemical analysis of three fluids used as killing agent was carried at the Project and Development Agency (PRODA), Enugu, Nigeria in order to determine their percentage components. The insects and their larvae were identified using Insect of Nigeria – Check List and Bibliography by Medler (1980). The identification of specimens was verified in the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The voucher specimens were also kept as reference point for further studies. Analysis of Variance (ANOVA) and Fisher's Probability Least Square Difference (F-LSD) tests were used for testing whether or not statistical differences existed between the day and night captures for all the treatment groups.

RESULTS

The percentage composition of the three fluids used for insect trapping is presented on Table 1, based on the chemical analyses carried out. Banana flavour essence had 48.95% water, 33.10% of essence (ester) and 17.95% propylene glycol while the 5% formalin was made up of 87.50% water and 12.50% formalin solution.

Table 1: Percentage composition of the three fluids in the traps

Selected killing agents	Contents	Percentage
Banana flavour Essence	Water	48.95
	Essence (ester	33.10
	Propylene glycol	17.95
Water	Water	100.00
5% formalin	Water	87.50
	Formalin	12.50

Table 2 shows the diurnal and nocturnal catches of the insect fauna obtained during the sampling period using pitfall catches containing 0.01% banana flavour essence in water. From the result of the Student t-test, there were statistical differences in the total diurnal catches and the total nocturnal catches obtained using 0.01 % banana flavour essence, ordinary water and 5 % formalin at t-values of 3.2133, 2.0841 and 3.000394 respectively at 5 % probability level.

Table 2: Diurnal and Nocturnal catches of insects obtained during sampling period using banana flavor essence, formalin and water

Insecta	Killing Agent used					
	Banana		Water		Formalin	
	D	N	D	N	D	N
Sminthurididae	3	24	5	12	12	41
Poduromorpha	64	154	20	92	43	112
Entomobryomorpha	10	6	-	4	8	9
Diptera	22	85	2	6	25	72
Coleoptera					1	
Staphylinidae	-	-	-	-		5
Coleoptera (others)	6	8	-	-	7	4
Dermaptera	2	3	-	1	6	5
Orthoptera						
Acheta lefevrel	19	32	8	9	22	59
Orthoptera (others)	3	3	2	3	3	3
Homoptera	4	5	-	-	10	12
Hemiptera	13	46	7	8	36	55
Aphididae	17	14	2	6	11	14
Hymenoptera	26	23	2	2	4	20
Formicidae						
***Acantholepsis* sp.**	72	217	10	44	62	162
***Pheidole* sp.**	6	6	-	2	20	15
***Paratrechina* sp.**	33	103	1	5	18	27
***Camponotus* sp.**	48	132	16	40	46	102
Insect Larvae						
Acheta lefevrei	-	-	1	-	3	1
Orthoptera (others)	5	12	-	2	5	15
Diptera	-	-	1	-	-	-
Coleoptera	1	7	1	2	11	15
t –value +	**3.213+**		**2.084 +**		**3.00394 +**	

+ Significant at 5% probability level; t –table = 2.021

Table 3: Comparison of diurnal and nocturnal catches of insect group with significant t - values

Insect Groups	Bimonthly Diurnal and Nocturnal catches							*Killing Agent	t value +	p-value
	I	II	III	IV	V	IV	VII			
Sminthurididae	0,3	0,3	0,3	1,4	1,4	0,4	1,3	B	-10.500+	<0.0001
Poduromorpha	13,20	11,25	9,19	12,36	6,13	4,15	9,24	B	-4.003+	0.0018
Diptera	2,10	1,10	1,6	4,10	2,18	7,18	5,13	B	-4.735+	0.0005
***Acantholepsis* sp**	18,52	26,26	3,14	7,38	5,24	6,31	7,32	B	-3.750+	0.0028
***Paratrechina* sp**	4,22	3,6	2,7	4,26	4,7	8,19	8,16	B	-3.129+	0.0087
***Camponotus* sp.**	2,19	16,16	7,16	3,14	12,19	3,19	5,19	B	-4.419+	0.00087
Poduromorpha	2,17	4,12	4,12	3,18	1,6	5,14	1,13	W	-6.423+	<0.0001
Entomobryomorpha	0,0	0,0	0,1	0,1	0,1	0,1	0,0	W	-2.828+	0.0152
***Acantholepsis* sp.**	1,7	1,2	1,3	1,12	1,5	2,9	3,6	W	-3.631+	0.0034
***Camponotus* sp.**	0,9	3,3	2,4	6,7	2,4	1,6	2,7	W	-2.1679+	0.0079
Sminthurididae	0,2	0,2	2,3	4,6	1,5	3,11	2,12	F	-2490+	0.0284+
Poduromorpha	1,7	6,12	2,12	14,33	10,25	5,14	4,9	F	-2.496+	0.0281
Diptera	1,3	7,5	2,4	4,20	4,12	4,21	3,7	F	-2.277+	0.0419
Acheta lefevrei	1,3	1,6	4,5	2,9	5,16	7,12	2,8	F	-2.810+	0.0157
Hymenoptera	0,4	0,2	1,3	0,4	0,0	1,4	2,3	F	-3.639+	0.0034
***Acantholepsis* sp.**	16,19	3,22	14,20	8,27	5,29	13,26	3,19	F	-5.516+	0.0001
Orthoptera (nymph)	0,2	1,0	1,2	1,2	1,2	1,2	0,5	F	-2.449+	0.0306

**Samples in the first row represent diurnal catches while those in the second row represent the nocturnal catches; *Killing agents used: B- banana flavour essence: W- ordinary water , F- 5% formalin; +Calculated t –values significant at 5% probability level at t >2.179 or when p- value < 0.05*

