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EFFECT OF AGE ON IMMUNE RESPONSE OF TRYPANOSOME-INFECTED RATS (*Rattus rattus*) FED DIETARY VITAMIN E AND SELENIUM

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ABSTRACT

This study was done to determine the combined effects of dietary supplementation of vitamin E and selenium on age-dependent immune response of Trypanosoma congolense-infected white rats (Rattus rattus, whiskers breed). Sixty rats were used in the study, 30 20-day old (newly weaned) rats and 30 90-day old (adult) rats. Four groups of rats with five rats of identical age per group were kept in wire-rat-cages. The cages were labeled G to J. Cage G contained adult rats (Control 1), while cage H contained newly weaned rats (Control 2). Cage I contained adult rats fed diet containing selenium and vitamin E (nutrient), while cage J contained newly weaned rats also fed diets containing selenium and vitamin E. Each treatment was replicated three times. Longevity (days of survival) and differential leucocyte counts which are functions of immune response of the rats upon infection with T. congolense were determined. At the end of the study, the longevity and differential leucocyte counts were analysed for significant differences using analysis of variance (ANOVA) and any differences were partitioned with the least significant difference (LSD) and the Duncan's Multiple Range Test (DMRT). The results revealed that there was no significant difference in longevity ($P > 0.05$) between the two control groups (newly weaned and adult rats) but there were significant differences between the longevity of each control group and the longevities of the rats given combined dietary supplementation of the nutrients. Longevity of newly weaned and adult rats given dietary supplementation of selenium and vitamin E were not different ($P < 0.05$). These results implied that age of the rats was not a contributory factor in improved immune response of the trypanosome-infected rats fed the combined dietary supplementation of selenium and vitamin E.

Keywords: Immune response, Newly weaned rats, Longevity, *Trypanosoma congolense*, *Rattus rattus*

INTRODUCTION

Food supplemented with vitamins and minerals had been suggested for control of trypanosomiasis (Van Dan, 1996; Bass, 1999) ranked among the first 10 diseases of man (Eisler *et al.*, 2001). Earlier researches demonstrated that supplemental vitamin E enhanced animals' immune response (Tengerdy and Brown, 1977; Hutchinson, 1999). Also, similar results had been reported for dietary supplement of selenium (Teige *et al.*, 1982; Nockel, 1986). The enhancement of animal humoral immunity by Vitamin E and by selenium may be due to the participation of both selenium and Vitamin E (nutrients) in similar

nutritional and biochemical pathways (Spallholz, 1980).

Feed supplements of 0.1 mg - 0.3 mg Selenium had been recommended for animals (FDA, 1987). The effect of each nutrient had been individually studied (Shukla *et al.*, 1988; Sidhu, *et al.*, 1993; Van Dan, 1996). Mgbenka and Ufele (2004) found that the combined dietary supplementation with 0.3 mg Selenium and 80 mg Vitamin E for adult white rats (*Rattus rattus*) significantly ($P < 0.05$) enhanced the resistance of *R. rattus* to trypanosome infection but not much is known on the effect of age on this resistance. Age-dependent effects on rats of disease-causing agent's challenge and of hormonal treatment had been reported (Antonini *et al.*, 2001; Jacobson, 2004).

In this study, age of individual rats is being checked as a factor in eradicating the disease using Vitamin E and Selenium as combined supplements. The specific objectives were to ascertain if there are synergetic effects of dietary supplementation of Vitamin E and Selenium on packed cell volume (PCV), differential leucocyte counts and longevity of trypanosome-infected white rats.

MATERIALS AND METHODS

Procurement and Management of *Rattus rattus* and *Trypanosoma congolense*: Male rats (20- and 90-day old) were purchased from the Animal Unit of the Department of Physiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The rats were held in stainless wire-rat-cages which were kept in the animal house. They were fed *ad libitum* with 25% crude protein chicks' mash diet (Top Feed Nigeria Ltd). The rats were given access to unlimited supply of water using drinkers. The faecal droppings in the tray were removed daily.

The rats were weighed before and after the experiment using a Mettler balance (electronic PC 2000). After initial weighing, each rat was differentially marked and kept five rats in each of four cages labelled G to J corresponding to four treatments. Each treatment set-up was replicated three times. One rat was first inoculated with *Trypanosoma congolense* isolated from other animals in NITR Veterinary Medicine Faculty. After 14 days, the level of parasitemia was determined to be 80,000 *Trypanosoma congolense* using a matching chart (Herbert and Lumsden, 1979). Tip of infected rat's tail was sterilised and a cut given to it using a sharp scissors. The blood of the infected rats used for inoculation was collected from the tip of the tail into a vial containing 1 ml of normal saline. This infected blood was used to inoculate other rats. Each experimental rat was given 0.1 ml of infected blood. Once infected, the rats were isolated and kept in cages.

Preparation of Diets: One kilogram of 25% protein chicks' mash was weighed into each of four clean containers labelled G – J to be fed to corresponding to Treatments G, H, I and J. Similarly, 0.3 mg Selenium and 80 mg Vitamin E were each weighed into containers labelled I and J, and the nutrients thoroughly mixed into the mash. In diet G (Control 1) and H (Control 2) Selenium and Vitamin E were not weighed in

and mixed into 1 kg of chicks' mash. Treatment G and I contained adult white rats while treatment H and J contained newly weaned rats. Each treatment was fed the corresponding diets for five weeks.

Estimation of Blood Parameters: Blood was collected weekly for estimation of total and differential leucocytes counts, and packed cell volume. For this purpose, absolute ethanol was used to sterilize rats' tails, sharp scissors was used to cut the tip of the tail, from which six drops of blood were drained into a vial containing two drops of EDTA. This was thoroughly mixed to avoid clotting. Each vial was labelled according to the number of animals and cages they belong to. Packed cell volume was determined using microhaematocrit method. In this method, microhaematocrit capillary tubes were $\frac{2}{3}$ filled with blood. One end of the capillary tube was sealed with plasticine after filling with blood. The tubes were spun at 10,000 rounds per minute for five minutes with microhaematocrit centrifuge. The results were read in percentage with haematocrit reader which was supplied with the centrifuge.

Total white blood cell count was determined using haemocytometer. Blood was drawn to 0.5 mark of the white cell pipette from haemocytometer and was used to mix 0.3 ml of diluting fluid of 1% glacial acetic acid mixed with a pinch of gentian violet. A firm pressure was used to slide in a cover glass into position on the counting chamber. The counting chamber was filled with mixed blood by holding a dropper which contained the mixture at an angle of 45° and lightly touching the tip against the edge of the cover glass. The chamber was placed on a microscope for five minutes for the cells to settle. The objective was focussed on each of the cover square millimetres and cells contained in them were counted. Cells touching the border lines on the top and right hand side of each square were included in the count, while those touching the border lines on the bottom and left hand side were disregarded. The final result was expressed as the number of cells per mm³ of blood. The diluting fluid helped to kill the red cells so that it was only the white blood cells that were seen and counted. The total white blood cells were calculated as follows: If N = number of cells counted in square mm, then N/4 = number of cells in square mm. The volume of each square mm = 1 x 1 x 10 mm³.

Table 1: Initial weight (g), final weight (g) \pm standard error of mean and longevity of white rats (*Rattus rattus*) fed combined dietary supplementation of 0.3 mg selenium (Se) and 80 mg of vitamin E in 5 weeks¹.

Treatment ²	Weight of rats (g)	
	Initial	Final
G	267.93 \pm 6.44 a	224.07 \pm 6.83 b
H	111.93 \pm 2.81 a	77.73 \pm 2.29 b
I	266.80 \pm 7.69 a	225.27 \pm 6.73 b
J	110.33 \pm 2.52 a	76.67 \pm 2.41 b

¹Means \pm SEM in a row with different letters are significantly different ($P < 0.05$). ²G, Adult white rats (Control 1); H, Newly weaned white rats (Control 2); I, Adult white rats fed 80 mg Vitamin E and 0.3 mg selenium supplemental diet; J, Newly weaned white rats fed 80 mg Vitamin E and 0.3 mg selenium supplemental diet.

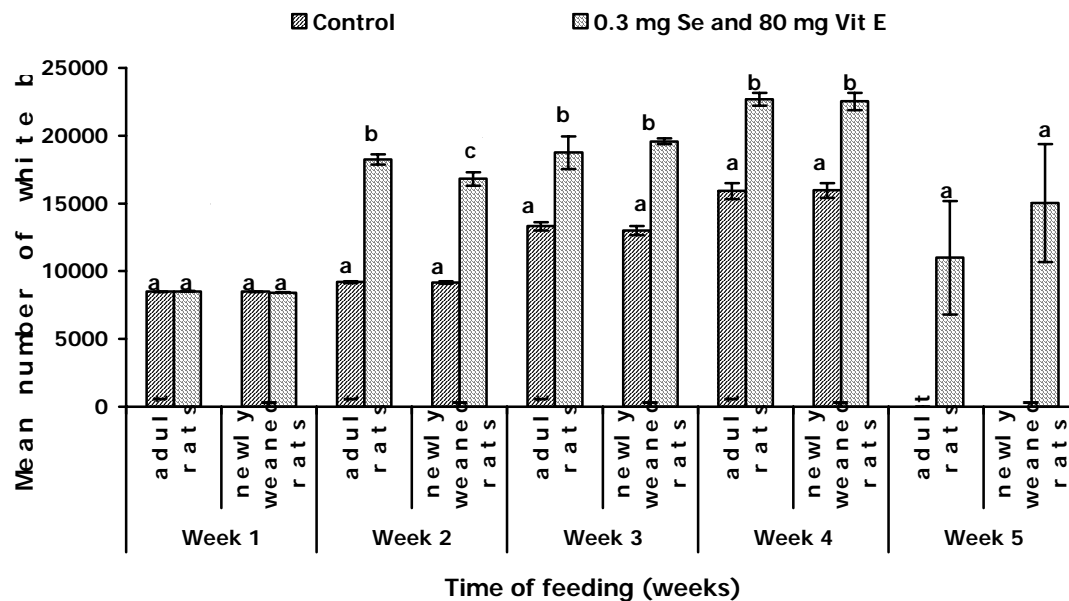


Figure 1: Weekly total white blood cells (mean \pm standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed dietary 0.3 mg selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks. Columns with different letters on top in a week are significantly different ($P < 0.05$).

Therefore, number of cells in $1 \text{ mm}^3 = N/4 \times 10$. The blood was diluted 1 in 20. The number of cells per mm^3 of undiluted blood = $N/4 \times 10 \times 20 = N \times 50$.

Procedure for differential leucocytes count was by dropping a fresh blood onto one end of clean, grease-free slide placed on a horizontal surface. Using a spreader, a little narrower than the slide, the drop was spread along the slide until the blood was smeared. When the blood film was made, drying was hastened by waving in air. It was stained immediately by Leishman technique, by adding 10 drops of Leishman stain on the dried smear and 20 drops of distilled water at pH 6.8. This was mixed by rocking the slide gently or with Pasteur pipette and allowed to stand for 10 to 15 minutes. The stain was washed with distilled water and flooded with tap water for 1 to 2 minutes. The slide was allowed to dry. The slide was later examined and the different cell

types counted under oil immersion objective at $\times 100$. The polymorphonuclear cells and mononuclear cells were counted separately.

The cells were counted in one complete longitudinal strip of the film. The different types of leucocytes observed were noted by scoring system. The cells counted were the polymorphonucleated cells comprising of neutrophils, basophils and eosinophils, and the mononucleated cells comprising of lymphocytes and monocytes. The results for each group of the leucocytes were expressed as a percentage of the total white blood cell count.

Blood parameters for each experiment were analysed for significant differences by descriptive statistics and by the one way analysis of variance (ANOVA) using SPSS computer package. Multiple comparisons on any detected significant differences were partitioned with the least significant difference (LSD) and the Duncan's Multiple Range Test.

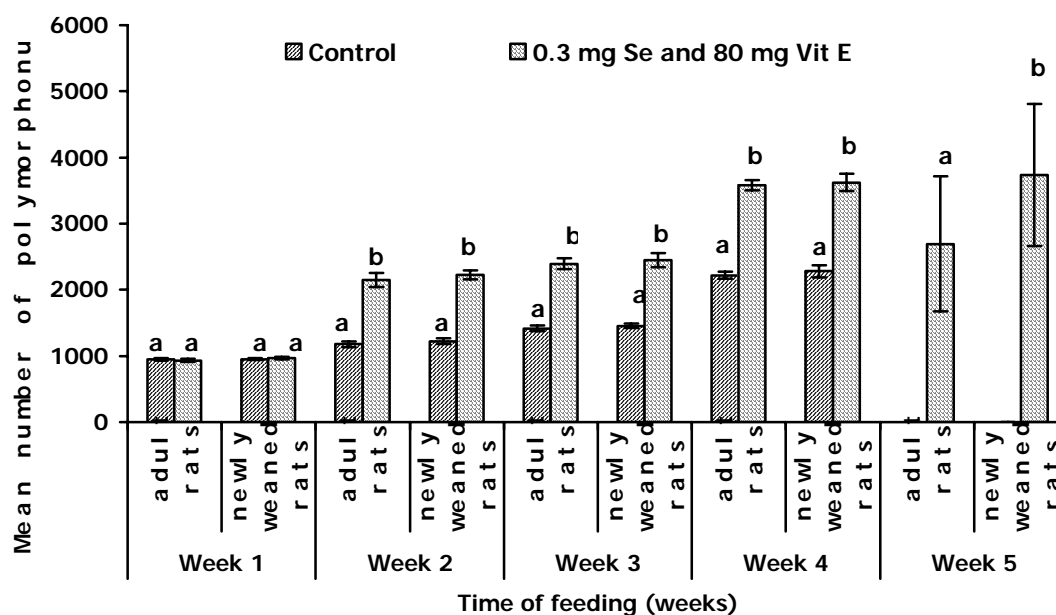


Figure 2: Weekly polymorphonucleated cells (mean \pm standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed dietary 0.3 mg selenium (Se) and vitamin E (Vit E) for 5 weeks. Columns with different letters on top in a week are significantly different ($P < 0.05$).

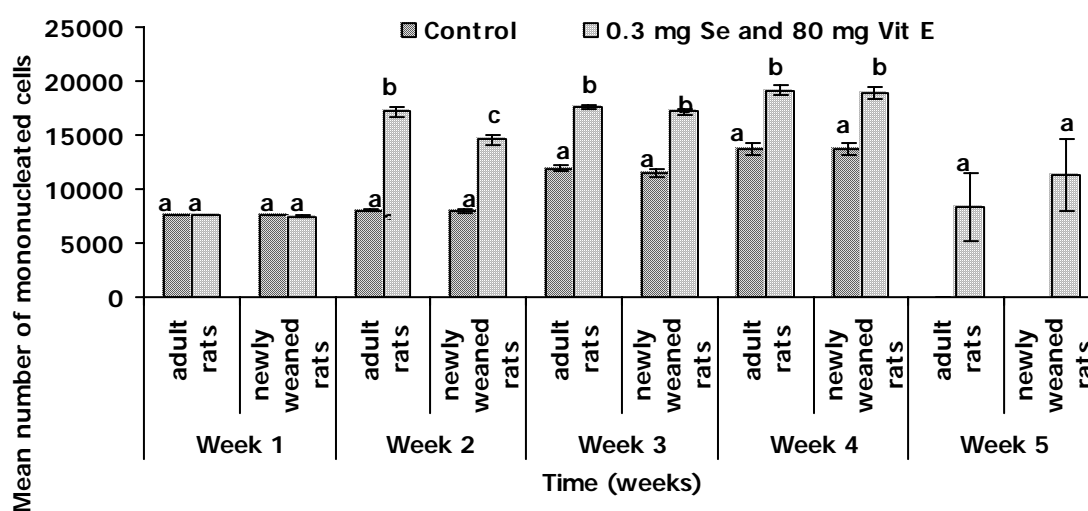


Figure 3: Weekly mononucleated cells (mean \pm standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed 0.3 mg selenium (Se) and 80 mg vitamin E (vit E) for 5 weeks. Columns in a week with different letters on top of columns are significantly different ($p < 0.05$).