Table 3 shows a comparison of the bimonthly diurnal and nocturnal catches of the insects collected during the sampling period using the Student t-test. From the result significant differences failed to exist in the diurnal land nocturnal catches of other insect groups except Sminthurididae, Poduromorpha, Diptera, *Acantholepsis* sp., *Paratrechina* sp., and *Camponotus* when 0.01% banana flavour essence was used as the killing agent, with more nocturnal activities recorded in all instances. In the use of ordinary water significant differences also existed in the nocturnal and diurnal catches of Poduromorpha, Entomobryomorpha, *Acantholepsis* sp., and *Camponotus* sp with more diurnal catches of Poduromorpha, Entomobyomorpha, *Acantholopsis* sp. and *Campoudus* sp with more diurnal activities also recorded. In the use of 5% formalin as killing agent however the insect groups which showed significant differences in their day and night captures included Sminthurididae, Poduromorpha, Diptera, *Acheta lefevrei* Hymenoptera, *Acantholepsis* sp., and nymphs of Orthoptera, with more nocturnal activities also recorded.

Table 4 shows the result of the ANOVA carried to determine whether or not statistical differences exist in the diurnal and nocturnal catches for the three fluids used. There was a significant effect of treatments (i.e. with the diurnal catches being significantly different from the nocturnal catches) for the traps containing flavour essence. There was also a significant effect of blocks with all the total diurnal catches obtained during the sampling period being significantly different from the nocturnal catches. The result of the Fischer's Least Significance Difference Test (F-LSD) is presented in Table 5.

The results show that there were significant differences between the pitfall catches of banana flavour essence and ordinary water, and between those of water and formalin. The total diurnal catches of the insects were also significantly different from those of the nocturnal catches obtained from the three fluids used.

Table 4: Analysis of variance (ANOVA) test to determine the significant difference in the diurnal and nocturnal catches obtained using the three killing agents

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F – Value	p-value
Treatments	2	10645.234	5322.616	4.895 +	0.0089
Blocks	1	8452.174	8452.174	7.773+	0.0061
Interaction	2	1472.217	736.109	0.677	0.5099
Residual	132	143534.696	1087.384		

Table 5: Fischer's Least Significance Difference (F-LSD) Test for the Treatments Investigated in Relation to Catches Made Using the Three Killing Agents

Treatment	Mean differences	Critical difference	Probability (p) value
B vs W	19.891	13.601+	0.0045
B vs F	2.848	13.601	0.6794
W vs F	-17.043	13.601+	0.0144
TD vs TN	-15.652	11.105+	0.0061

B- Pitfall catches of traps with .01% banana flavour essence; W- Pitfall catches of traps with ordinary water; F- Pitfall catches of traps with 5% formalin; TD- Total nocturnal catches of the three sets of traps; + Critical differences significant at 5% probability level

DISCUSSION

The pitfall catches made in this study, suggested rhythmic activity among insect (Davis *et al.,* 1990, Ewuim, 1998). The level of activity also showed diurnal cycle (Southwood 1996, Ewuim 1998).

There was also evidence that suggested these insects to be largely nocturnal in their activity (Ewuim, 1998), hence their increased activity was reflected in the night catches. The trapping of the various insect taxa in difference proportions was also suggestive of the differences in their relative abundance (Ewuim 1998).

The statistical difference existing in the use of pitfall traps containing water with 0.01% v v banana flavour essence in the trapping of the Sminthuridídae, Poduromorpha, Diptera, *Acantholepsis, Paratrechina,* and *Camponotus,* when the diurnal and nocturnal catches were compared, indicated more nocturnal catches, as a reflection of the increased activity of these insects at nights. It may also be a reflection of the favourable nature of the habitat. Past cultivation at this site might have favoured the activities of some of these insect taxa, as observed for *Paratrechina* in earlier studies in similar habitats (Ewuim, 1996, 1997; Ewuim and Ezenwugo 1997; Ewuim 1998).

It was also established that the members of Diptera, which ordinarily are not surface-active forms might have been attracted by the banana flavour and formalin. We may further suggest that propylene glycol has attractive properties for insects as observed in their preponderance in the banana flavour essence pitfall catches.

The attractive powers of propylene glycol invariably exceeded that of formalin. In an earlier study, Adis (1979 had implicated a related preservative, ethylene glycol, as having attractive properties. These observations were also in line with the results obtained by Weeks and McIntyre (1997) using propylene glycol solution in pitfall traps. Week and McIntyre (1997) observed that propylene caught more insect species than did live pitfall traps. It was therefore obvious that the presence of propylene glycol in the banana flavour essence incidentally influenced the catches observed in the traps.

In the comparison of the diurnal and nocturnal catches, the statistical differences observed in the trapping of Entomobryomorpha, Poduromorpha, *Acantholepsis* and *Camponotus* with higher nocturnal catches recorded in all the cases using pitfall traps containing ordinary water (Table 3) is possibly an indication of the higher activity – density of these group at night, even though water may be repellant (Aids, 1976; 1979 Ewuim and Nwuba, 2002).

In the comparison of the diurnal and nocturnal catches the statistical difference in the trapping of Sminthuridídae Poduromorpha, Diptera, Hymenoptera (other then formicids) and Orthoptera (nymphs), *Acantholepsis* and *Acheta, lefevrei* with more nocturnal catches made in all the cases is suggestive of increased nocturnal activity of these insect taxa an the efficiency of formalin as a killing agent. Formaldehyde solutions (3.5%), for example are however known to have attractive properties (Adis, 1976; Ewuim and Nwuba, 2002). In fact, 5% formalin also had more insect groups that showed significant differences in their day and captures, with nocturnal captures being higher in all the instances.