Means and standard error of means (SEM) from results of descriptive statistics analyses were used for production of figures and tables of the various blood parameters.

RESULTS

Total White Blood Cells: The mean weekly differential total white blood cells and packed cell volume of *Trypanosoma congolense*-

infected *R. rattus* of different ages fed diets containing the same level of Selenium and Vitamin E is shown in Figure 1. Total white blood cells were highest in Week 4 but declined rapidly thereafter. The rats died in the control groups by Week 5. Treatment J (newly weaned, nutrients-supplemented diet rats) had the highest though not significantly different ($P < 0.05$) total white blood cells. Figure 1 shows that there were no significant differences ($P >$

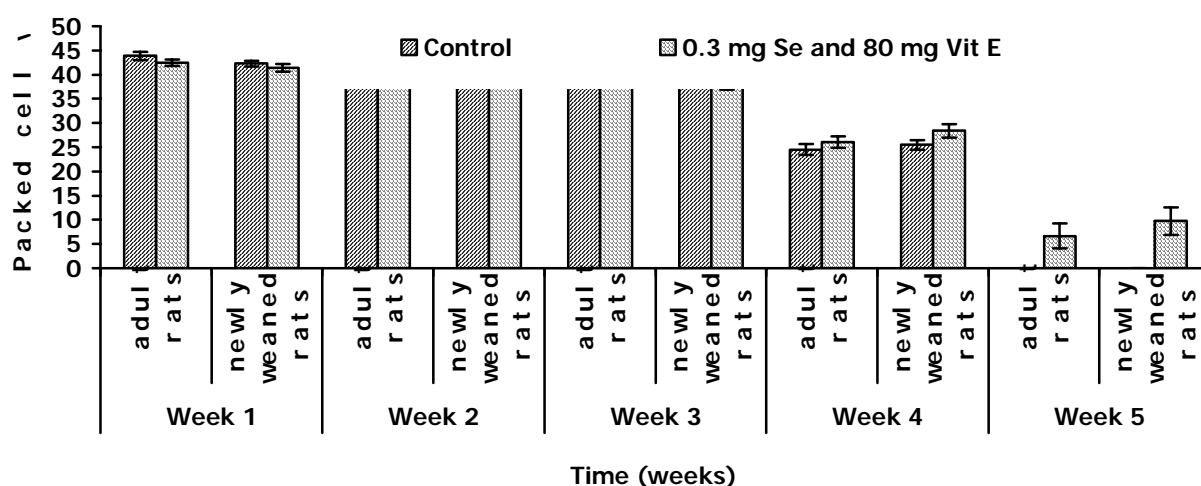


Figure 4: Weekly packed cell volume (mean \pm standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed 0.3 mg dietary selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks. Columns with different letter on top in a week are significantly different ($P < 0.05$).

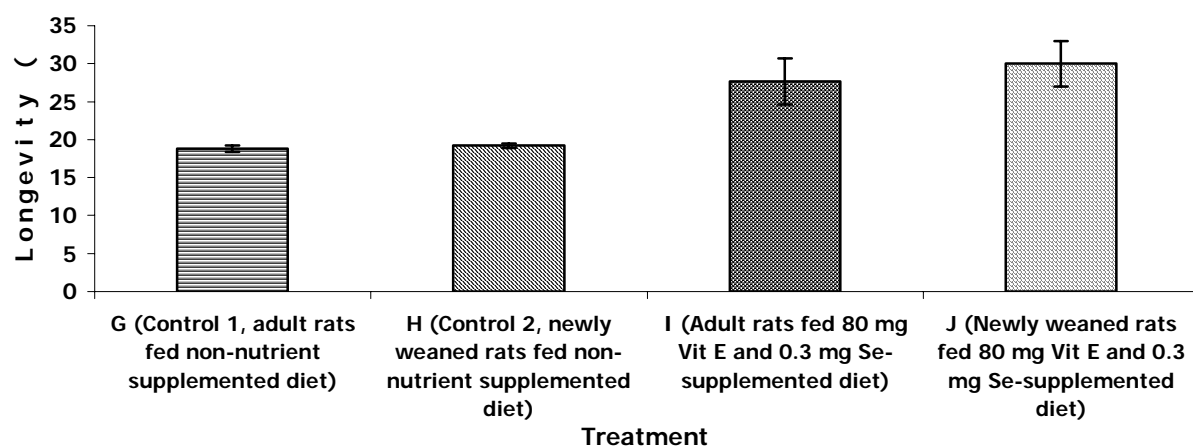


Figure 5: Longevity \pm standard error of mean (days) of white rats (*Rattus rattus*) fed dietary supplementation of 0.3 mg selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks.

0.05) among the treatments in total white blood cells by Week 1. From Weeks 2 to 5, there were significant differences ($P < 0.05$) in total white blood cells between the control treatments and nutrients-supplemented treatments. But for Week 2, there were no significant differences ($P < 0.01$) between Treatments G and H, and Treatments I and J in each week.

Polymorphonucleated Cells: The mean weekly polymorphonucleated cells of *Trypanosoma congolense*-infected *R. rattus* of different ages fed diets containing the same level of combined dietary selenium and Vitamin E was highest in Week 4 (Figure 2). There was no significant difference ($P > 0.05$) between the treatments (groups) at Week 1, while from Weeks 2 to 5, there were significant differences

among treatments in polymorphonucleated cells ($P > 0.05$). Comparison of the polymorphonucleated cells among the treatments through the weeks revealed that it had the same pattern as the total white blood cells. Namely, there were no significant differences in polymorphonucleated cells of rats of both ages among the control treatments and among nutrients-supplemented treatments but highly significant differences ($P < 0.001$) were observed when the controls were compared with the treatment group.

Mononucleated Cells: Figure 3 shows that mean weekly mononucleated cells of a *Trypanosoma congolense*-infected *R. rattus* of both ages fed diets containing the same level of selenium and Vitamin E was highest at Week 4.

Treatment I had the highest number of mononucleated cells at Week 5.

There was no significant difference ($P > 0.05$) between the treatments at Week 1 with respect to the mononucleated cells. From Weeks 2 to 5 there were highly significant differences ($P < 0.01$) in the number of mononucleated cells among the treatments. Figure 5 shows that there was no significant difference in mononucleated cells ($P > 0.05$) in Week 1 among the groups except in Treatments I and J (nutrients-supplemented groups) where significant difference ($P < 0.05$) existed.

Packed Cell Volume (PCV): Figure 4 shows that mean weekly packed cell volume of *Trypanosoma congolense*-infected *R. rattus* of different age fed diets containing the same level of selenium and vitamin E declined as the weeks progressed. By Week 4 the value of packed cell volume declined sharply due to death of the rats. There were significant differences ($P > 0.01$) in packed cell volume between nutrient supplemented groups and control groups from Weeks 2 – 5.

Longevity (Days) of Rats: Figure 5 shows the longevity of the rats. From Figure 5, Treatment J (newly weaned, nutrients-supplemented diet rats) had the highest mean longevity (days), followed by Treatment I (adult rats, nutrient-supplemented). There was highly significant difference in longevity ($P < 0.01$) between the groups fed nutrient supplemented diets and the control groups.

Weight (g) of Rats: Table 1 shows the weight difference of *R. rattus*. From this table, it was observed that there was significant difference ($P < 0.01$) between the initial and final weights of rats in each age category.

DISCUSSION

The significantly higher ($P < 0.05$) total white blood cells in the nutrient-supplemented diet groups beyond Week 1 in this study (Figure 1) implies that immune response whereby different types of white blood cells multiplied in numbers was elicited by infection of the rats with *T. congolense*. That Treatments I and J (adult and newly weaned rats fed nutrients-supplemented diets) had significantly higher ($P < 0.05$) numbers of polymorphonucleated cells compared to controls of both ages from Weeks 2 – 5 implies that the polymorphonucleated cells were secreted more as the infection intensified

and that the nutrients effected the immune response of the rats irrespective of their age groups. This agrees with the finding of Tengerdy and Brown (1977) with supplemental Vitamin E and that of Nockel *et al.* (1986) with supplemental selenium that each nutrient used singly boosts immune response. Using polymorphonucleated cells as a parameter, the non-significant difference ($P > 0.05$) among the treatments in Week 1 shows that the rats had the same immune response at the beginning of the study.

The non-significant difference in mononucleated cells ($P > 0.05$) in Week 1 among the groups while significant differences ($P < 0.01$) existed in later weeks indicated that under normal circumstances there is no age-dependent difference in the number of mononucleated cells. The non-significant difference in PCV ($P > 0.05$) between treatments G and H and between treatments I and J, while in other comparisons there were significant differences among the treatments ($P < 0.05$) implies that treatments of identical nutrient supplementation had the similar physiological depression.

The highly significant difference ($P < 0.01$) in the weight of animals before and after the experiment implies that there was weight loss due to trypanosomiasis. Despite the improvement of the immune response with 0.3 mg selenium and 80 mg Vitamin E dietary supplementation, as is evidenced by the significant increases ($P < 0.05$) in leucocyte counts in *T. congolense*-infected rats, trypanosomiasis caused the weight loss. Also, age of the rats did not have any effect on the weight loss. It was concluded that there was loss of weight associated with the trypanosome infection irrespective of the age of the rats. Physiological stress has been incriminated for weight loss during trypanosome infection (Tizard, 1985; Ogwu *et al.*, 1986; Stephen, 1986).

In summary, the lack of significant differences ($P > 0.05$) in mean weekly total white blood cells, mononucleated cells and polymorphonucleated cells among rats of different age groups treated alike in this study implies that unlike in the studies of Antonini *et al.* (2001) who recorded age-dependent respiratory defense mechanism in bacteria-infected rats and Jacobson and Ansari (2004) who found age-dependent reaction of rats to leuteinising hormone releasing hormone, age of *R. rattus* did not hinder or aid the effects of trypanosomes in *R. rattus*. Furthermore, the

combined dietary supplementation of the nutrients in this study enhanced the immune system of the rats in resistance to the trypanosomiasis, irrespective of age, as evidenced by the fact that some trypanosome-infected rats lived more than the mandatory 20 days (Figure 5). This is in agreement with earlier finding of Mgbenka and Ufele (2004) that combined dietary supplementation with 0.3 mg selenium and 80 mg Vitamin E enhanced immune response of *Trypanosoma Congolese*-infected *R. rattus* leading to living beyond 20 days

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PREVALENCE OF URINARY SCHISTOSOMIASIS IN OZUITEM, BENDE LOCAL GOVERNMENT AREA OF ABIA STATE, NIGERIA

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ABSTRACT

*Studies on the prevalence of urinary Schistosomiasis were carried out in Ozuitem, Bende LGA between May and September, 1998. Urine collections from villagers were examined using centrifuge and filtration technique. A total of 1173 urine samples were collected and examined, of which 496 (42.3%) were positive for Schistosomiasis. Visible haematuria was their predominant presenting symptom. Of the total, 370 (74.6 %) were excreting under 100 eggs per 10 ml urine sample with 250 (75.0 %) males and 120 (72.6 %) females, while 3(0.6 %) were excreting more than 500 eggs in 10 ml of urine samples with 2(0.6 %) males and 1(0.6 %) females. A chi square analysis showed that intensity of infection and frequency of water contact were significantly higher in persons under 20 years of age than in persons 20 years and above ($P < 0.05$). Of the 496 infected persons, 333 (67.1 %) were males, while 163 (32.9 %) were females. Overall peak infection (59.4 %) occurred in the 11-20 years age group. Infection varied significantly among different villages, ages and sex in the study area ($P < 0.01$). *Schistosoma* intermediate host snails collected in routine malacological survey include *Bulinus globosus*, *B. forskalii*, *B. truncatus*, *Lymnaea natalensis* and *Melanoides tuberculata*. Only *B. globosus* was found to shed furcocercous cercariae believed to be human schistosomes.*

Keywords: *Schistosoma haematobium*, Urinary schistosomiasis, Urine, Bende

INTRODUCTION

Schistosomiasis is an infection of man caused by a parasitic trematode known as *Schistosoma haematobium*, the disease is characterized by haematuria.

Of all the parasitic helminth infections of man, urinary Schistosomiasis remains the most important health problems in the world, despite efforts made towards its control (WHO, 1985). Record of this disease showed that its health and socioeconomic impact is only outstripped by malaria. Over 200 million people in tropical and subtropical regions are infected by the disease. Seventy four countries in Africa, Middle East, India, South and Central America are endemic for Schistosomiasis (WHO 1985). They reported that over 38 million people are infected in sixteen African countries with Nigeria having a very high endemicity. Consequent on this, the Nigerian Government in September 1987 set up a "National Schistosomiasis control programme" with mandate to free Nigeria of Schistosomiasis by the year 2010. She empowered the health sector of the economy to retarget her health

priority to preventive health services such as the Schistosomiasis prevalence survey workshop.

Transmission patterns in Schistosomiasis have been studied using infection in snails as the intermediate host of *S. haematobium* especially the *Bulinus* species (Okafor, 1990, Emejulu *et al.*, 1992).

Various physical and chemical factors collectively have an effect on the abundance of snails under natural conditions. Okafor, (1990) explained how rainfall affects the quality of the habitat making it suitable or unsuitable for the snail with time. According to him, the absence of snails in most flowing water habitat during the heavy rains may be attributed to flooding which increases the volume and speed of water. In addition, *Bulinus* species in Nigeria exhibit a preference for stagnant or slow moving waters and thus common inhabitants of streams and irrigation systems (Okafor, 1990).

The focus of this research was to determine the epidemiology of this infection within Ozuitem local population in Bende LGA.

The information so obtained may provide the basis for the developments of cost-

effective control measures of the disease in the study area.

MATERIALS AND METHOD

The Study Area: The study area is a rural town (Ozuitem) in Bende LGA of Abia State. The area is located in the Northern part of the state. Nine villages were used for the study. The area is a rural settlement with farmers and few civil servants. There is no pipe borne water, electricity and good social infrastructures in the area. The main source of water is provided by the streams which are stagnant or slow moving within rice plantations, thus the village have unlimited use of their streams.

Collection of Samples: Labeled specimen bottles were given to participants for urine collection. Prior to the collection, name, age, sex and occupation of each randomly selected sampling individual was recorded. The participants were advised to collect their samples between 9.00 am and 2.00pm on the said day. Okafor (1990) reported urine collection in this time to be rich in ova of *S. haematobium*. Sample bottles were retrieved, taken to the laboratory and examined for ova in urine using the centrifuge and filtration technique (Mott *et al* 1989). Some samples that were not analyzed on that day were preserved in the laboratory refrigerator till the next day. 10ml of the samples is poured each into the test tube and spined in the centrifuge for 5 minutes at 1000 rpm. 5 drops of the sediments were poured onto a microslides covered with coverslides and observed under the electronic microscope for the ova of *Schistosoma haematobium*. The colour of the urine samples were examined and the mean egg count was calculated.

Malacological Survey: The Ozuitem area has many streams which were jointly used by the different villages in the area. The streams visited and samples were Uhu, Iyintagbo, Iyiagu, Idei, Iyidei, Uchiyi and Idei Uzomba. Snails were searched using long handle rectangular scoop net. The net was lowered into water and then a scoop was taken towards the bank, collecting emergent vegetation for snail search. After the sampling, snails collected were taken to the laboratory where they were

washed, sorted out identified and placed according to their source using the format of Okafor (1990). They were checked for cercariae after exposure to bright illumination for 2 hours in specimen tubes. This was repeated for 3 days. After each days exposure, the snails were taken back into a glass aquarium put in the dark and fed with crushed lettuce leaves and the water changed at intervals. Snails which did not shed any human type *Schistosoma bifid* cercariae within the interval of exposure were labeled as not being infected.

RESULTS AND DISCUSSION

During the study on the prevalence of *S. haematobium* infection in Ozuitem, a total of 1173 persons selected randomly from the communities and their primary schools were examined, of these 496% persons were infected giving a prevalence rate of 42.3%. This result is fairly high which corresponds with some studies in some eastern regions of Nigeria. Udonsi (1990) around Igwun River Basin recorded a prevalence rate of 30%, Anigbo and Nworgu (1990) working in Amagunze Enugu state recorded a total prevalence of 48.4% and Emejulu *et al* (1992) working around Agulu lake area recorded a prevalence rate which ranged from 5.96 % to 54.00 % among towns around the Agulu lake. Some other studies in Northern areas of Nigeria recorded fairly lower prevalence rates. Akogun (1986) recorded 39.05 % in Gamau district in Bauchi state, in Toro LGA of Bauchi State Anosike *et al* (1992) reported 25.4 % prevalence of patent and clinical severe infection with *S. haematobium* and noted that

Table 1: Prevalence of *S. Haematobium* in the various villages in Bende

S/no	Villages	Number examined	Number infected	Percentage infection
1.	Umuameri	129	54	41.9
2.	Ndianya	72	28	38.9
3.	Amaeke	87	41	47.1
4.	Ofiaru	60	28	46.7
5.	Umuokube	201	90	44.8
6.	Eluama	84	32	38.1
7.	Isiegbu	342	173	50.6
8.	Amankwo	105	29	27.6
9.	Isiori	63	21	33.3
	Total	1173	496	42.3

Table 2: Sex and age related intensity of *S. haematobium* infection in Bende

Age group	Number examined	Males Number infected (%)	Mean egg / 10 ml urine	Number examined	Females Number infected (%)	Mean egg / 10 ml urine
0-10	174	87(50.0)	68.8	111	33 (29.7)	66.0
11-20	270	150(55.5)	94.0	114	78 (68.4)	64.1
21-30	24	9(37.5)	60.0	39	15 (38.5)	60.0
31-40	96	42(43.8)	69.0	33	9 (27.3)	45.0
41-50	105	25(23.8)	30.0	45	12 (26.7)	35.0
51-60	45	15(33.3)	23.5	48	15 (31.3)	30.0
60+	21	5(23.8)	15.0	48	7 (14.6)	16.0
Total	735	333(45.3)		438	163 (37.2)	

visible haematuria was their predominant presenting symptom. Awogun (1990) at Ilorin (western Nigeria) recorded a prevalence of 23 %. A chi-square analysis showed that the prevalence of *S. haematobium* was significantly different among the various villages sampled ($P < 0.05$). Details are shown in Table 1.

The prevalence of urinary Schistosomiasis according to age and gender is shown in table 2. Of the 735 males and 438 females examined 333(45.3 %) males and 163 (37.2 %) females were infected. Although more males than females were infected, there was no significant variation among sexes ($P > 0.05$) as was reported by Emejulu *et al* (1992) that sex did not play a significant role in prevalence and intensity of infection rather host age. Prevalence rate was significantly higher in persons within 0 – 20 years than in those 21 years and above ($P > 0.05$). This is because of this age groups frequent contact with water as in swimming. Though it is also fairly high among 21 – 30 age group and 31 – 40 males because this people are mostly the farmers who work more in the rice plantations. The sex-age related intensity analysis in Table 2 showed that intensity of infection was found to be statistically independent of the sex. The mean egg count / 10 ml urine sample increased within

0 – 40 years and decreased within 41 – 60⁺ in the males while in females, it decreased as the years increased, Table 2.

Identification of the snails collected from various water bodies showed five snail species; they include *Bulinus globosus*, *B. forskalii*, *B. truncatus*, *Lymnaea natalensis* and *melanoides tuberculata*. On the whole 395 snails were collected, and 327 were identified to be *B. globosus* which were the only species found to be infected. Table 3 shows the distribution of *B. globosus* in different freshwater system in the area. Of the 327 *B. globosus* collected, 69(21.1 %) were infected that is shedded cercariae.

CONCLUSION

Studies in Bende LGA revealed that persons within 0 – 20 age group are important in the spread of this disease as they perform water related activities such as swimming though age groups within 21 – 40 who work in the farms go to bath in the streams after the days work.

The relative abundance of *Bulinus* species in the stagnant water could be attributed to better adaptability of *Bulinus* species to local ecological factors in the stagnant water. Thus, less use of stagnant water bodies is recommended and Government should install pipe borne water in this area.

Table 3: Distribution of *B. globosus* in various fresh water systems in Bende

Type of Habitat	Name of Habitat	Number of Snail collected	Number of snail infected	Infection rate
Flowing habitat	Iyintagbo	19	0	0
	Iyiagu	11	0	0
	Idei	4	0	0
Stagnant habitat	Iyidei	108	37	34.3
	Uchiyi	86	14	16.3
	Idei	99	18	18.2
	uzomba			
	Total	327	69	21.1

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EMBRYONIC DEVELOPMENT IN *Clarias gariepinus* (BUCHELL, 1822) UNDER LABORATORY CONDITIONS

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ABSTRACT

The embryonic development in Clarias gariepinus was studied under laboratory conditions. The development stages of eggs starting from first cleavage to hatching were examined microscopically. The accurate timing and detailed description of each stage were recorded. Photomicrograph of important stages, segmentation, blastulation, differentiation of embryo and hatching, was taken. The result shows that the blastodisc (polar cap) appeared 35±1 minutes after fertilization. The first cleavage dividing the blastodisc into two blastomeres occurred 15±0.5 minutes after the polar cap formation. The larva emerged from the egg case 22 hours after fertilization at a water temperature of 25.1±1.5 °C. This result will assist in better management of C. gariepinus, enhance their survival to fry and increase the supply of fingerlings in Nigeria.

Keywords: Embryonic development, *Clarias gariepinus*

INTRODUCTION

Knowledge of fish development process is important because of its role in life history studies and in fish culture practice. Sule and Adikwu (1999) reported that embryology unfold many features of evolutionary relationship. Much of the details of *C. gariepinus* embryology remain yet to be fully understood in spite of the fact that *C. gariepinus* is a better animal model for developmental and embryological studies. The merits include lack of pigment, ova transparency and convenient size. These facilitate observation of change in organs and tissues under the microscope (Sule, 2001).

A generalized account of embryonic development in fish are based largely on the works of (Battle, 1944; Carr, 1942) who indicated that in *Clarias anguilaris* the first cleavage of the fertilized eggs occurs 22 minutes after fertilization.

Despite the growing interest in the culture of African catfishes, the early life cycle of the group has not been thorough investigated for *Clarias gariepinus*.

Detailed embryological development of *Heterobranchus longifilis* has been reported (Olufeagba, 1999) and in *Clarias anguilaris* by Aluko, (1994). Detailed information on ontogeny

of *Lucania parva* has been reported by Crawford and Balon (1994) and in *Tilapia zilli* by Omotosho (1989). In all these, continuous process of development was reported from the fertilized egg to hatching and free swimming stage.

The aim of this study was to investigate the embryonic development in *Clarias gariepinus* under laboratory conditions.

MATERIALS AND METHODS

Breeders of *Clarias gariepinus* were obtained locally from Lake Alau, Maiduguri. The males have an average weight of 450.0±0.3g with females having an average weight of 350.0±0.8g. The females were injected with ovaprim (Gonadotropic hormone) at 0.5 ml per kilogram of fish body weight after 10 hours latency period; the females were stripped by applying slight pressure on the abdomen. This led to running out of the eggs which were collected in a plastic container. The males were sacrificed to expose the testes which were removed and squeezed to let out the milt for fertilizing the eggs. Dry fertilization was carried out by mixing the milt and the eggs in a plastic bowl using feather. Measurements of diameter

of the egg were made to the nearest 0.01 mm using a microscope with micrometer.

Fertilized eggs were removed from the container and put in well aerated water in 60 x 30 x 30 cm³ glass aquaria at a temperature of 25.1±1°C. Monitoring of the development stage of the fertilized eggs immediately after fertilization until the free – swimming stage was carried out.

Fifty fertilized eggs were removed randomly from incubating tank into a Petri dish in 5 batches of 10 eggs per batch. Selected eggs were viewed under the photomicroscope immediately after fertilization and at 5 minutes intervals for the first 3 hours, later at an hourly and two hourly intervals until hatching. The development stages of eggs starting from first cleavage to hatching were examined microscopically at a magnification of x 1000 for 22 hours.

Photomicrograph was used to take important stages of segmentation, blastulation, differentiation of embryo and hatching. The film of the photograph was developed and prints of each stage produced. The accurate timing and detailed description of each was done.

The newly hatched larvae were reared for 21 days in indoor aquaria. The fish larvae were fed with zooplankton mostly monia harvested from fertilized ponds. They were later transferred to the outdoor tanks (2 x 2 x 1.m³) and reared until they attained mean weight of 5.0 g.

RESULTS

Figure 1 shows the visible embryogenetic chronology of *Clarias gariepinus* immediately after fertilization up to the commencement of free-swimming of larva. The stages of embryonic development and time after fertilization are explained in table 1.

Fertilized Eggs: The fertilized eggs were brownish in colour spherical and adhesive. The mean egg size before fertilization was 0.65±0.02 mm and 1.01±0.19 mm after fertilization (Figure 1a)

Embryonic Development (Cleavage): The blastodisc (polar cap) appeared about 35 minutes after fertilization (Figure 1b) and the first cleavage dividing the blastodisc into two blastomeres occurred 15 minutes after polar cap formation (Figure 1c), The second cleavage perpendicular to the first, followed within 55 minutes after fertilization (Figure 1d). The eight

cell stage was reached later in 10 minutes (Figure 1c). The fourth cleavage, which is parallel to the second one occurred 2 hours 5 minutes after fertilization and the 16 – cell stages was obtained (Figure 1f). The 32 cell stage followed by the sixth cleavage in another 4 minutes later. The Morula followed in 2 hours 9 minutes after fertilization (Figure 1g) Blastula was reached in 1 hour 3 minutes later (3 hours 17 minutes after fertilization) It was observed that as successive cleavage occurred the blastomeres decreased in size.

Formation of Embryo: The blastoderm cells started spreading over the yolk mass 6 hours 10 minutes after fertilization. Yolk mass invasion progressed considerably 6 hours 30 minutes after fertilization. The eggs reached the late gastrula stage and yolk mass invasion was completed. Gastrulation was completed (Figure 1f) after another 40 minutes.

Differentiation of Embryo: Early stages of somite formation started 7 hours 10 minutes after fertilization. Advanced form of somite formation was observed 13 hours later. Head and tail were clearly differentiated. 15 somites were counted while the embryo became C – shaped. In another 1 hour, myotomes appeared, the embryo developed further and looked like a girdle over the yolk mass (Figure 1i). The embryo elongated and the tail became separate from the yolk mass. In another 1 hour 30 minutes, the head separated completely from the yolk mass and the heart beat was noticed. The tail further elongated, while the embryo developed further and movements of the body could be observed approximately 20 hours after fertilization. In another 2 hours later, the yolk mass further reduced and the tip of the tail extended nearer to the head. 21 hours after fertilization, the embryo was fully differentiated and was about to hatch.

Hatching: The elongated tip of the tail struck against the head end of the egg shell causing the later to break, the head came out first. The larva shook off the shell and emerged completely from the egg case at about 22 hours after fertilization at a water temperature of 25.1°C (Figure 1j).

Larval Development: The newly emerged larva had unpigmented eyes and no fin buds, the mouth is not formed and the anus situated posterior to yolk mass. The yolk sac projected anteriorly near end of the larva.

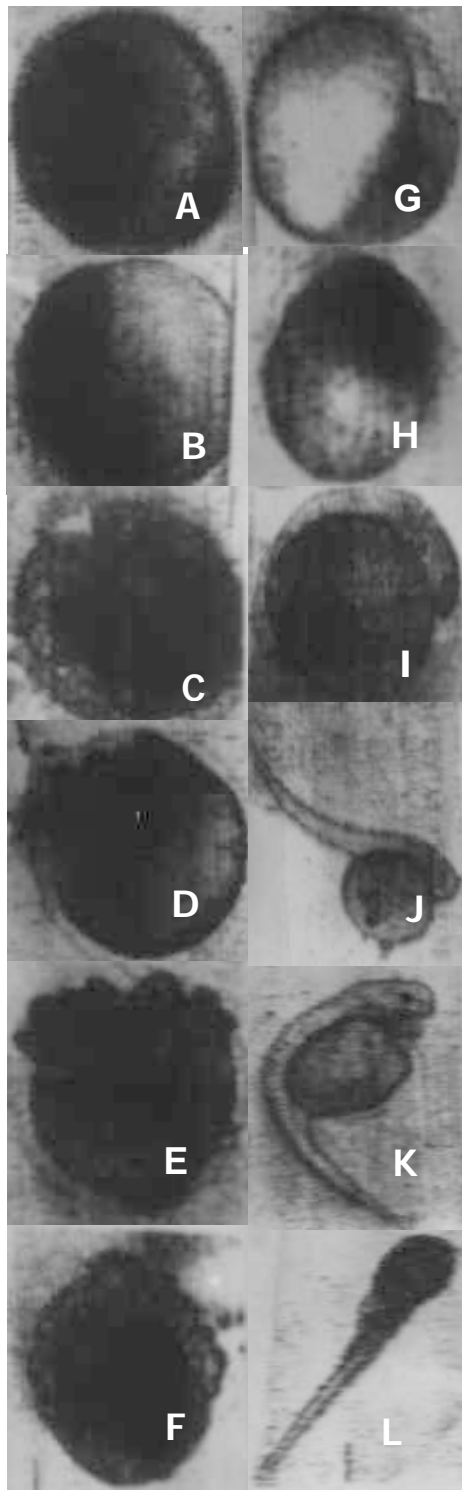


Figure 1: Stages in embryonic development in *Clarias gariepinus* under laboratory condition ($27 \pm 1.5^\circ\text{C}$). A = Fertilized egg, B = Animal and vegetal pole, C = Two cells at first cleavage, D = Four blastomeres, E = Eight blastomeres, F = 16-32 cell stage, G = Morula/Blastula, H = Gastrulation, I = Advanced stage of somite formation, J = Hatchling (Day1), K = Hatchling (Day 2) and L = Hatchling (Day 3).

Black pigmented cell were noticed in the fin fold except the tip of the caudal region. Pigments were also scattered in the yolk mass and on the head and body of the larva. The larva swims slowly up to the surface of the water and then gradually drops down and remains suspended in the column of water in an oblique position with head down in the Petri dish in an inclined posture (Figure 1k).

The day old larva had yolk sac that was considerably reduced. Faint pigmentation of the eyes was observed to have commenced. The hind gut was clearly visible. Melanophores were scattered on the head and trunk and also on the dorsal fin fold.

The yolk sacs of the two day old larva were very much reduced. The eyes were fully pigmented and pectoral fins elongated. The mouth was fully formed and the oesophagus was distinctly visible. There was pigmentation on the dorsal part of the body which was denser on the caudal peduncle region. There were a few melanophores over the posterior portion of the gut. The remnant of the yolk sac was observed as a small streak. The mouth was opened and the intestine was fully formed. The optic lens was shiny. The fry made slow directed movements with occasional jerks and moved on the surface as well as at the bottom of the water column (Figure 1l).