The approximate night-day capture ratios of 2:1 obtained in this study is also suggestive of higher locomotary activities of these insects at night and an asymmetrical day to night response of these insects (Ewuim, 1998), and in relation to environmental cues in their diel activity in this fallow plot. There is also no doubt that the activity-density of these insects can serve as an appropriate index for measuring their biological rhythms, which is circadian using the pitfall technique (Ewuim, 1998).

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LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF *Clarias gariepinus* AND *Tilapia zillii* IN LAKE ALAU AND MONGUNO HATCHERY, BORNO STATE, NIGERIA

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ABSTRACT

Length-Weight relationship and condition factor of Clarias gariepinus and Tilapia Zillii were studied in lake Alau and Monguno hatchery, both in Borno State of Nigeria, for a period of two weeks. A total of 98 C. gariepinus and 140. T. zillii were measured. The length-weight regression coefficient (b) for both fishes in lake Alau were not significantly different from the hypothesized value 3, but for both fishes in Monguno hatchery (b) differed significantly from the hypothesized value. Isometric growth of both fishes was recorded in lake Alau while a comparative decline in weight in relation to specific length of fishes was recorded in Monguno hatchery. Furthermore, condition of C. gareipinus in lake Alau revealed that all size groups of the fish grew better than those in Monguno hatchery, while the condition of T. zillii in Monguno hatchery was better than that in lake Alau. Although our results suggest that C. gariepinus in lake Alau grew faster than that cultured in Monguno hatchery, the study is not conclusive as abiotic, biotic, and sampling error might have interplayed. The reverse is also true for the growth potentials of T. zillii in Monguno hatchery when compared to that in lake Alau.

Keywords: *Clarias gariepinus*, *Tilapia zillii*, lake, Hatchery pond

INTRODUCTION

The global demand for animal protein has increased because of geometrical growth in human population along with decline agricultural productivity. Fisheries is an important contributor to animal protein needs. The rational and scientific management of fisheries depend on a fundamental understanding of fish biology and ecology. Among the various biological aspects of fish, the length-weight relationship and the condition factor of fish are of importance in the management of both culture and captive fisheries. The yield of fish is usually studied using weight as a measure of size. Fish grows both in length as well as in bulk, and length is easier to measure and so often used along with weight in growth studies.

Length and weight are related by a power relationship and the equation relating length to weight gives some indication of the growth pattern of fish in a population. The length-weight relationship has both applied and pure applications in the fisheries industry. Market sampling of fish of commercial importance often measures the length, as fish are usually gutted and life weight cannot be measured with certainty. An estimate of it can be obtained using predetermined length-weight regression.

Results of the studies on length-weight relationship of individual fresh-water fish are important and are applied in culturing, managing and developing individual fishery.

Against this background, an attempt was made in this study to determine and compare the length-weight relationship and condition factor of *Clarias gariepinus* and *Tilapia Zillii* from lake Alau in Konduga Local Government Area and a hatchery in Monguno Local Government both in Borno State of Nigeria. *Clarias* and *Tilapia* are probably the most abundant groups of fish in Nigeria freshwater. A major proportion of the total fish in Maiduguri fish market is constituted by these groups of fish. These groups, therefore, have been selected for this study.

Clarias is a fish genus belonging to the family clariidae and it comprises ten species in Nigeria waters, namely, *C. gariepinus, C. anguillaris, C. jaensis, C. Macromystax, C. albopunctatus, C. agboyiensis, C. buthupogon, C. ebriensis, C. pachynema, and C. camerunensis* (Olaosebikan and Raji, 1998). *C gariepinus* and *C. anguillaris* are capable of growing up to one meter or more in total length and more than 7 kilograms in weight (Holden and Reed, 1972). *C. gariepinus* is the main species of the genus found in Borno fisheries.

Tilapia is a fish genus belonging to family cichlidae and at least four species are found in Nigeria, namely*, T. zillii, T. mariae, T. dageti,* and *T. guineensis* (Olaosebikan and Raji 1998). *Tilapia* is the best known fish group in Nigeria. *I. zillii* is the most attractive and widely distributed species of the group. The species grows to an adult size of about 20 centimeters in total length (Holden and Reed, 1972). This species is common and abundant in lake Alau. Monguno hatchery pond and lake Alau are the main sources of fish supply to Borno State capital fish market.

Table 1: Values of loga, regression coefficient (b), correlation coefficient (r) with t-test, and mean condition factor of *Clarias gariepinus* and *Tilapia zillii* of groups from Lake Alau and Monguno hatchery

Study organism	Study Area	$\log_a$	b	t-test Ho: $\beta = 3$	r	t-test Ho: (P = 0)	Mean condition factor
Clarias gariepinus	Lake Alau	-2.18805	2.9999	P > 0.05	0.996	P < 0.001	0.7637
	Monnguno hatchery	-0.1718	1.4173	P < 0.001	0.9423	P < 0.001	0.7036
Tilapia zillii	Lake Alau	-17193	2.9696	P > 0.05	0.9823	P < 0.001	1.8666
	Monnguno hatchery	–0.4459	2.0243	P < 0.001	0.8621	P < 0.001	3.1216

Table 2: Values of loga, regression coefficient (b), correlation coefficient 9r) with t-tests, and mean condition factor of *Clarias gariepinus* and *Tilapia zilli* of comparable size range from Lake Alau and Monnguno hatchery

Study organism	Length range (in cm)	Study area	Loga	b	t-test Ho: $\beta = 3$	r	t-test Ho: {P = 0)
Charias gariepinus	17.0-30.2	Lake Alau	-2.222	3.0318	P > 0.05	0.99	P < 0.001
		Monnguno hatchery	-1.175	2.1528	P < 0.005	0.903	P < 0.001
Tilapia zillii	10.7-14.3	Lake Alau	-1.757	3.0042	P < 0.005	0.934	P 0.001
		Monnguno hatchery	-0.17	2.2603	P < 0.05	0.828	P < 0.001

The present work compares the length-weight relationship and condition of *Clarias gariepinus* and *Tilapia Zillii* in these two breeding habitats, natural and artificial habitats.