DISCUSSION

The embryology of *Clarias gariepinus* is similar to that of fishes like *Clarias anguilaris* (Kamler *et al.*, 1994) *Clarias macrocephalus* (Mollah and Tan, 1983). The completion of cleavage and hatching within 22 hours at 25.1°C agrees with the reports of Freund *et al.* (1995), for *Heterobranchus longifilis* and Kamler *et al.* (1994) for *Clarias gariepinus*.

The early development of the optic vesicles is an indication that the eye was functional before the larva started active swimming. This will also help the larva to detect and identify their food well in advance of mouth formation and start of exogenous feeding. Similar observation was made in *Sarotherodon niloticus* (Omotosho, 1989). Head and blood vessel formation at twenty hours after fertilization suggest their functional significance as systems were laid ahead of time as embryo increase in size to allow for proper nutrient circulation in the embryo. The rate of heart beat continue to increase as the embryo matures, there is also an increase in somatic contraction and swimming activities noticed is in agreement with

Table 1: Embryonic development and time after fertilization in *Clarias gariepinus* under laboratory condition

No	Stages of development	Time after fertilization	Description
	Unfertilized egg	0 min.	The unfertilized egg of <i>C. gariepinus</i> with a mean diameter of 0.65 ± 0.02 mm
A.	Fertilized egg	0 min.	The fertilized eggs expand few seconds after fertilization with a mean diameter of 1.01 ± 0.19 s.
B.	Animal and Vegetal pole (Blastodisc)	35 min.	Expansion of the yolk away from the membrane and accumulation of cytoplasm at the anterior to form animal and vegetal pole.
C.	2 – cell stage	40 min.	This was observed as a vertical division of the animal pole producing two cells of equal sizes
D.	4 – cell stage	55 min.	Second line of division was perpendicular to the first line producing 4 cells which were still of equal sizes.
E.	8 – cell stage	1 hr. 5 mins.	Cells were seen as heaps on top of the “round yolk”.
F.	16 – cells stage 32 – cell stage	2 hrs. 5 mins.	Cells were seen as irregular in size and could be difficult to count. Further division of cells. Some cells tend to lie on another cell. Many further division producing many cells, irregular in size.
G.	Morula stage Blastula stage	2 hrs 9 mins. 3 hrs. 17 mins	Further division of cells produce many more but the morula size decreased. Further division producing mass of cell elevated over the general outline of the yolk mass (like a dome-shaped)
H.	Gastrulation stage	7 hrs 10 mins	Embryo develops germ rings. Cephalic and caudal edges which were formed at advanced stages of the blastula.
I.	First wriggling movement	21 hrs	The long somite started from both sides within the chorion wall. It started with 1 movement in 25 seconds, but this rate gradually increased with time.
J.	Hatchling	22 hrs.	Violent movement of tail to either side against the chorion wall, followed by contraction. As a result of which the chorion wall breaks and hatching occurred

the report of Freund *et al.* (1995) for *H. longifilis*. The structured appendages for swimming like fin rays and the skeletal structure were developed in preparation of the larva entry into the swimming stage.

Apart from sex organs all the organs and system of the fish were already formed in the fry by the time of final yolk absorption. Time of yolk absorption is very significant as exogenous feeding must commence forty eight hours after hatching that is, before final yolk absorption to enable the fry to get used to natural food. This practice has been found to reduce high 4th day mortality noticed in routine

hatchery management due to complete yolk absorption (Madu, 1989).

Kamler *et al.* (1994) reported observed temperature induced changes of early development and yolk utilization in the African catfish, *Clarias gariepinus* and *C. macrocephalus*. Generally, low temperature slows down the rate of embryo development. Egg to fry survival in the sea trout has been reported by Rubin and Glimsater (1996). They identified four critical phases, which are spawning phase, incubation phase, and emergence of the alevin phase and growing of the fry.

This study on embryonic development of *C. gariepinus* has bridged a gap in the knowledge of developing eggs and larvae morphology. Furthermore, useful information is provided for routine fish hatchery operators. This will allow for better management of fertilized egg to fry stages for higher survival and increased *C. gariepinus* fingerlings supply in Nigeria.

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THE EFFECT OF ACTELIC 25 EC ON MINERAL COMPOSITION ON CURED FRESH WATER FISH: *Heterobranchus longifilis*, *Heterotis niloticus* AND *Chrysichthys nigrodigitatus*

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ABSTRACT

A study to evaluate the effects of the preservative, Actellic 25 EC solution, on the mineral composition of the three freshwater fish species was carried out. Pieces of fish (samples) were analysed for mineral composition before and after traditional smoke drying and smoke drying after Actellic treatment. The investigations were carried out using fresh water fish, Heterobranchus longifilis, Heterotis niloticus and Chrysichthys nigrodigitatus. The result showed that Actellic 0.03 % solution greatly reduced the sodium content of the smoked dried fish species. Furthermore, Actellic 25 EC eroded the magnesium (Mg) content of C. nigrodigitatus and also reduced slightly the naturally high iron content of H. longifilis, H. niloticus and C. nigrodigitatus. The implications of these results are discussed.

Keywords: Actellic, Mineral, Freshwater fish

INTRODUCTION

The amino acid composition of fish protein compares favorably with the amino acid composition of milk, meat and egg. Some of the essential amino acids like lysine and methionine which are lacking in tuber-based or cereal-based diets are present at higher levels in fish than in meat (Borgstrom, 1962). This makes fish protein highly valuable in many developing countries like Nigeria where the staple diet consists of starchy foods like cassava, yam, potatoes, rice, sorghum and millet. The lack of sufficient live stock and fresh meat products in Southern part of Nigeria, makes fish production all the more essential (Ikeme, 1986; Nwuba, 1996 and Latunde-Dada, 1999).

Besides essential amino acids and proteins, fish, also contains fats, oils, carbohydrates and fibre. Fish is also a very good source of essential vitamins and minerals (Nwuba, 1996; Nwuba, 2002; Pearson 1976; Onukuozor, 1996). Nwuba (2002) has reported minerals in freshwater fish to include, phosphorus, magnesium, sodium, potassium and iron. This study focuses on the extent of buildup or erosion of these mineral elements by Actellic 25 EC solution.

Furthermore, the objective of this study is to note such effects and alert food scientists, nutritionists, dieticians, scientists, medical personnels and the fishers. The food scientist when equipped with such information will now know how to replenish the loss by addition of certain food supplements.

The fishers need be informed of the buildup or erosion of mineral nutrients in fish due to their processing methods through seminars and workshops. This knowledge will help them achieve sustainability in their occupation and reduced disorders/ailments arising from mineral deficiency.

MATERIALS AND METHODS

A total of 12 fresh *Heterobranchus longifilis* (Boulenger) 12 *Heterotis niloticus* (Clupisudis & Cuvier) and 20 *Chrysichthys nigrodigitatus* (Lacepede) were procured from a fish landing site at the bank of river Niger in Onitsha, Nigeria (Mariner" water side) (Figure 1). Each fish species was then cut into 108 pieces of about 96.84 ± 0.12 mg mean weight. This was divided into three treatment batches (I – III) of 36 pieces of each species samples per batch. The treatment of 36 pieces of fish per fish specie was replicated thrice. Fish samples in

batch I served as the control for mineral analyses of the fresh fish specimen. Fish samples in batch II were smoke dried only. While fish samples in batch III were washed thoroughly and submerged in 10 litres of Actellic 25 EC solution for 15 seconds and smoke dried for 6 hours. After which they were sprayed with ½ litre of Actellic 25 EC solution and sun dried for 3 hours. Samples from the three batches were taken and analysed for mineral components. The results from batches I to III were compared to determine the effect of the Actellic 25 EC solution on fish mineral composition.

Preservative: The insecticide solution, 0.03% Actellic 25 EC used in treating the fish samples in batch III was prepared by adding 12.0 ml of Actellic 25 EC to 10 litres of water.

Smoking Oven: A traditional smoke drying mud-oven of the dimension 120 cm x 60 cm x 30 cm (length, width and height) was used in the study. The same smoking oven and smoking process were adopted for fish pieces in batches II and III.

Mineral Assay: The fish samples were first solubilised by the wet-oxidation involving perchloric acid (HClO₄) digestion. The filtered digested solution was used in the determination of minerals (AOAC, 1980).

Phosphorus (P) was determined colorimetrically by ascorbic acid method. The blue colour developed was read at 882 nm using a VP-10-12 spectrophotometer. Sodium (Na) and potassium (K) in the digest were determined using by flame photometry (AOAC, 1980). Iron (Fe) and Zinc (Zn) contents were determined using Atomic Absorption Spectrophotometer (AAS). Magnesium and calcium (Ca) contents were also determined (AOAC, 1980).

RESULT AND DISCUSSION

The mean values of percentage phosphorus, calcium, magnesium, sodium, potassium, iron and zinc contents of fresh, smoke dried and Actellic treated and smoke dried samples of *H. niloticus*, *H. longifilis* and *C. nigrodigitatus* are recorded as in Table 1.

Actellic 0.03 % solution treatment increased the phosphorus content mean value of *C. nigrodigitatus* greatly but slightly decreased phosphorus content of *H. longifilis* and *H. niloticus*. The calcium content was

decreased in *H. longifilis* and *C. nigrodigitatus* but increased in *H. niloticus*. The mean calcium value in this work were 0.123 ± 0.001 % in *H. longifilis*, 0.12 ± 0.002 % in *H. niloticus* and 0.121 ± 0.001 % in *C. nigrodigitatus*. These mean values were not significantly different at $P = 0.05$ and agrees with the values recorded by Nwuba (2002).

The mean values of magnesium contents were 0.046 ± 0.0005 %, 0.023 ± 0.0008 and 0.00 % in *H. longifilis*, *H. niloticus* and *C. nigrodigitatus* respectively. The statistical analyses showed significant difference at $P > 0.05$. The sodium content mean value of *C. nigrodigitatus* was almost eroded completely by Actellic 25 EC treatment. Iron content of cured fish using preservative was reduced when compared with the content in the fresh fish.

The mean value of potassium only increased in *H. longifilis* treated with Actellic. The mean potassium values were reduced in *H. niloticus* and *C. nigrodigitatus* treated with Actellic. The preservative was observed to retain the zinc contents of the three fish species used in this study. Thus confirming the results of Nwuba (2002).

In general, nutrient changes in food occur at several stages during harvesting, preservation, processing, distribution and storage. Heat processing does result in some losses of nutrients, particularly labile nutrients such as ascorbic acid. But modern heat processing equipment and techniques can minimize such losses. All heat treatments should be optimized for nutrient and quality retentions and microbial destruction (Uwaegbute and Ikeme, 1988).

Conclusion and Recommendation: The major discoveries in this study showed that:

- Actellic treatment eroded the magnesium content of *C. nigrodigitatus* by 100 %.
- Sodium content of *C. nigrodigitatus* was also eroded by approximately 98%.
- The mean value of iron content was decreased by approximately 99 %, in *C. nigrodigitatus*. Actellic treatment greatly increased the potassium content of *H. longifilis*.

In the light of the above information, it would appear that Actellic should be avoided in the preservation treatment of the fish *C. nigrodigitatus*. But if it happens to be the only available preservation, necessary steps could be

Table 1: Percentage mineral content of three fresh water fish species smoked dried after Actellic 25 EC dehydration treatment

	Fresh Fish			Smoke Dried Fish			Actellic Treated and Smoked Dried Fish		
	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>
Phosphorus									
Range	0.044-0.005	0.059-0.064	0.065-0.068	0.033-0.39	0.048-0.05	0.055-0.057	0.022 – 0.024	0.05 – 0.055	0.06 – 0.616
Mean	0.047	0.061	0.036	0.036	0.049	0.066	0.23	0.52	0.154
SE	0.0011	0.0008	0.0011	0.0011	0.004	0.0004	0.0004	0.009	0.092
Calcium									
Range	0.25-0.29	0.035-0.05	0.2-0.25	0.22.25	0.05-0.06	0.07-0.09	0.12 – 0.126	0.11 – 0.125	0.119 – 0.124
Mean	0.272	0.043	0.22	0.233	0.053	0.083	0.123	0.12	0.0121
SE	0.0060	0.0021	0.0093	0.0042	0.0021	0.0032	0.0010	0.0022	0.001
Magnesium									
Range	0.094-0.097	0.552-0.0576	0.045-0.048	0.081-0.088	0.05-0.522	0.034-0.04	0.045 – 0.048	0.021 – 0.026	0.00
Mean	0.096	0.558	0.048	0.086	0.512	0.037	0.046	0.23	0.00
SE	0.0005	0.0037	0.0011	0.0011	0.0029	0.0010	0.0005	0.0006	0.00
Sodium									
Range	0.0021-0.0023	0.0018-0.003	0.0018-0.0024	0.0018-0.0024	0.0014-0.0015	0.042-0.0048	0.0021– 0.00023	0.0013 – 0.0015	0.00010– 0.00012
Mean	0.0022	0.0022	0.0051	0.0021	0.0014	0.0045	0.0022	0.0014	0.00011
SE	0.65E-05	0.0002	0.0001	9.55E-05	2.5E-05	8.33E-05	3.33E-05	4.94E-05	3.33E-06
Potassium									
Range	0.01111-0.011	0.0078-0.077	0.0102-0.0105	0.011-0.013	0.0077-0.008	0.0103-0.0104	0.125 – 0.44	0.0045 – 0.0047	0.0012 – 0.0014
Mean	0.011	0.030	0.0104	0.0118	0.0079	0.0103	0.13	0.0046	0.0013
SE	4.77E-05	0.014	4.47E-05	0.004	4.94E-04	1.54E-04	0.0022	4.01E-05	2.17E-05
Iron									
Range(x 10 ⁵)	25.28	27.29	530-550	25-28	26.28	430-450	31 – 33	43 – 45	17 – 19
Mean (x 10 ⁵)	27	28	540	27	27	440	32	44	18
SE(x 10 ⁵)	0.583	0.4	3.74	0.51	0.374	40	0.074	0.316	0.447
Zinc									
Range(x 10 ⁵)	4.9-55	45.55	4.8-5	2.8-55	3.3-4.4	5-5.5	5.5 – 5.6	5.0 – 5.5	5.5 – 6.0
Mean(x 10 ⁵)	5.2	5.04	4.96	5.06	40	6.18	5.54	5.26	5.74
SE(x 10 ⁵)	0.114	0.199	0.04	0.117	0.182	0.111	0.245	0.108	0.08

taken to replenish the loss of mineral nutrients in diets cooked with *C. nigrodigitatus*.

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EFFECTS OF AQUEOUS LEAF EXTRACT OF *VERNONIA AMYGDALINA* ON BLOOD GLUCOSE AND TRIGLYCERIDE LEVELS OF ALLOXAN-INDUCED DIABETIC RATS (*Rattus rattus*)

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ABSTRACT

The effect of Vernonia amygdalina aqueous leaf extract on serum glucose and triglyceride level of diabetic rats were investigated. The aqueous extract was administered to alloxan-diabetic rats. The blood glucose and serum triglyceride levels were estimated at time intervals post oral administration of the extract (80 mg/kg). The extract caused significant ($P < 0.05$) and progressive time dependent reduction of blood glucose and serum triglyceride levels in both normoglycaemic and alloxan-induced diabetic rats, with similar time course of action. In conclusion, the significant reduction in blood glucose and serum triglyceride level observed in this study may help in alleviating some of the complications associated with diabetic conditions.

Keywords: *Vernonia amygdalina*, Leaf extract, Blood glucose, Serum triglyceride, Diabetic rats

INTRODUCTION

Differences in the lipid profile between diabetic and non-diabetic individuals are now apparent (Garg and Grundy, 1990; Siegel *et al.*, 1996) and lipid abnormalities are common in patients with diabetes mellitus (Siegel *et al.*, 1996). Dyslipidaemia has been identified as one of the major risk factors for macrovascular complications in diabetes mellitus (Stamler *et al.*, 1984; Kannel, 1985; Stern and Haffner, 1991; ADACS, 1993). In Non-Insulin Dependent Diabetes Mellitus (NIDDM) the most common form of dyslipidaemia results in elevated plasma triglyceride levels, high low density lipoprotein cholesterol (LDL-C) total cholesterol and low high-density lipoprotein cholesterol (HDL-C) (Isseb *et al.*, 1996). The prevalence of dyslipidaemia in NIDDM varies among different populations (Stern *et al.* 1989, Garg and Grundy 1990). Elevated serum lipids are associated with a higher risk of coronary heart disease (CHD) for patients with diabetes as they are for non-diabetes (Lehto *et al.*, 1997). In addition hyperlipidaemia has been implicated in the progression of glomerulosclerosis and renal insufficiency (Stevenson and Kaysen, 1999; Philip *et al.*, 1999). There is a higher mortality

rate in diabetics with hyperlipidaemia than those with hypolipidaemia. A significant association between hyperlipidaemia and poor glycaemic control has been reported (Akbar 2001). Coronary heart disease morbidity is about four times higher in diabetics than in non-diabetics, and the mortality rate from CHD is up to 100 % higher in diabetic patients than in non-diabetics (Lehto *et al.*, 1997). There is evidence that cardiovascular complication rate associated with diabetes can be reduced considerably through adequate treatment of hyperlipidaemia (Pyorala *et al.*, 1997; Golderg *et al.*, 1998). Therefore, adequate treatment of diabetes dyslipidaemia through diet and weight control is critical in reducing these complications.

Vernonia amygdalina, Del (bitter leaf) is a common medium sized shrub with abundant bitter principle in every part of the plant. It is a widely used local plant in Nigeria for both therapeutic and nutritional purposes, where it serves as the main ingredient in 'bitter leaf soup'. Fresh extract of the leaf has been reported to contain alkaloids, saponins, tannins, flavonoids and proteins (Akah and Okafor 1992), as well as vitamins and minerals (Fafunso and Basir, 1977).

In addition to its numerous uses, as in malaria and stomach disorders (Dalziel, 1937; Bever, 1960), the leaf decoction of the plant is popular in traditional medicine as an antidiabetic remedy; the potency and safety of which has been documented (Iwu, 1980; Akah and Okafor, 1992; Akah *et al.*, 2002). The aim of this study was to determine the effect of *Vernonia amygdalina* Del. (Compositae) aqueous leaf extract on triglyceride level of diabetic rats and to relate this to its anti diabetic effect.

MATERIALS AND METHODS

Plant Material: Fresh leaves of *V. amygdalina* were collected in December 2000 from Umuahia, Abia State, Nigeria. Botanical identification was done by a curator, Botany Department, University of Nigeria, Nsukka and voucher specimen deposited in the University Herbarium.

Preparation of the Aqueous Extract: After washing, the leaves were sun-dried for seven days and milled to coarse powder using mortar and pestle. The powder (250 g) was soaked in 500 ml of distilled water. The mixture was allowed to stand for 24 hours with intermittent shaking. Following filtration, the filtrate was freeze-dried to afford a solid residue (48.7 g; 19.5 % yield). The extract was reconstituted in distilled water in appropriate concentration before administration.

Animals: Adult Wistar albino rats (*Rattus rattus*) (180 – 250 g) of either sex inbred and maintained in the Animal Unit of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used in the study. The animals were maintained under standard laboratory conditions and were allowed free access to food (grower mash Guinea Feed) and water before the beginning of the experiment.

Induction of Diabetes Using Alloxan: Normal healthy albino rats were fasted overnight with free access to water prior to and throughout the duration of the experiment. At the end of the fasting period, blood was withdrawn from the media canthus of the eyes by occipital puncture using heparinized capillary tube, and the fasting blood sugar (FBS) level determined using o-toluidine method (Bauer *et al.*, 1974). Serum triglyceride level was also estimated using the method of Onyesom and Atakuo (1998). Rats with FBS and serum

triglyceride between 100 – 130 mg % and 150 – 200 mg % respectively were used. Forty mice were then divided into four groups of 10 rats each. Groups 1 and 2 were the normal (non-diabetic) rats. Groups 3 and 4 were given alloxan monohydrate (120 mg/kg ip), freshly prepared as a 10 % solution in distilled water, (Iwu 1980). The animals were allowed free access to food and water for 7 days. On the 8th day, the animals were fasted for 12 hours prior to the estimation of their blood glucose and serum triglyceride levels determined (0 hr). The treatment groups employed were;

Group 1 Normal (non-diabetic) control,
Group 2 Normal (non-diabetic) treated,
Group 3 Diabetic control
Group 4 Diabetic treated

Groups 2 and 4 were given *V. amygdalina* extract (80 mg/kg) as a single dose, (this dose was previously determined as the optimum anti-diabetic dose (Akah and Okafor, 1992); while groups 1 and 3 received distilled water (2 ml/kg). Blood glucose and serum triglyceride levels were determined for each rat in each group 2, 4, 8, and 24 hours post treatment.

Statistical Analysis: Values are expressed as means (mg %) \pm SEM and two-way ANOVA and F-LSD was employed to test the significance of difference between means at $p = 0.05$.

RESULTS

Effect of the Extract on Blood Glucose Level: In alloxan-diabetic rats (Group 4) *V. amygdalina* extract (80 mg/kg) caused a significant ($P < 0.05$) reduction in blood glucose level (Table 1). Blood sugar was reduced from an initial value of 291.1 ± 9.4 to 194.5 ± 2.0 in 8 hours, i.e. a 33.2 % reduction after 8 hr. The reduction, which became significant by the second hour persisted to the 8th hour. Similar pattern of effect was also observed in non-diabetic animals treated with the extract; thus there was a reduction from 125.4 ± 5.0 to 81.3 ± 6.1 in 8 hours, i.e. 35.6 % reduction after 8 hr.

Effect on Serum Triglyceride Level: The effect of the extract on serum triglyceride is shown in Table 2. The extract caused a prolonged and significant reduction ($P < 0.05$) in serum triglyceride level of diabetic rats. There was about 56 % reduction of serum triglyceride by the 8th hour in diabetic group. In non-diabetic rats, the extract also evinced significant ($P < 0.05$) reduction in serum triglyceride level. There was 29.4 % reduction in serum triglyceride by the 8th hour for the non-diabetic rats.

Table 1: Effect of *V. amygdalina* aqueous leaf extract on blood glucose of diabetic rats

Groups	Dose of extract	Blood glucose (mg %) at hr after treatment ^a					% Reduction after 8 hr
		0 hr	2 hr	4 hr	8 hr	24 hr	
Non-diabetic (control)	DW (2 ml/kg)	123.6±2.4	125.1±1.6	121.8±3.1	126.3±9.5	124.4±7.3	-2.0
Non-diabetic (treated)	80 mg/kg DW	125.4±5.0	114.5±4.4*	100.6±3.0*	81.3±6.1*	103.1±1.5*	35.6
Diabetic (control)	(2 ml/kg)	298.7±6.5	278.1±5.0	281.5±3.2	311.6±7.2	326.5±4.2	-4.3
Diabetic(treated)	80 mg/kg	291.1±9.4	265.0±8.6*	209.5±4.1*	194.5±2.0*	231.6±5.1*	33.2

^a Values are means ± SEM, (n=10), *P < 0.05; DW = distilled water

Table 2: Effect of *V. amygdalina* aqueous leaf extract on serum triglyceride level of diabetic rats

Groups	Dose of extract	Serum triglyceride (mg %) at hr after treatment ^a					% Reduction after 8 hr
		0 hr	2 hr	4 hr	8 hr	24 hr	
Non-diabetic (control)	DW (2 ml/kg)	193.6±2.4	192.8±4.1	195.1±10.6	193.2±7.7	191.4±2.1	0
Non-diabetic (treated)	80 mg/kg DW	192.5±5.1	161.4±4.5*	150.7±5.4*	136.4±2.5*	160.5±1.6*	29.1
Diabetic (control)	(2 ml/kg)	386.9±6.5	407.1±10.2	404.8±5.2	381.7±4.2	403.8±17.8	1.3
Diabetic(treated)	80 mg/kg	355.5±6.0	333.5±5.3	188.3±4.2*	158.0±3.5*	231.6±5.7*	55.6

^a Values are means ± SEM; (n=10), *P < 0.05, DW = distilled water

DISCUSSION

The hypoglycaemic potentials of medicinal plants have been documented (Akah *et al.*, 2002). The result of this study confirms our earlier report (Akah and Okafor, 1992) on the hypoglycaemic effect of the leaf extract of *V. amygdalina* in rabbits. Although several biologically active constituents were reported present in the extract (Fafunso and Basir, 1977; Akah and Okafor, 1992), it was not demonstrated which of the groups of phytochemical were responsible for the effect and the mechanism of action. The prompt and remarkable reduction in blood glucose in both the fasting normal rats and alloxan diabetic rats (with blood sugar levels comparable to total pancreatectomy) point to a mechanism of action different from that of sulphonylureas, and unrelated to insulin secretion from pancreatic β -cells. In diabetes, the causes and sites of intervention in the biochemical process are diverse (Larner 1985), and high serum total triglyceride level has been implicated (Anaja 1995).

It is now widely believed that an important signal for insulin secretion may be the

link between glucose and lipid metabolism; and long-term exposure of islet cells to high levels of fatty acids may result in β -cell dysfunction (lipotoxicity), and diminished glucose-stimulated insulin secretion (Krolewski *et al.*, 1994, Haffner *et al.*, 1998). It has been established that hyperlipidaemia does not only increase the risk of ischaemic heart disease (IHD) in diabetic patients, but also may impair glycaemic control, accelerates the progression of renal insufficiency and increases mortality (Akbar, 2001). Moreover, an alarming proportion of diabetic patients with dyslipidaemia is not aware of the problem and only a small fraction receives lipid-lowering therapy (Isseb *et al.*, 1996). Since dyslipidaemia occurs in most diabetic patients (Isseb *et al.*, 1996), the utilization of lipid-lowering drugs is now advocated for diabetic patients.

In the present study, *V. amygdalina* leaf extract evinced a potent lowering of serum triglyceride level in both normoglycaemic and alloxan-induced diabetic rats. The effect on blood glucose and serum triglyceride level followed the same time course and peaked by the 8th hour. Adequate treatment of diabetes dyslipidaemia through diet is critical in reducing risk and complications, and the role of medicinal plants in the treatment of diabetes is emerging.

A high fiber content that reduces insulin secretion was used in the management of hyperlipidaemia in diabetic patients (Paisey *et al.*, 1984). Furthermore, the effectiveness of the seed extract of *Trigonella foenum*, L, as a cholesterol-lowering agent has been reported (Sharma *et al.*, 1990). The seed was reported to contain soluble fibres, which decrease cholesterol absorption and bile acids reabsorption by disrupting the intraluminal formation of micelles (Sharma *et al.*, 1990). We suspect similar mechanism of action for *V. amygdalina* extract due to its high fibre content. *V. amygdalina* has been reported to decrease weight gain in rats (Ene-Obong and Amadi 1986). Although the mechanism of the weight reduction was not explored, it may be related to its lipid lowering effect as shown in the present investigation. The ability of the extract to reduce both blood glucose and serum triglyceride levels is remarkable. The effect of *V. amygdalina* on serum triglyceride is an added advantage towards effective glycaemic control. *V. amygdalina* is very abundant and relatively cheap, thus recommended as dietary inclusion for diabetics.

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CYTOTOXICITY OF FRACTIONS OF *Pistia stratiotes* L. ON LARVAE OF *Culex* MOSQUITO AND *A. salina*

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ABSTRACT

Crude chloroform and aqueous extracts of the duckweed, Pistia stratiotes L., were bioassayed at various concentrations for larvicidal activities against larvae of Culex mosquito and Artemia salina (brine shrimp). The crude ($LC_{50} = 159.50^{\mu}g/ml$) and chloroform extracts ($LC_{50} = 0.0909^{\mu}g/ml$) exerted mortality at $40^{\mu}g/ml$ of 16.67 % and 90.0 % respectively on Culex mosquito larvae, while the aqueous extract ($LC_{50} = >1000^{\mu}g/ml$) at $200^{\mu}g/ml$, resulted in 3.33 % mortality. The crude ($LC_{50} = 2524.22^{\mu}g/ml$) was moderately toxic on A. salina larvae at $1000^{\mu}g/ml$ which killed 30.00 % of the test organisms. Whereas the chloroform extract showed lower activity on brine shrimp larvae (3.3 % mortality, $LC_{50} > 1000^{\mu}g/ml$). The aqueous extract demonstrated no activity on brine shrimp at all concentrations tested. The study showed that the chloroform extract of P. stratiotes selectively exerts cytotoxic effect on Culex mosquito larvae resulting in high mortality with $LC_{50} = 0.0909^{\mu}g/ml$ than on the brine shrimp larvae at $LC_{50} > 1000^{\mu}g/ml$. It is therefore recommended that these extracts of P. stratiotes L. should be tested for adulticidal and/or mosquitocidal activity as well as toxicity in higher animals up to man. This may yield a more base line data valuable for use in the development of a microbially active chloroform fraction of the plant for possible use in modern medicine.

Keywords: Toxicity, *Pistia stratiotes*, Chloroform fractions, Aqueous fractions, *Artemia salina*, *Culex* mosquito.

INTRODUCTION

Phytochemical constituents analysis of indigenous medicinal plants can assist in identifying those components that have positive therapeutic values. Important also will be the identification of any toxic element or injurious fractions whose use should be actively discouraged (Mahjuba, 1995). The bioassay of plants used as medicine covered a large number of plants (Mchlaughlin, 1996) but still, there are numerous, other plants including *P. stratiotes* that need to be microbiologically and chemically investigated and evaluated thoroughly.

Cytotoxicity is the ability of a compound to kill a cell. Guided by the brine shrimp lethality test, the cytotoxicity values of several plants extracts such as *Xylopiia aromatica*, *Euphobia poisnii*, *Lantana camara*, *Fusarium proliferatum* e.t.c. have been evaluated (Colman and McLaughling, 1994).

Test for cytotoxic effect of plant extracts on mosquito larvae is also currently accepted as additional pharmacognostic step towards elucidating the lethality and/or safety aspects of a candidate plant extract being investigated (Fatope, 1995). Presently however, there has been paucity of literature on cytotoxicity of *P. stratiotes* on insect larvae.

P. stratiotes, referred to as the tropical duck weed, is one of the most dominant aquatic weeds in fresh water, polluted water and streams of Nigeria. The plant is raised in fishponds as a shelter for certain edible shrimp species. Its usage as salad in swine feeds as well as its preference as foliage by buffalos have been reported. However, it has been reported to exert a poisonous effect on rabbits (Mukhtar and Hafiz, 2001).

Herbalist recommends its use as concoction for relieving nervous disorders and fever. It was also reported to have antagonistic effect on intestinal bacteria. While its leaves are

used locally for the treatment of lice (Mukhtar and Huda, 2003).

The various uses of *P. stratiotes* in the treatment of stomach disorder, throat, and mouth inflammation have been documented. The tissues of *P. stratiotes* when in contact with the mucous membrane are exceedingly irritating (Mukhtar and Tukur, 2000). It was reported that ethanol and hot water fractions of the plant exert antimicrobial action on a few pathogenic bacteria. It was also observed that chloroform fraction of the same plant possess antifungal and antibacterial activities on some pathogens (Mukhtar and Huda, 2003).