MATERIALS AND METHODS

Study Areas: Lake Alau is a natural water body while Monguno hatchery is an earthen cultured fish pond. Lake Alau is situated in the southeastern part of Maiduguri, 10 kilometers off Maiduguri-Konduga road the junction of which is at kilometer 15 from Maiduguri. The lake lies on approximately latitude 12°5' N and longitude 13°6' E. It receives annual delivery of water from the Ngadda and Yedzeram river systems and covers an area of about 22330 square meters (20 acres). It provides fish protein to people of Maiduguri and its suburbs.

Monguno hatchery is managed by the Directorate for Food, Roads, and Rural Infrastructure (DFRRI), Borno State. The pond is located in Monguno town, northern part of Maiduguri, about 85 kilometers from Maiduguri, Monguno town lies on latitude 12°40' N and longitude 13°30' E. The pond covers an area of 22.30 square meters (0.20 acre) fish in this pond live on natural food items.

Length-Weight Parameters: Life fish were collected and the total length and weight of individual fish were measured using a one-meter long rule and a triple-beam balance respectively. Lengths were measured in centimeter and to 0.1 cm while weights were taken in gram and to 0.1g. Weights were taken after wiping off water with a dry towel.

The relationship between total weight (W) and total length (L) of the fish were estimated using the equation: $W = aL^b$, where 'a' is a constant and 'b' is a regression coefficient relating weight (W in grams) and length (L in cm), and was estimated by ordinary least square regression. After transforming the weight and length to logarithms the above equation was applied as follows: $\log W = \log_a + b \log L$, calculated by the method of least square. Condition factor of the fish was calculated using the formula: $K = W \times 100 / L^3$ Where K= condition factor, W= fish weight in grams, L= total length of fish in centimeter.

The total length and total weight data of the fishes were subjected to statistical analysis according to Zar (1984). In order to verify if calculated 'b' was significantly, different from 3, the student's t-test was employed (Ezenwaji, 2004)

RESULTS AND DISCUSSION

Length-weight relationship was calculated for a total of 238 fishes made up of 70 *Clarias gariepinus* and 110 *Tilapia zillii* from lake Alau, and 28 *C. gariepinus* and 30 *T. zillii* from Monguno hatchery. The results of length-weight relationship and condition factor are shown in Tables 1 and 2.

Correlation coefficients for both *Clarias* and *Tilapia* in the study areas revealed a strong correlation between length and weight of the fishes (P<0.001) in lake Alau than in Monguno hatchery fishes (Table 1). This was also true when fish of the same size range from the two study areas were compared (Table 2). A similar strong correlation between length and weight of *Channa obscurus* in Nigeria freshwater was reported by Umeham (2001). Figures 1 and 2 represent the length-weight relationship (LWR) of *C. gariepinus* and *T. zillii* of comparable size range respectively in the study areas.

LWR of Clarias in lake Alau conforms with already recorded LWR of all species of the genus in Nigerian freshwater systems (Ezenwaji, 2004).

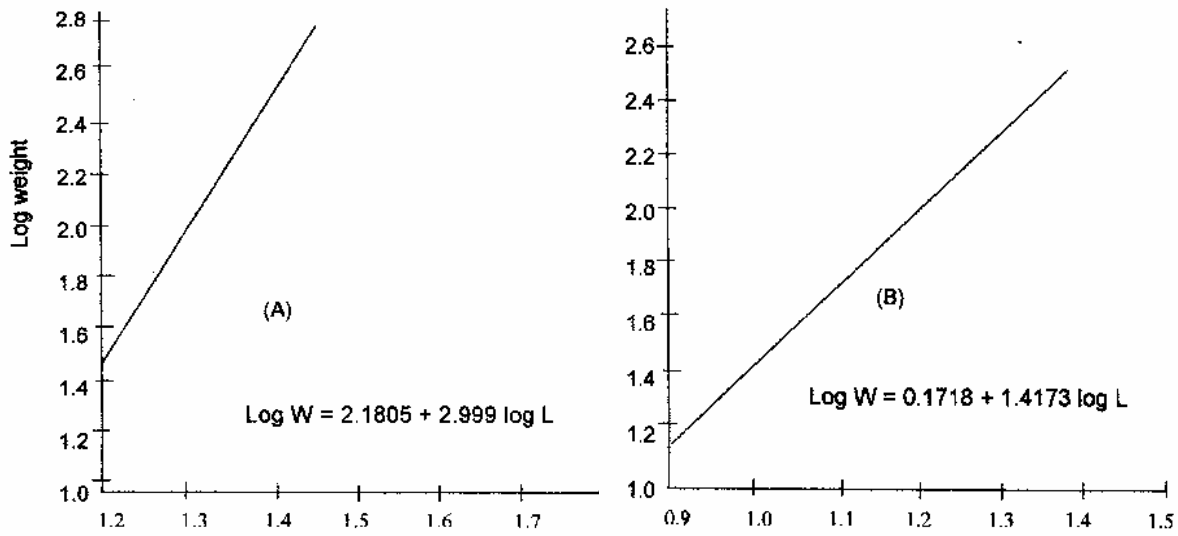

Figure 1: length-weight relationships using log length and log weight of *Clarias gariepinus* from (A) Lake Alau and (B) Monguno hatchery