The objectives of the present study were: (1) to determine whether extract(s) of *P. stratiotes* can be cytotoxic to larvae of mosquitoes and brine shrimps. (2) to assess what extracts of *P. stratiotes* can be reputed as pesticides/insecticides and as safe potential source of antimicrobial agents.

MATERIAL AND METHODS

The Plant Material: Whole plant of *P. stratiotes* L. was collected by hand-picking on 12th August, 2003 at Kofar Naisa ponds along BUK Road and identified in the Department of Biological Sciences, Bayero University, Kano using the keys after Arber (1972).

Extraction: The leaves of the plant material were air dried and ground into powder using pestle and mortar. 200g of the powder was percolated with 4 litres of 95% ethanol for 2 weeks. The percolate was filtered and the solvent evaporated using a rotary evaporator at 40°C (Fatope et al, 1993). The residue (F0₁) was utilized as the crude extract for the subsequent steps.

Fractionation of the Crude Extract (F0₁): The crude extract was dissolved in 200ml water and Trichloroform CHCl₃ in the ratio of 1:1 mixture, shaken for about 15 minutes, and left to stand overnight. The mixture was partitioned into distinct water – soluble layer, which was drained and labeled (F0₂), and the chloroform soluble layer (F0₃). These were all evaporated and preserved (Fatope et al, 1993).

Brine Shrimp Lethality Bioassay: A portion of instant sea water was poured into a hatching chamber and charged with about 250 *A. salina* shrimp eggs. These were allowed to hatch and mature for two days (Fatope et al, 1993).

The vials for testing were prepared and tested initially at 10,000 µg/ml. Subsequently, a concentration of 1000 µg/ml and 100 µg/ml were prepared for each fraction respectively. Three vials for each concentration were prepared for a total of 6 vials per fraction plus a control. The solvent was evaporated at room temperature, overnight. The vials for the control contain non-of the plant extracts. 2 drops of DMSO (Dimethyl Sulphuroxide) were added to each vial plus 4 ml of the sea water followed by addition of 10 shrimps. The volume of the liquid in each vial was adjusted to 5ml with instant sea water. After 24 hours, the numbers of survivors were counted and the LC₅₀ was determined at 95% confidence interval using regression analysis (Arias and Mulla, 1975).

Collection and Rearing of Culex Mosquitoes:

The eggs of *Culex* mosquitoes were identified by their appearance as they always fastened together vertically in batches of about 100 – 300 forming raft like structure which can float (Sarosini, et al 1979). These were collected by scooping from gutters around the Bayero University, Kano (Old campus). The eggs were placed in a container of sterile water to which 0.3g/L of ascorbic acid have previously been added in order to create a low oxygen tension required to facilitate rapid and simultaneous egg hatching. The larvae were harvested and transferred to several beakers of sterile water to which a few grains of baker's yeast were daily added. Every 2 - 3 days a Pasteur pipette was used to suck, so as to remove the faecal and dead matter as well as changing the water (Arias and Mulla, 1975).

As the larvae turn to pupae, it was removed and placed in fresh beakers of sterile tap water and transferred into 'mosquitoes', which is in a laboratory fume chamber covered with net to prevent flying adults from escaping or stray mosquitoes entering. The mosquitoes, prior to pupal introduction were sterilized by subjection to perpetual ultra-violet radiation for 48 hours, in addition to thorough cleansing with 'Dettol' disinfectant. Within a day or two, the pupae hatch out into imagoes that were fed with glucose solution.

A mouse (for blood meal) was placed in the mosquitoery and left to stand overnight. After successful mating some females proceeded to lay eggs in containers of sterile water.

The containers were daily examined and any batch of eggs laid were immediately transferred to fresh beakers of water containing a little amount of ascorbic acid to stimulate egg

hatching. Emergent larvae were extracted, placed in fresh beakers of water and fed with baker's yeast. The larvae were daily examined and the first instar larvae were harvested for bioassay (Gerberg, 1970).

Preparation of Concentrations of the Plants Extracts for the Test: Four solutions for each of the different fractions were prepared such that a final concentration of 400, 200, 40 and 0 $\mu\text{g/ml}$ in distilled water was obtained. 10 larvae were then added in each case. 0.0 $\mu\text{g/ml}$ of test sample served as control. Test at each dosage was carried out in triplicates at room temperature. After a period of 24 hours, the survivors were counted and the percentage mortality at each dosage was determined. The LC_{50} was determined at 95% confidence interval (Fatope *et al*, 1993).

RESULTS

Some physical parameters (weight, pH, colour and transparency) of crude, chloroform and aqueous extracts of *P. stratiotes* are presented in Table 1.

Table 1: Some physical parameters of residues recovered from chloroform and aqueous extracts of *P. stratiotes*

S/N	Fraction	Weight (g)	pH	Colour
1	Crude	5.11	6.85	Dirty green
2	Chloroform	4.10	7.0	Dirty green
3	Aqueous	3.90	5.6	orange

Toxicity of the crude, chloroform and aqueous extracts on *Culex* larvae at various concentrations respectively were shown in Tables 2 - 4. The crude extract (LC_{50} , 159.5 $\mu\text{g/ml}$) exerted larvicidal activity on *Culex* which is directly proportional to the concentration of the extract. For example, mortality rate was 16.6% at concentration 40 $\mu\text{g/ml}$. 50% died after exposure to 200 $\mu\text{g/ml}$ and it was 80% mortality when the larvae were exposed to 400 $\mu\text{g/ml}$ (Table 2).

The larvicidal effect of the chloroform extract on the *Culex* larvae was high with 96.6% mortality rate at 400 $\mu\text{g/ml}$. At 200 $\mu\text{g/ml}$ the death rate was 93.3% and at 40 $\mu\text{g/ml}$, the mortality rate was up to 90% (Table 3). The aqueous extract shows a low activity with

mortality rate at of 13.3% at 400 $\mu\text{g/ml}$, 3.3% at 200 $\mu\text{g/ml}$ and no death at 40 $\mu\text{g/ml}$. the LC_{50} was too large thus cannot be defined within the limit of this work (Table 4).

The activity of the extracts on *A. salina* (brine shrimp) is presented in Tables 5 - 7. There was 30% mortality even at 1000 $\mu\text{g/ml}$ concentration of the crude extract, corresponding to LC_{50} of 2524.22 $\mu\text{g/ml}$ (Table 5). The chloroform extract (FO_2) $\text{LC}_{50} > 1000 \mu\text{g/ml}$ killed 3.3% at 1000 $\mu\text{g/ml}$. There was no observed death at 100 $\mu\text{g/ml}$ concentration (Table 6). The aqueous extract did not exert lethal effect on brine shrimp at all concentrations (Table 7).

DISCUSSION

The bioassay showed that chloroform soluble extract of *P. stratiotes* $\text{LC}_{50} = 0.0909 \mu\text{g/ml}$ exerted highest lethal activity even at lower concentrations on *Culex* mosquito larvae. This was followed by the crude extract (LC_{50} : 159.5 $\mu\text{g/ml}$). The lowest activity was found in the aqueous extract at much higher dose of 400 $\mu\text{g/ml}$, the LC_{50} of which proves to be too large. Thus, the most mosquitocidal component of the plant could be said to be carried in chloroform soluble compartment.

The extracts of *P. stratiotes* have low activity on Brine shrimp larvae. The crude fraction was moderate at 1000 $\mu\text{g/ml}$. this confirmed some few reported works, which stated that the crude fraction of *P. stratiotes* L. was moderately toxic to brine shrimp (Adoum *et al*, 1997). Comparatively, the chloroform extract of the plant showed a very low toxicity at 1000 $\mu\text{g/ml}$, while the aqueous extract did not show any activity on brine shrimp at all. Perhaps, this may partly be the reason why some shrimps prefer to associate with the plant in their sea environment (Mukhtar and Hafiz, 2001).

Effects such as delayed mortality, reduced survivorship of mature insects, reduction in the production of viable eggs and reduction in fecundity, example in mosquitoes were however, outside the scope of the current study. An interesting observation was that the plant extracts that have shown high activity on *Culex* mosquito larvae have shown low activity on brine shrimp larvae, despite the brine shrimp being more primitive than the mosquitoes. Additionally, an investigation carried out on the toxicity of extracts of *P. stratiotes* in rats has

Table 2: Larvicidal effect of crude extracts (FO₁) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	400	10	7	9	8	3	1	2	80	159.50
2	200	10	4	5	6	6	5	4	50	
3	40	10	2	0	3	8	10	7	16.67	
Control	0	10	0	0	0	10	10	10	0	

Table 3: Larvicidal effect of chloroform extracts (FO₃) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	400	10	9	10	10	1	0	0	96.6	0.0909
2	200	10	4	10	8	0	0	2	93.3	
3	40	10	9	8	10	1	2	2	90.0	
Control	0	10	0	0	0	10	10	10	0	

Table 4: Larvicidal effect of aqueous extracts (FO₂) of *P. stratiotes* on culex mosquito larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	400	10	2	1	1	8	9	9	13.33	> 1000
2	200	10	0	0	1	10	10	2	3.33	
3	40	10	0	0	0	10	10	10	0	
Control	0	10	0	0	0	10	10	10	0	

Table 5: Larvicidal effect of crude extracts (FO₁) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial NO. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	1000	10	4	4	1	6	6	9	30	2524.22 or >1000
2	100	10	0	0	1	10	10	9	3.3	
Control	0	10	0	0	0	10	10	10	0	

Table 6: Larvicidal effect of chloroform extracts (FO₃) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial No. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	1000	10	1	0	0	9	10	10	3.3	>1000
2	100	10	0	0	0	10	10	10	0	
Control	0	10	0	0	0	10	10	0	0	

Table 7: Larvicidal effect of aqueous extracts (FO₂) of *P. stratiotes* on *A. salina* larvae

Expt.	Conc. of extract μ g/ml	Initial NO. of larvae	Total deaths in each test compartment			Total survivors in each test compartment			% mortality	LC ₅₀ μ g/ml
			1	2	3	1	2	3		
1	1000	10	0	0	0	10	10	10		>1000
2	100	10	0	0	0	10	10	10	0	
Control	0	10	0	0	0	10	10	10	0	

shown adverse effects but no lethality was recorded (Mukhtar and Hafiz, 2001).

CONCLUSION

The crude extract of *P. stratiotes* was cytotoxic on both brine shrimp and *Culex* mosquito larvae. Where but, the chloroform and aqueous extracts were active on *Culex* mosquito larvae only. The chloroform fraction appeared with highest potential of acting as anti-mosquito larvae because of its high cytotoxicity with LC_{50} of $0.0909 \mu\text{g/ml}$ than all the other tested fractions of the plant. It can be recommended that the extracts be further assayed to ascertain their potential use as antibacterial, antifungal or as insecticides, so that its economic values can be harnessed.

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THE EFFECTS OF PRESERVATION METHODS ON PROXIMATE COMPOSITION, INSECT INFESTATION AND ORGANOLEPTIC PROPERTIES OF *Heterobranchus longifilis*, *Heterotis niloticus* AND *Chrysichthys nigrodigitatus*

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ABSTRACT

A study to evaluate the effects of insecticide, Actellic 25 EC and salt solutions on proximate composition, preservation of organoleptic properties and reduction of insect infestation on traditionally smoked dried fish samples was carried out using three freshwater fishes, Heterobranchus longifilis, Heterotis niloticus and Chrysichthys nigrodigitatus. The various dehydration and smoking treatments had effect on the proximate composition. The highest moisture contents were recorded in the batch I (fresh) fish pieces, while the lowest moisture content occurred amongst the Actellic dehydrated and smoked dried fish pieces. The fat content of fish pieces dehydrated and smoked dried showed that the non dehydrated and non smoked dried fish pieces (fresh fish) had the highest fat content. The highest fibre content was recorded in the batch I (fresh fish) fish species and the lowest was recorded among the fish pieces dehydrated in salt solution before smoke drying. The protein content of fish pieces variously dehydrated and smoked dried revealed that the Actellic 25 EC dehydrated smoked dried fish pieces had the highest protein content while the lowest protein contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions. The highest carbohydrate content was recorded in the batch I (fresh fish) while the lowest occurred among Actellic 25 EC dehydrated smoked dried fish pieces. Two insects, Dermestes sp and Necrobia sp were identified to attack dehydrated and smoked dried fishes. The smoked dried fishes had comparatively higher insect attack than the salted and / or Actellic dehydrated smoked dried fish pieces. Fish pieces preserved with Actellic had the overall best organoleptic properties while acceptability of the dried fish was best for salted smoked dried fish pieces. The relevance of this study to humanity is discussed.

Key words: Deltamethrin, Freshwater Fish, Fish salting, Fish Smoking, Fish Storage, Chemical Properties, Organoleptic Properties

INTRODUCTION

Fish supplies a significant part of the protein nutrient of animal origin in the diet of man. From the nutritional point of view, fish is one of the most important animal proteins available in many of the less developed countries (James, 1984). FAO (1985) estimated that 53% of the world's fish harvest by developing nations is consumed locally. Unfortunately, most of these catches are lost because of lack of adequate technology to prevent post harvest losses in the third world countries (Osuji, 1976).

In Nigeria, available data show that the artisanal fishers contribute more than 95% of the local fish production and over 50% of the total fish supply (Eyo, 1992). Nwuba (1997)

established that 80% of the artisanal fishers were women. Thus indicating the predominant role women play in post harvest handling, processing and preservation of fish.

The agents of spoilage include insects, bacteria, fungi and autolytic enzymes. These agents operate under certain optimum conditions. This paper is concerned with the effect of salt and Actellic 25 EC solution on the shelf life and organoleptic properties of smoke cured *Heterobranchus longifilis*, *Heterotis niloticus* and *Chrysichthys nigrodigitatus*, against insect attack. These fish species are of high commercial value in Nigeria. Actellic 25 EC is used because of its recommendation (FAO, 1985).

Fish preservation is a process of keeping the fish close to its fresh state by minimizing changes in its physical appearance, taste and smell. The prevailing methods of preserving fish in Nigeria are still traditional curing of fish by sun-drying, smoking with or without presalt treatment or a combination of these methods (Awoyemi, 1990; Akande and King, 1997).

MATERIALS AND METHODS

Fish Procurement: A total of 864 fish pieces of about 96.84 gm average weight of fish samples – *H. longifilis*, *H. niloticus* and *C. nigrodigitatus* were used in this study. The fish samples were procured fresh from landing sites at Mariner water side and nearby Ose market, all at Onitsha bank of the river Niger.

Fish Processing: The fish samples were cut into uniform pieces as much as possible and divided into four batches. This is important for valid conclusions to be drawn from reliable results. Each batch contained 216 fish pieces made up of each of the three fish species.

Fish samples in batch I (fresh specimen) served as control for mineral and proximate analyses. Fish samples in batch II were traditional smoke dried and stored. Fish samples in batches III and IV were either salted or treated in 0.03 % Actellic 25 EC solution before smoke drying and storage.

25.4 % salt solution was prepared by dissolving 2.54 grams of salt in 10 litres of distilled water. 0.03 % Actellic 25 EC solution was prepared by dilution of 12.00 ml of Actellic 25 EC in 10 litres of distilled water. Batches III and IV fish pieces were dehydrated in either salt or Actellic 25 EC solutions respectively before smoke drying.

Batch III fish pieces were washed and soaked in 10 litres of brine solution for 3 hours, removed and soaked in distilled water for 15 minutes. They were subsequently drained and smoke-dried for 12 hours, wrapped in paper and stored in bamboo basket for 12 weeks under tropical ambient condition. Batch IV fish pieces were washed and soaked in 10 litres of Actellic solution for 15 seconds, drained, smoke-dried for 6 hours, sprayed with 0.5 litre of the solution, sun-dried for 3 hours, wrapper in paper and stored in bamboo basket for 12 weeks under tropical ambient condition.