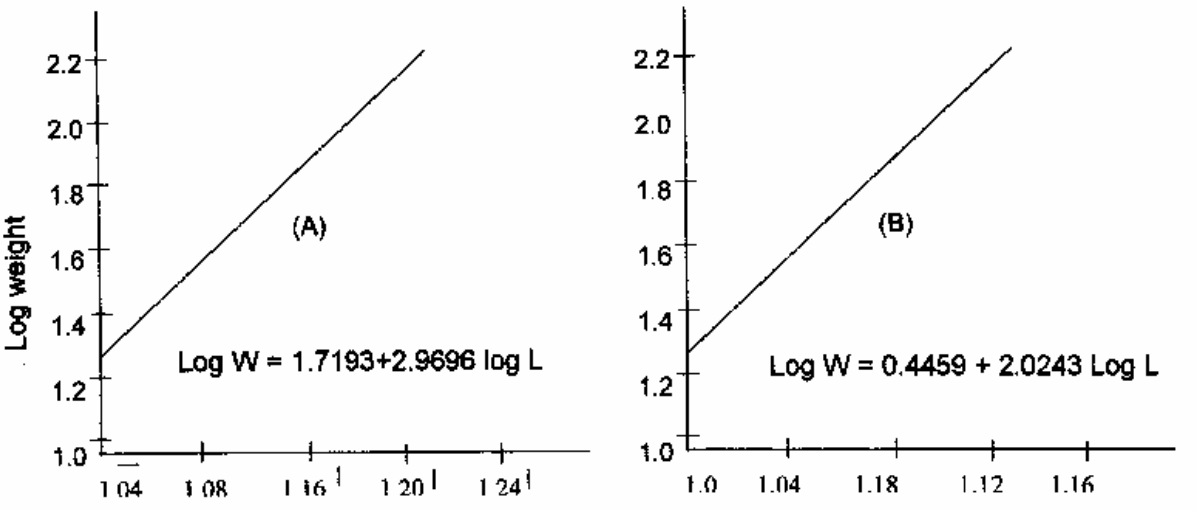

Figure 2: length-weight relationships using log length and log weight of *Tilapia zillii* from (A) Lake Alau and (B) Monguno hatchery

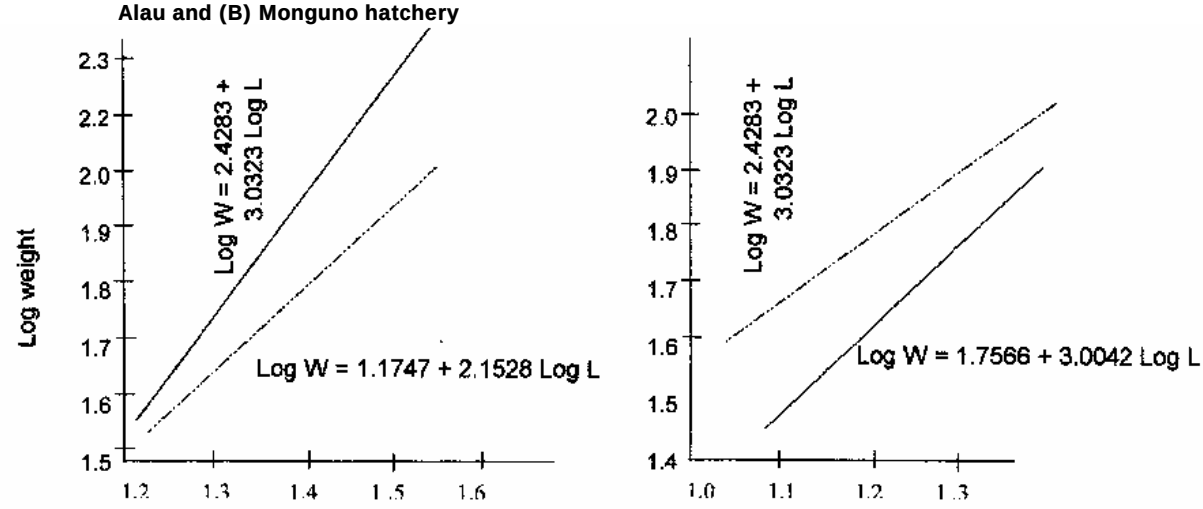

Figure 3: Regression line drawn between log length and calculated log weight of (A) *Clarias gariepinus* and (B) *Tilapia zillii* in lake Alau and Monguno hatchery

The same is true for LWR of T*. zillii* of this study in lake Alau with reference to LWR records in Nigeria (King, 1996 a, b).

The values of regression coefficient of *Clarias* and *Tilapia* in lake Alau did not differ significantly from 3 at 5 percent level of significance. Furthermore, fish of the same size range in Monguno hatchery differed significantly from 3 at 1 percent level of significance (Tables 1 and 2). Figure 3a showed the regression lines drawn between log length and calculated log weight for comparable size range of *Clarias* in both study areas; while the regression lines of *Tilapia* in the study areas are shown in Figure 3b.

Clarias of lake Alau showed higher values of mean condition factor than those of Monguno hatchery for all size groups (Table 1); whereas *Tilapia* of Monguno hatchery showed higher values of condition factor than lake Alau Tilapia.

Both Clarias *gariepinus* and *Tilapia zillii* from lake Alau were larger than those from Monguno hatchery. The difference in size of the fishes may be due to limited food supply to the hatchery resulting in poor pond productivity. The correlation coefficients for both species in the two study areas were found to be significant ($P<0.001$). This proves that there as strong relation between length and weight of all fish populations. But all fishes from lake Alau showed stronger relation than those of hatchery.