To ensure that the fish samples did not deplete the active ingredients in the individual solution, 10 litres of each preservative solution

was used to ensure that all fish pieces were totally submerged in the solution.

Proximate Analysis: Specimens were collected from batch I - IV for proximate analysis to determine crude protein, fats, fibre, ash and carbohydrate values (AOAC, 1980).

Insect Infestation: Visual observation, collection and counts of all insects at the end of the storage period were done. All insects collected were preserved and identified to their species level.

Organoleptic Analysis: A panel of 10 independent judges was arranged to examine, tastes and score fish pieces from batch II – IV for odour, colour, texture and acceptability. A 9 point range score card was used thus: (1 = extremely –ve, 2 = very –ve, 3 moderately –ve, 4 = slightly –ve, 5 = intermediate 6 = slightly + ve; 7 moderately + ve; 8 = very + ve and 9 = extremely + ve).

Statistical Analysis: The proximate values arising from fish pieces in batches I – IV were analyses for their range values, mean and standard errors. F-LSD was used to separate treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results on the proximate composition of the fish pieces from the three freshwater fish variously dehydrated and smoked dried are presented on Table 1. The highest moisture content was recorded in the batch I (fresh fish) fish species thus; *H. longifilis* - 70.01 ± 0.027 , *H. niloticus* - 69.29 ± 0.053 and *C. nigrodigitatus* - 68.06 ± 0.014 . The lowest moisture content were recorded among the fish pieces dehydrated in Actellic 25 EC before smoke drying thus; *H. longifilis* - 8.89 ± 0.034 , *H. niloticus* - 10.11 ± 0.025 and *C. nigrodigitatus* - 16.62 ± 0.083 . Other moisture content values were thus; *H. longifilis* - 10.34 ± 0.071 , *H. niloticus* - 14.64 ± 0.034 and *C. nigrodigitatus* - 29.62 ± 0.033 for salt dehydrated and smoked dried and *H. longifilis* - 20.41 ± 0.092 , *H. niloticus* - 18.64 ± 0.060 and *C. nigrodigitatus* - 19.63 ± 0.046 for smoked dried fish pieces respectively. Akande and King (1997) reported similar ranges in moisture contents of West Africa sardines *Sardinella maderensis* using Actellic 50 EC solution.

The ash content of fish pieces variously dehydrated and smoked dried revealed that the

Table 1: Percentage proximate composition of pieces of three freshwater fish species variously dehydrated and smoked-dried

	Fresh			Smoke-dried			Salted & Smoked-dried			Actellic & Smoked dried		
	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>
Moisture												
Range	69.90 - 70.11	69.03 - 69.12	68.02 - 68.12	20.11 - 20.64	18.61 - 18.99	19.54 - 19.80	10.12 - 10.62	14.55 - 14.76	29.50 - 29.70	8.79 - 9.0	10.0 - 10.17	16.3 - 16.9
Mean	70.01	69.29	68.06	20.41	18.64	19.63	10.34	14.64	29.62	8.89	10.11	16.62
SE	0.027	0.053	0.014	0.092	0.06	0.046	0.071	0.034	0.033	0.034	0.025	0.083
Ash												
Range	1.0 - 1.2	0.99 - 1.20	0.09 - 1.10	6.13 - 6.15	6.89 - 6.91	10.00 - 12.00	14.98 - 15.22	4.7 - 5.3	4.80 - 5.12	5.9 - 6.03	3.95 - 4.05	2.96 - 3.14
Mean	1.1.00	1.03	0.983	6.15	6.9.00	11.00	15.03	5.02	5.01	5.99	4.00	3.03
SE	0.036	0.033	0.031	0.005	0.004	0.365	0.039	0.079	0.046	0.019	0.013	0.03
Fat												
Range	13.00 - 32.00	23.09 - 24.01	11.00 - 12.00	8.00 - 10.00	16.16 - 16.31	9.55 - 11.00	15.14 - 15.99	21.00 - 21.80	13.00 - 13.45	3.49 - 3.68	3.0 - 3.10	2.99 - 3.01
Mean	22.80	23.69	11.28	9.16	16.11	10.07	15.72	21.31	13.22	3.60	3.03	3.00
SE	4.10	0.143	0.153	0.306	0.053	0.199	0.121	0.113	0.074	0.025	0.015	0.003
Fibre												
Range	0.40 - 0.45	1.40 - 1.56	1.00 - 1.10	1.00 - 1.01	1.00 - 1.02	0.18 - 0.25	0.08.0.1	0.30 - 0.33	0.24 - 0.27	0.33 - 0.37	0.58 - 0.61	0.28 - 0.33
Mean	0.428	1.51	1.04	1.00	1.01	0.207	0.092	0.317	0.253	0.35	0.602	0.30
SE	0.007	0.024	0.015	0.002	0.004	0.01	0.003	0.005	0.004	0.007	0.005	0.007
Protein												
Range	15.36 - 15.55	10.66 - 1.07	17.72 - 17.78	61.11 - 61.22	45.33 - 54.34	54.27 - 54.31	55.30 - 55.42	55.22 - 55.40	40.10 - 40.20	50.31 - 50.55	58.36 - 58.90	67.23 - 67.44
Mean	15.46	10.68	17.75	61.15	52.83	54.29	55.36	55.34	40.15	50.42	58.64	67.32
SE	0.03	0.006	0.01	0.016	1.50	0.006	0.021	0.026	0.017	0.035	0.07	0.028
Carbohydrate												
Range	9.22 - 9.30	14.00 - 14.07	8.77 - 8.99	3.14 - 3.18	3.44 - 3.46	3.80 - 4.00	3.33 - 3.36	3.22 - 3.44	12.22 - 12.33	2.15 - 2.20	2.66 - 2.80	1.40 - 1.47
Mean	9.25	14.04	8.89	3.16	3.45	3.93	3.34	3.33	12.29	2.18	2.71	1.44
SE	0.012	0.01	0.03	0.006	0.004	0.034	0.005	0.028	0.016	0.007	0.022	0.01

non dehydrated but smoked dried fish pieces had the highest ash content thus; *H. longifilis* – 6.15 ± 0.005 , *H. niloticus* – 6.90 ± 0.004 and *C. nigrodigitatus* – 11.00 ± 0.365 . The lowest ash contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions and not smoked dried thus; *H. longifilis* – 1.10 ± 0.036 , *H. niloticus* – 1.03 ± 0.033 and *C. nigrodigitatus* – 0.983 ± 0.031 . Other ash content values recorded in this study were thus; *H. longifilis* – 15.03 ± 0.039 , *H. niloticus* – 5.02 ± 0.079 and *C. nigrodigitatus* – 5.10 ± 0.046 for salt dehydrated and smoked dried and *H. longifilis* – 5.99 ± 0.019 , *H. niloticus* – 4.0 ± 0.013 and *C. nigrodigitatus* – 3.03 ± 0.030 for Actellic 25 EC dehydrated smoked dried fish pieces. Our result is not in variances with Akande and King (1997) who observed almost similar range values in ash contents of West Africa *Sardinella maderensis* dehydrated with Actellic 50 EC before smoked drying and Nwuba (2002) who reported similar range values for three freshwater fishes dehydrated with salt before smoke drying.

The fat content of fish pieces dehydrated and smoked dried showed that the non dehydrated and non smoked dried fish pieces (fresh fish) had the highest fat content thus; *H. longifilis* – 22.80 ± 4.10 , *H. niloticus* – 23.69 ± 0.143 and *C. nigrodigitatus* – 11.28 ± 0.153 . The lowest fat contents were recorded among the fresh fish pieces dehydrated in Actellic 25 EC solution and smoked dried thus; *H. longifilis* – 3.60 ± 0.025 , *H. niloticus* – 3.03 ± 0.015 and *C. nigrodigitatus* – 3.0 ± 0.003 . Other fat content values recorded in this study were thus; *H. longifilis* – 9.16 ± 0.306 , *H. niloticus* – 16.11 ± 0.053 and *C. nigrodigitatus* – 10.07 ± 0.199 for smoked dried fish pieces and *H. longifilis* – 15.72 ± 0.121 , *H. niloticus* – 21.31 ± 0.113 and *C. nigrodigitatus* – 13.22 ± 0.074 for salt dehydrated smoked dried fish pieces (Table 1). Our result was not in variances with Akande and King (1997) who reported fat contents in West Africa *Sardinella maderensis* dehydrated with Actellic 50 EC before smoked drying to be within the experimental range values of this study.

The results on the fibre composition of the fish pieces from the three freshwater fish variously dehydrated and smoked dried are presented on Table 1. The highest fibre content was recorded in the batch I (fresh fish) fish species thus; *H. longifilis* – 0.428 ± 0.007 , *H. niloticus* – 1.51 ± 0.024 and *C. nigrodigitatus* – 1.04 ± 0.015 . The lowest fibre content were recorded among the fish pieces dehydrated in

salt solution before smoke drying thus; *H. longifilis* – 0.092 ± 0.003 , *H. niloticus* – 0.317 ± 0.005 and *C. nigrodigitatus* – 0.253 ± 0.004 . Other fibre content values were thus; *H. longifilis* – 0.35 ± 0.007 , *H. niloticus* – 0.602 ± 0.005 and *C. nigrodigitatus* – 0.30 ± 0.007 for Actellic dehydrated and smoked dried and *H. longifilis* – 1.00 ± 0.002 , *H. niloticus* – 1.01 ± 0.004 and *C. nigrodigitatus* – 0.207 ± 0.01 for smoked dried fish pieces. Nwuba (2002) reported similar range values for three freshwater fishes dehydrated with salt before smoke drying.

The protein content of fish pieces variously dehydrated and smoked dried revealed that the Actellic 25 EC dehydrated smoked dried fish pieces had the highest protein content thus; *H. longifilis* – 50.42 ± 0.035 , *H. niloticus* – 58.64 ± 0.07 and *C. nigrodigitatus* – 67.32 ± 0.028 . The lowest protein contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions and not smoked dried thus; *H. longifilis* – 15.46 ± 0.03 , *H. niloticus* – 10.68 ± 0.006 and *C. nigrodigitatus* – 17.75 ± 0.01 . Other protein content values recorded in this study were thus; *H. longifilis* – 55.36 ± 0.021 , *H. niloticus* – 55.34 ± 0.026 and *C. nigrodigitatus* – 40.15 ± 0.017 for salt dehydrated and smoked dried and *H. longifilis* – 61.15 ± 0.016 , *H. niloticus* – 52.83 ± 1.50 and *C. nigrodigitatus* – 54.29 ± 0.006 for non dehydrated but smoked dried fish pieces. The protein content of batch I fish pieces differed significantly from batches II to IV ($P > 0.05$). Our result is not in variances with Akande and King (1997) for West Africa *Sardinella maderensis* dehydrated with Actellic 50 EC before smoked drying and Nwuba (2002) for three freshwater fishes species variously dehydrated before smoke drying.

The results on the carbohydrate composition of fish pieces from either *H. longifilis*, *H. niloticus* or *C. nigrodigitatus* variously dehydrated and smoked dried are presented on Table 1. The highest carbohydrate content was recorded in the batch I (fresh fish) fish species thus; *H. longifilis* – 9.25 ± 0.012 , *H. niloticus* – 14.04 ± 0.01 and *C. nigrodigitatus* – 8.89 ± 0.03 . The lowest carbohydrate content were recorded among batch IV fish pieces, dehydrated in Actellic 25 EC before smoke drying thus; *H. longifilis* – 2.18 ± 0.007 , *H. niloticus* – 2.71 ± 0.022 and *C. nigrodigitatus* – 1.44 ± 0.01 . Other carbohydrate content values were thus; *H. longifilis* – 3.34 ± 0.005 , *H. niloticus* – 3.33 ± 0.028 and *C. nigrodigitatus* –

Table 2: Insect infestation of pieces of three freshwater fish species variously dehydrated and smoked-dried

	Fresh			Smoke-dried		
	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>
<i>Dermestes sp</i>						
Range	0.00	0.00	0.00	10.00 - 25.00	60.00 - 72.00	55.00 - 63.00
Mean	0.00	0.00	0.00	17.00	66.00	59.00
SE	0.00	0.00	0.00	2.00	0.50	1.05
<i>Necrobia sp</i>						
Range	0.00	0.00	0.00	32.00 - 44.00	61.00 - 74.00	55 - 61
Mean	0.00	0.00	0.00	36.00	66.00	59.00
SE	0.00	0.00	0.00	3.60	2.00	2.50
	Salted & Smoked-dried			Actellic & Smoked dried		
	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>	<i>Heterobranchus longifilis</i>	<i>Heterotis niloticus</i>	<i>Chrysichthys nigrodigitatus</i>
<i>Dermestes sp</i>						
Range	20.00 - 24.00	22.00 - 28.00	12.00 - 18.00	12.00 - 15.00	6.00 - 11.00	6.00 - 9.00
Mean	22.00	25.00	17.00	13.00	5.50	7.00
SE	1.00	2.00	2.50	1.00	2.50	1.50
<i>Necrobia sp</i>						
Range	22.00 - 25.00	15.00 - 17.00	12.00 - 18.00	10.00 - 13.00	6.00 - 9.00	4.00 - 8.00
Mean	23.00	16.00	16.00	12.00	7.00	6.00
SE	1.00	1.00	2.00	1.00	1.50	2.00

12.29 \pm 0.016 for salt dehydrated and smoked dried and *H. longifilis* – 3.16 \pm 0.006, *H. niloticus* – 3.45 \pm 0.004 and *C. nigrodigitatus* – 3.93 \pm 0.034 for smoked dried fish pieces. The protein content of batch IV fish pieces differed significantly from batches I to III ($P > 0.05$). Similar ranges values in carbohydrate contents have been reported for West Africa sardines *Sardinella maderensis* (Akanke and King, 1997).

Two insects, *Dermestes* sp and *Necrobia* sp were identified to attack dehydrated and smoked dried fishes. The smoked dried fishes had comparatively higher *Dermestes* sp attack when compared to either salt and / or Actellic dehydrated smoked dried fish pieces thus; *H. longifilis* – 17.00 \pm 2.00, *H. niloticus* – 66.00 \pm 0.50 and *C. nigrodigitatus* – 59.00 \pm 1.05 (Table 2). Actellic treated fish pieces had the least number of *Dermestes* thus; *H. longifilis* – 13.00 \pm 1.00, *H. niloticus* – 5.5 \pm 2.50 and *C. nigrodigitatus* – 7.00 \pm 1.50. Similarly, the smoked dried fish pieces had higher *Necrobia* sp attack when compared to either salt and / or Actellic dehydrated smoked dried fish pieces thus; *H. longifilis* – 36.00 \pm 3.60, *H. niloticus* – 66.00 \pm 2.00 and *C. nigrodigitatus* – 59.00 \pm 2.50 (Table 2). Actellic

dehydrated and smoked dried fish pieces had the least number of *Necrobia* sp thus; *H. longifilis* – 12.00 \pm 1.00, *H. niloticus* – 7.00 \pm 1.50 and *C. nigrodigitatus* – 6.00 \pm 2.00. The insect attack on Actellic treated fish pieces (batch IV fish pieces) differed significantly from fish pieces batches I to III ($P > 0.05$). Esser *et al* (1990), reported low insect infestation arising from the use of insecticides to protect salt-dried marine catfish during processing and storage.

The results of the sensory evaluation, texture and taste are shown in Figure 1. The texture of the dried fishes in batch IV were the best followed by those in batches III and II. The fish samples in batch I were not assayed for organoleptic properties. Evaluation of the odour showed that the fish pieces in batch II had the worse odour after storage while those stored after dehydration and smoking, batches (III and IV) had the better odour. Acceptability of the dried fish was best for those in batch III.