Regression coefficient for *C. gariepinus* and *T. zillii* from lake Alau did not differ significantly from the hypothesized value 3. This result indicated that both fishes from the lake satisfy the cube law, as they grew isometrically. Fishes from Monguno hatchery showed b- values significantly less than the hypothesized value 3 and less than those of their counterparts in lake Alau. Low b-values of hatchery fishes revealed poor growth of the fishes, as they got relatively thinner as they grew. The regression lines computed between log length and log weight (Figure 3) for comparable size ranges from both *Clarias* and *Tilapia* populations also revealed the same result.

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EFFECT OF pH ON THE GROWTH PERFORMANCE OF *Heterobranchus bidorsalis* (♂) X *Clarias gariepinus* (♀) HYBRID JUVENILES

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ABSTRACT

Twelve plastic basins each filled with 6 litres of dechlorinated tap water at pH 6, 7 (control), 7.5 and 8 such that each pH treatment was replicated three times in a Latin square design were used for the study. The tanks were randomly stocked with 10 four-week-old Clarias gariepinus (♀) x Heterobranchus bidorsalis (♂) hybrid juveniles (mean weight 3.32 ± 0.05 g) and fed 30.25% crude protein diets for five weeks. Fish growth was measured by weighing the juveniles every week and the weight differences, specific growth rate (SGR) and food conversion ratio (FCR) determined. Fish raised at pH treatment 7.0 recorded significantly higher weight gain (P < 0.05) than other pH treatments. Weight gain of fish raised at pH 6.0 was however not different (P > 0.05) from that of fish raised on pH 8.0 treatment. There was significant difference (P < 0.05) in the SGR of the juveniles raised at pH 6 and pH 8. There was no significant difference (P > 0.05) between the SGR of fish raised at pH 7 and pH 7.5 but there was significant difference between the SGR of fish raised at pH 7 and fish raised at other treatments. Fish showed reduced growth when raised at pH 6 and pH 8 and positive growth when raised at pH 7 and 7.5 though there was no significant difference (P > 0.05) in FCR of fish cultured at all pH treatments. Our results showed that the optimal pH range for raising the hybrid catfish juveniles was between 7.0 – 7.5 pH.

Keywords: *Heterobranchus bidorsalis* x *Clarias gariepinus* hybrid, Optimal pH range

INTRODUCTION

As the primary sources of animal protein became depleted, more expensive and usually beyond the rich of a substantial number of people, especially in developing countries of the world, fish became increasingly one of the less expensive and readily available sources of dietary animal protein (Kapetsky, 1981). Besides, fish contains large amounts of quality amino acids including lysine, methionine and tryptophan as well as substantial quantities of vitamins although poor in vitamins A and C (Lovell, 1989; Benitez, 1999). Over-fishing in many tropical countries has however resulted in scarcity of fish species in the wild highlighting the need to explore more avenues to satisfy the high demand for fish and fish products. Aquaculture is often recommended as a solution to the scarcity of fish protein.

It has been recognized that the abiotic and biotic environment profoundly affect the distribution of animals in different habitats. The physical environment also embraces everything that is not directly associated with the presence of other animals including fish. The life patterns and activities of animals in a given ecological system are also influenced by a number of factors which could be endogenous (body size, activity, reproductive cycle pattern, nutritional status), or exogenous (hydrogen ion concentration (pH), salinity, temperature, oxygen concentration and photoperiod, among others). The hydrogen ion concentration (pH) of a solution is among the many abiotic factors that affect the survival, growth, reproduction and distribution of aquatic animals. The similarity of the effects of the pH and carbon IV oxide (CO_2) tension on the oxygen-carrying capacity of the blood has also been noted (Cameron and Randall, 1972; Lagler *et al*., 1977). Thus, it is envisaged that increased pH is accompanied with increased rate of respiration.

It has been recognized that three distinct processes, namely, cell division, assimilation and cell expansion contribute to the physiological process of growth – a process which acts as an integrator of a variety of physiological end-products and which may be classified into reproductive and somatic phases. While the reproductive growth phase involves increase in the sizes of the reproductive structures, the somatic aspect entails increase in body size. In both processes growth is often determined by change in weight and/or length. Fish growth incorporates the larva, fry, fingerling and adult stages. Most researchers have focused on the acid and alkaline pH limits at which fish grow and reproduce rapidly because fishes have a narrow tolerance pH range (De Silva and Anderson, 1995). In a study, Boyd (1982) noted that the acid and alkaline death points for fish are about pH 4 and pH 11, respectively. However, if waters are more acidic than pH 6.5 or more alkaline than pH 9.0 for long periods, reproduction and growth will diminish. Furthermore, during periods of rapid plant growth in ponds, pH values in these ponds have reached 12 or more and led to death.

One of the suggested causes of fish death in very acidic water is failure to regulate their internal ion content. Both influx and efflux of sodium and chloride through the gills and kidneys are affected by this. Sodium influx rates appear to be largely dependent of pH.

Empirical evidence has also shown that fish blood, muscle and cellular parameters are altered by pH (Brown and Sadler, 1989).

Research observations have shown that *Clarias gariepinus* species do not grow as large and as quickly as *Heterobranchus bidorsalis*. On the other hand, *Heterobranchus* species do not possess the high survival rates of *Clarias gariepinus* (Madu *et al.*, 1999), hence the strong urge in aquaculture for hybrid of *Clarias gariepinus* (♀) and *Heterobranchus bidorsalis* (♂) commonly called *Heteroclarias* in which the characteristics of *Heterobranchus bidorsalis* is dominant. *Heteroclarias* is voracious and capable of interbreeding.

The pH tolerance range varies for different species. For example, the tolerance ranges are: for sticklebacks, pH 4.0 - 5.0; cichlids, 6.5 – 9.2; perch, 4.6 – 9.5; *Clarias gariepinus,* 6.5 – 8.0 (Gaunder, 2005). The objective of this study was to determine the growth performance of the *Heteroclarias* juveniles and more specifically the optimum pH range that supports optimum growth rate.

MATERIALS AND METHODS

Experimental Fish: Prior to the collection of the experimental fish, 12 plastic basins (12 L capacity), were bought, washed and filled with dechlorinated tap water. Artificial diet was formulated and prepared from artificial ingredients (Table 1) as described by Eya and Mgbenka (1990). The standard diet was dried at 60° C for 2 days, finely reground and stored in airtight container prior to feeding to fish. Two hundred and ten four-week-old *Heteroclarias* (mean weight 3.32 ± 0.05 g) produced in the Fisheries and Hydrobiology wet laboratory within the Zoological garden, University of Nigeria, Nsukka were used for the study. The juveniles were divided into 10 plastic culture tanks each filled with 6 litres of water and acclimatized for two weeks. After acclimatization, using a randomized Latin square design, the catfish were then divided into 12 plastic fish experimental tanks comprising four treatment groups replicated three times and 10 juveniles per tank. During the period of acclimation, the juveniles were fed the reground formulated catfish diet (Table 1) at 4% body weight twice daily. During a five-week experimental period, the water temperature was measured with mercury-in-glass thermometer 5 cm below the surface. To minimize the risk of fungal and algal growth, the culture tanks were cleaned weekly and covered with plastic mosquito nettings held in place by plastic rubber bands to prevent the fishes from jumping out. The remaining catfishes were kept in a reservoir tank to serve for replacement in cases of mortality. The pH of the tap water prior to pH dilutions was measured with a hand-held pH meter (ExStick™ pH Meter, Expects Instrument Corp., USA) and was 7.0. This served as control.

pH Dilutions: For stock solution for acid, 2% hydrochloric acid was prepared by measuring 2 ml concentrated hydrochloric acid and 98 ml of distilled water into a beaker, mixed thoroughly with a clean glass rod and stored in a reagent bottle. For the base, 0.01M sodium hydroxide (NaOH) was prepared by weighing 0.4 g of sodium hydroxide granules and dissolving in 100 ml of distilled water. This served as control. Various pH readings employed were 6, 7, 7.5 and 8. To get pH 6, drops of the 2% hydrochloric acid were added to get the most stable reading of 6. To get pH greater than 7.0, drops of 0.01M sodium hydroxide were added to obtain the most stable reading as displayed by the pH meter.

Growth of the Hybrid Juveniles: The growth was measured by weighing the juveniles every week for the 5 weeks that the study lasted. Weight differences were determined. Also estimated were specific growth rate (SGR) and food conversion ratio (FCR) (De Silva and Anderson, 1995), thus: SGR = $\ln(W_2) - \ln(W_1) / t_2 - t_1 \times 100$ where W_1 – Initial weight (g), W_2 = Final weight (g), t = time (days), ln = natural log. FCR = mass of feed offered to fish (g)/ increase in weight of fish (g).

Statistical Analysis: The data collected were analysed for significant differences ($P \leq 0.05$) by the analysis of variance (ANOVA) using a computer Statistical Package for Social Sciences (SPSS). Determined differences were partitioned by the least significant difference (LSD) and the Duncan's New Multiple Range Test (DNMRT).

RESULTS

The effect of various pH on the growth performance of *H. bidorsalis* (♂) x *C. gariepinus* (♀) hybrid juveniles is shown in the weight gain and loss corresponding to the different pH values (Table 2). During the experimental period which lasted for five weeks, the water temperature ranged from 23.9° C – 29.4° C. The pH of the water at the same period prior to treatment ranged from 6.87 – 7.00. At the end of the culture period, pH treatment 7.0 (control) recorded significantly higher weight gain or loss ($P < 0.05$) than other treatments (Table 2). The weight difference of juveniles of fish raised at pH 6.0 was however not different ($P > 0.05$) from that of fish on pH 8.0 treatment. pH treatment 7.5 also indicated weight gain, while pH 6.0 and 8.0 showed weight reductions.

The specific growth rate of the hybrid catfish juveniles at the different pH treatments showed that the juveniles at pH 7.5 treatments had higher mean specific growth rate (2.90 ± 0.47 g/d) when numerically compared with the hybrid catfish fed at pH 7.0 (1.63 ± 0.31 g/d) and other pH treatments (Table 3). There was significant difference ($P < 0.05$) in the SGR of the juveniles fed at pH 6 and pH 8. There however was no significant difference ($P > 0.05$) between the SGR of fish fed at pH 7 and pH 7.5. The food conversion ratio of the hybrid catfish juveniles at different pH treatments (Table 4) indicate that the catfish in pH treatment 7.0 (0.17 ± 0.04) showed greater but non-significant ($P > 0.05$) ability to convert food energy for growth when compared with other pH treatments.

Table 1: Composition (%) of the standard diet with estimated percent crude protein content

Ingredient	% ingredient	Estimated % crude protein in ingredient	Estimated % crude protein
Yellow corn	35.0	9.8	3.43
Soybean meal	50.5	40.0	20.20
Fishmeal	10.0	57.0	4.62
Blood meal	3.0	60.0	2.00
Vitamin and mineral premix (Vitalyte)[1]	1.2	-	-
Vitamin C	0.3	-	-
Total:	100.00		30.25
Proximate composition			
Ash	10.70		
Crude protein	29.95		
Fats	11.60		
Fibre	15.25		
Moisture	14.60		
Nitrogen free extract	17.90		

[1]*Vitamin and mineral premix provided the following constituents diluted by cellulose (mg/kg of diet): Vitamin, A; Vitamin, B_2; Vitamin, B_6; Vitamin, B_{12}; Vitamin, C; Vitamin, D_3; Vitamin, E, Vitamin, K; Panthothenic acid, 5,350; Lysine, 15,000; Methionine, 10,000; Lactose, 1,000; Potassium chloride, 87,000; Sodium chloride, 50,000; Sodium sulphate, 212,000; Magnesium sulphate, 12,000; Copper sulphate, 12,000; Zinc sulphate, 12,000.*

Table 2: Weight gain and loss of hybrid *Heterobranchus bidorsalis x Clarias gariepinus* juveniles reared at different pH values

Treatment	Weight of fish (g)					
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
pH 6.0	7.03 ± 1.35	4.43 ± 0.05	4.17 ± 0.08	3.73 ± 0.30	3.47 ± 0.43	3.17 ± 0.58
pH 7.0	6.10 ± 0.34	6.96 ± 0.21	7.33 ± 0.02	7.47 ± 0.50	7.73 ± 0.18	8.06 ± 0.35
pH 7.5	5.23 ± 0.48	5.70 ± 0.25	6.07 ± 0.06	6.40 ± 0.11	6.70 ± 0.26	7.06 ± 0.44
pH 8.0	6.13 ± 0.96	4.77 ± 0.28	3.97 ± 0.12	3.77 ± 0.33	3.77 ± 0.22	3.36 ± 0.43

Table 3: Specific growth rate (g/d) of hybrid (*Heterobranchus bidorsalis* x *Clarias gariepinus*) juveniles raised at different pH for five weeks

Ph	Week 1	Week 2	Week 3	Week 4	Week 5	Mean
6.0	-6.57 ± 1.19	-7.57 ± 0.69	-9.00 ± 0.03	-10.04 ± 0.60	-11.43 ± 1.24	-9.94 ± 0.75
7.0	0.54 ± 0.54	1.26 ± 0.18	1.54 ±0.04	2.11 ± 0.24	2.69 ± 0.53	1.63 ± 0.31
7..5	1.29 ± 0.81	2.18 ± 0.36	3.00 ±0.05	3.60 ± 0.35	5.43 ± 0.77	2.90 ±0.47
8.0	-3.57 ± 1.50	-6.14 ±0.22	-6.86 ±0.15	-7.71 ± 0.57	-8.57 ±1.00	-6.57 ± 0.69

Table 4: Food conversion ratio of hybrid (*Heterobranchus bidorsalis* x *Clarias gariepinus*) juveniles raised at different pH for five weeks[1].

pH	Week 1	Week 2	Week 3	Week 4	Week 5	Mean
6.0	-0.03 ± 0.00	-0.03 ± 0.00	-0.02 ± 0.00	-0.02 ± 0.00	-0.02 ± 0.07	-.024 ± 0.00
7.0	0.37 ± 0.10	0.16 ± 0.00	0.13 ± 0.02	0.10 ± 0.03	0.07 ± 0.05	.166 ± 0.04
7..5	0.16 ± 0.04	0.09 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	.086 ± 0.02
8.0	-0.06 ± 0.10	-0.03 ± 0.00	-0.03 ± 0.00	-0.03 ± 0.00	-0.02 ± 0.01	-.034 ± 0.02

[1]*There is no significant difference (P > 0.05) in feed conversion efficiency for the juveniles raised at different pH.*

While the hybrid catfish in pH treatment 7.5 were next in converting their feed to energy needed for growth, the fish in pH treatments 6.0 and 8.0 were relatively poor feed-energy for growth transformation. The non-significant difference (P > 0.05) in feed conversion ratio of the juveniles fed at different pH implies that the juveniles apparently transformed the feed to energy with values close to each other at the different pH values.

DISCUSSION

Weatherly (1990) described fish growth as the end product and an integrator of the reactions involving the intrinsic and extrinsic factors (including the aquatic medium) in which the fish finds itself.

It has been established that specific features of the catfish environment are of primary importance in determining the growth and survival of the species in varying degrees. Naturally, most organisms possess well–defined range or pH tolerance. The results of the study indicate that *Heterobranchus bidorsalis* (male) x *Clarias gariepinus* (female) hybrid juveniles would have their tolerance pH range at 6.0 to 8.0 since death was not observed at this pH range, and an optimum pH range of 7.0 to 7.5 as maximum growth performance was recorded within this range (Fig. 1). The results obtained are in conformity with those of Boyd (1982) and Gaunder (2005) who had observed that the acid and alkaline death points for fish are about pH 4 and 11 respectively with reproduction and growth diminishing with increasing acidity or alkalinity.

The reduction in weight in pH treatments 6.0 and 8.0 recorded in the study (Table 4) could be attributed to imbalance in homeostasis since low or high pH that is not directly lethal only affects fish growth and reproduction (Kimmel, 1993). Also, Wilkie and Wood (1991) observed that if the pH falls below the tolerance range death would ensue as a consequence of the disturbance of the balance of sodium and chloride ion in the blood of the fish and the inhibition of ammonia excretion through the gills during high pH situations.

The weekly growth performance (Table 2) indicate that at pH 7.5 the catfish added satisfactory amount of growth compared to the optimal amount added by fish at the control treatment (pH 7.0). While the optimum pH for this fish is pH 7.0, a satisfactory range is pH 7.0 to 7.5. The results for the food conversion ratio (another parameter used to determine growth performance) show that catfish in pH treatment 7.0 had the highest food conversion ratio (Table 4). This indicates that the catfish in pH treatment 7.0 had greater ability to convert their food to energy necessary for growth since their environment was devoid of acid and base. In other words, the growth of the catfish which is largely dependent on their ability to convert food to energy for growth is, to a large extent, dependent on the pH of their surrounding medium.

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