When all the parameters used in the judgment are put together and analyzed, the overall performance of the various treatments can be readily seen. For instance, the fish pieces preserved with Actellic had the overall best performance (6.49 \pm 0.14).

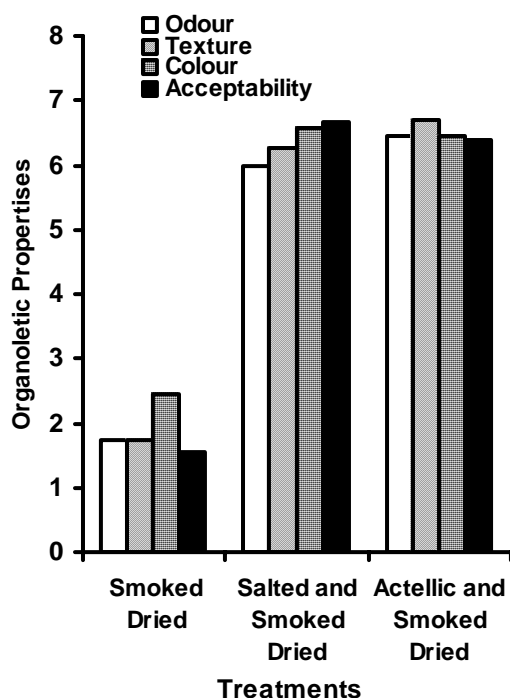


Figure 1: Organoleptic propertise of varoiusly dehydrated and smoked dried freshwater fish pieces.

This was followed closely by batch III (6.37 ± 0.14), while the batch II fish pieces had the overall worst performance (1.87 ± 0.14). Ikeme (1985) reported the extension of shelf life and preservation of organoleptic properties in salted smoked dried fish. The results of the study showed that for better performance of smoke dried fish, application of insecticide before smoke-drying yielded better products.

Finally, despite our results in this study, it is suggested that alternative method such as the use of natural preservatives and physical screening of processed fish from insect be used instead of chemicals. Furthermore, the recommendation of FAO/WHO safe level for food preservatives and insecticides should be strictly adhered to for health reasons.

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GROWTH PERFORMANCE OF MONO-SEX AND MIXED SEX POPULATION OF *Oreochromis niloticus* FED SIMILAR DIET

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ABSTRACT

The growth performance of all-male, all-female and mixed sex population of Oreochromis niloticus fed similar diet was carried out. The fingerlings used in the study were of relatively similar weight ranges (24.8 g – 26.6 g) with initial mean weight of 25.7±1.3 g and initial mean total length of 3.8 ± 1.5 cm. The mean increase in weight for the all-male Oreochromis was significantly higher than the values for the all-female Oreochromis and the control (P < 0.05). The food conversion ratio (FCR) was best in the all-male Oreochromis, while that of all-female was better than that of the mixed population. There was no significant difference between the food conversion ratio of all-male Oreochromis and those of all-female and mixed population (P > 0.05). The percentage survival of all-male O. niloticus was 94 % and that of all-female was 88 %, while that of the mixed population was 74 %. All-male O. niloticus grew better than the all-female under the same experimental conditions. It is therefore recommended that the culture of all-male O. niloticus species by fish farmers should be encouraged for increased fish production in Nigeria.

Keywords: Mono-sex culture, All-male, All-female, *Oreochromis niloticus*

INTRODUCTION

Nile Tilapia, *Oreochromis niloticus* is an important culturable species and it is highly accepted by the consumers in Nigeria. The species have a disadvantage of prolific reproduction under mixed sex culture in ponds. They attain sexual maturity in 2 -3 months from fry stage. They can breed as often as once a month under favourable conditions. This characteristic results in the production of large numbers of stunted fish which cause overcrowding in the pond and does not appeal to consumers when harvested for sale (Sule *et al.*, 1996). It therefore becomes imperative to curb or eliminate completely this unwanted reproduction and its resultant consumers non preference.

The existing methods in use to salvage this problem include: Cage culture (Coche, 1976), irradiation (Nelson *et al.*, 1976), combined stocking with piscivorous fish (Shell, 1967), hybridization (Allison *et al.*, 1976, sex reversal (Guerrero, 1975) and monosex culture (Bardach *et al.*, 1972). Kirk, (1972) discussed *Tilapia* with special reference to those aspects of their physiology and breeding behaviour which were relevant to their culture in fresh and brackish water. Details on husbandry techniques of *Tilapia* have been described in Bardach *et al.*, (1972). Their Biology and culture with particular

reference to Africa have also been reported by Balarin (1979).

Improved growth capacity and high fish production are the major economic aims of fish farmers. Comparative studies on growth and survival of fingerlings of hybrids of *Tilapia* species have been carried out by different workers in Nigeria, notably, Eyo, (1996), Madu *et al.* (1996), and Omoniyi and Fagade (2003). Comparative growth studies on mono-culture and mixed population of *O. niloticus* has not been extensively studied in Nigeria. This study was therefore carried out to investigate the growth responses of all-male, all-female and mixed population of *Oreochromis niloticus* using 25% crude protein fish feed.

MATERIALS AND METHODS

Differentiation of Sexes: The distinctive feature of the genitalia of male and female *Tilapia* as described by Maar *et al.*, (1966), was used in separating the males from the females. The fingerlings used for this experiment were obtained from the Institute's production pond.

Stocking of Fish Fingerlings: One hundred (100) fingerlings each of all-male, all-female and randomly selected mixed population with initial mean weight of 28.6 ± 1.30 g were stocked in 30 m² earthen ponds located at Dadin Kowa,

Gombe State, after pond preparation. The ponds were marked A₁ – A₃, B₁ – B₃ and C₁ – C₃ respectively for all male, all females and mixed sex populations.

Feeds and Feeding: Feeding of fish fingerlings started immediately after stocking at established feeding spots. The fish were fed with 25 % crude protein feed at 5 % body weight twice daily; 900 and 1600 hours. The feed was formulated from local feed ingredients as described by Eyo (1989). The proximate composition of the diet is presented in Table 1. Length and weight measurements of the experimental fish were taken fortnightly and the feed adjusted to the new body weight. The experiment was monitored for 42 weeks.

Table 1: Ingredients and proximate composition of experimental diet

Ingredients (g/100g dry matter)	Weight (g)
Guinea corn	54.4
Groundnut cake	20.0
Blood meal	11.8
Fish meal	8.3
Bone meal	0.5
Vitamin premix	2.0
Starch	1.5
Vegetable oil	1.0
Common salt	0.5
Total	100
Calculated crude protein level (%)	25
Analyzed nutrient content (% dry matter)	
Protein level (%)	24.9±0.4
Moisture	6.8±0.7
Ash	8.3±0.5
Crude fibre	4.9±0.3
Crude lipid	4.5±0.3
Energy content (KJg ⁻¹)	19.0

Mean Growth Rate: The mean growth rate (MGR) was computed using the method of Wayne and Davis (1977) as: $MGR = \frac{W_2 - W_1}{0.5(W_1 + W_2) \times T} \times 100/1$. Where: W₂ = Final weight, W₁ = Initial weight, T = Culture period in days, 0.5 = Constant

Food Conversation Ratio: Food conversation ratio (FCR) was computed as: $FCR = \frac{\text{Dry weight of feed fed}}{\text{Gain in fish weight}}$.

Percentage Survival: Percentage survival was calculated as: $\% \text{ survival} = \frac{\text{Number of fish survival}}{\text{Number of fish stocked}} \times 100/1$.

Physico-Chemical Parameters: The dissolved oxygen, temperature and pH of pond water were monitored fortnightly. Dissolved oxygen was determined by Winkler's method, water temperature by glass thermometer and the pH by Lovibond comparator.

Statistical Analysis: Growth data were tested using the Analysis of Variance (ANOVA). Means were analysed for significant difference using the multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The growth performance of all-male, all-female and mixed population of *Oreochromis niloticus* fed similar diet is shown in Table 2. At the end of the experiment, the mean total weight increase for the all-male *Oreochromis niloticus* was 206.0 ± 1.3 g and that of the female was 170 ± 1.0 g while that of the control (Mixed population) was 132.0 ± 0.8 g respectively. The mean increase in length for the all-male and all-female *Oreochromis niloticus* were 2.6 ± 0.3 cm and 1.8 ± 0.1 cm respectively. The mean increase in weight for the all-male *Oreochromis* were significantly higher than the values of the female *Oreochromis* and the control ($P < 0.05$). This result is supported by an earlier observation of Abella, *et al.*, (1990) in which higher growth rate was reported for male (*Oreochromis aureus*).

The food conversion ratios (FRC) were 0.45 in the all-male *O. niloticus* and 0.44 in all-female, and were better than those of the mixed population (0.33). There was no significant difference between the food conversion ratio of the all-male and those of the all-female and the mixed sex population ($P > 0.05$). This agrees with the report of Guerrero (1985) where no difference was observed in the food conversion of all-male *Sarotherodon galilaeus* and that of the all-female and mixed sexes. The percentage survival of all male *O. niloticus* was 94 % and that of the female was 88 % while that of the mixed population was 74 %. The comparison between the all-male and all-female *O. niloticus* in this experiment shows that growth and survival of all-male was better than those of the all-female and the control. This result is in line with Chervinski (1982) who reported that male *Tilapia* grows better with higher survival rate than the all-female when the sexes are cultured separately.

Table 2: Growth performance of all-male, all-female and mixed population of *Oreochromis niloticus* fed similar diet

Treatments	Initial mean weight (g)	Final mean weight (g)	Mean weight gain (g)	mean growth rate g/day	mean initial length (cm)	Mean final length	mean length increase	feed conversion ratio	% survival
Males	24.0	230.0	206	0.57	3.6	6.2	2.6	0.45	94
Mixed (control)	25.5	157.0	132.0	0.36	3.8	4.6	0.8	0.33	74
Females	28.6	198.6	170.0	0.47	4.0	5.8	1.8	0.44	88

The result of water quality parameters show that laboratory water temperature ranged from 27.5 - 32.5 °C with a mean of 29.95 ± 1.95 °C during the period of the experiment. The dissolved oxygen ranged between 5.2 mg l⁻¹ – 5.7 mg l⁻¹ with a mean of 5.65 ± 0.39 mg l⁻¹. The pH has a range of 6.0 - 7.5 with a mean of 7.0 ± 0.53. These parameters has no negative effect on fish growth, since they fall within the range reported by Boyd (1976). It has been clearly shown from this experiment that all-male *O. niloticus* grew better than the all-female under the same experimental conditions. It is therefore recommended that for the purposes of table size *Oreochromis* production, the culture of all-male *Oreochromis* species by fish farmers should be encouraged for increased fish production from aquaculture in Nigeria.

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EFFECT OF PERMETHRIN ON SURVIVAL AND REPRODUCTION OF *Bulinus globosus* MORELET 1868 AND *Bulinus truncatus* AUDOUIN 1827

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ABSTRACT

The effects of permethrin on reproduction and survival of Bulinus globosus and Bulinus truncatus are reported. Serial dilutions of the chemical were used in 96 h exposure tests on the molluscs, followed by postexposure maintenance in the laboratory for 8 weeks. There was significant decrease in oviposition with increase in pesticide concentration. There were significant differences between treatment-means for both egg mass and embryo counts for both species of molluscs. The F-LSD values at 5% alpha level for egg mass counts were 2.81 and 2.97 respectively for B. globosus and B. truncatus; 49.60 and 55.72 for the embryo counts in that order. The chemical did not produce an appreciable adverse effect on snail survival and longevity.

Keywords: Permethrin, Fecundity, Survival, *Bulinus globosus*, *Bulinus truncatus*

INTRODUCTION

Various reports indicate that sublethal doses of molluscicides and radiation do cause some impairment of survival and reproductive capacity in freshwater snails (Haroun *et al.*, 1996; Rondelaud and Dreyfuss, 1996; Abdel-Hafez, *et al.* 1997. Motta and Melo, 1997). Okafor (1990) reported that the xenobiotic, ivermectin at concentrations above 0.01 µg/ml is toxic to freshwater snails, resulting in mortality. But, below this concentration, this chemical does not evoke any major behavioural changes. Rather, it brings about significant reductions in egg output and no mortalities. Snail deaths as reported here can be attributed to an acute toxic effect and impaired oviposition to a chronic effect. This suggests that for the freshwater molluscs, a given toxicant may prove non-toxic or sublethal superficially and yet could indirectly affect their reproductive capacity adversely possibly by way of chronic toxicity. This, for the medically important, pulmonate snails may constitute an avenue for regulating those trematode infections transmitted by such molluscs (Okafor, 1990), given that even low level contamination of freshwater systems with such toxicants could reduce snail populations as much as to interrupt transmission.

Ecotoxicological studies with Permethrin (a non-cyanated pyrethroid) within the context of the West African Onchocerciasis Control Programme (OCP), demonstrated severe

adverse effects by this insecticide on benthic invertebrate density following 15 weekly applications (Calamari *et al.*, 1998). That the changes in faunal density and diversity only became apparent after repeated application of the substance suggests that the mechanism of toxicity was more of the chronic than the acute type. This hypothesis is supported by the fact that the fauna recovered to almost pre-treatment levels 1 month from stoppage of insecticide spraying (Calamari *et al.*, 1998).

Having hypothesised that depressed fecundity is possibly a chronic response to sublethal doses of toxicants in freshwater snails, coupled with the fact that the ecotoxicological findings of Calamari *et al.* (1998) do suggest chronic toxicity for permethrin as well, it becomes exigent to investigate such responses on intermediate host snails, specifically. Therefore, the objective of this study was to determine whether or not permethrin has adverse effect(s) on postexposure survival, longevity and fecundity of the schistosom-transmitting bulinids – *Bulinus globosus* and *B. truncatus* –under laboratory conditions.

MATERIALS AND METHODS

Molluscs were exposed to 19 dilutions of permethrin (ranging from 0.01 – 150mg/l) prepared by measuring predetermined quantities of the dust formulation into IL plastic bottles. These were topped with deionised

water and shaken to uniform consistency. All molluscs survived the 96h exposures and were subsequently maintained singly for 8 weeks (mid-September to mid-November 2002) in 300ml of borehole water in 400ml plastic bowls. The controls were set up parallel in distilled water. Survival was assessed as the proportions of previously exposed molluscs that remained alive at weekly intervals postexposure (Okafor and Anya, 1991; Giovanelli *et al.*, 2002). The percentage of molluscs that survived to week 8 postexposure was taken as a measure of fecundity.

Four dilutions: 0.09, 0.9, 20 and 150mg/l were used to study the effect of concentration on egg-laying. After allowing for ovipositing to stabilise, 20 such individuals were exposed per insecticide dilution to serve as the experimental group. Twenty unexposed ovipositing individuals served as the control group. Both the control and experimental groups were monitored for oviposition for a fortnight. Two fecundity measures were adopted: (1) number of egg masses and (2) number of embryos (Okafor, 1991; Okafor and Anya, 1991 and Giovanelli *et al.*, 2002). Egg mass and embryo counts were done every four days to coincide with water changes, with the aid of a hand lens. For the fecundity calculations, the 10 best ovipositing molluscs were chosen per insecticide dilution and same number for the control batch. The same control batch was used for comparison against all treatments.

Statistical Analysis: In order to ascertain whether or not there were significant differences in postexposure fecundity means, data was analysed by one-way analysis of variance (ANOVA), using the LSD to separate the means that were statistically different. Survival and longevity data were transformed into percentages.

RESULTS

Response of Adult Snails to Exposure: Of a total of 190 *B. globosus* adults treated to various concentrations of permethrin none was killed. None of the control individuals died during the period of exposure. Similar results were obtained for *B. truncatus*.

Effects on Survival: No significant differences were obtained for post-exposure survival between the experimental and control snails for both species. The results for the 150 mg/l dose are summarised in Figures 1 and 2.

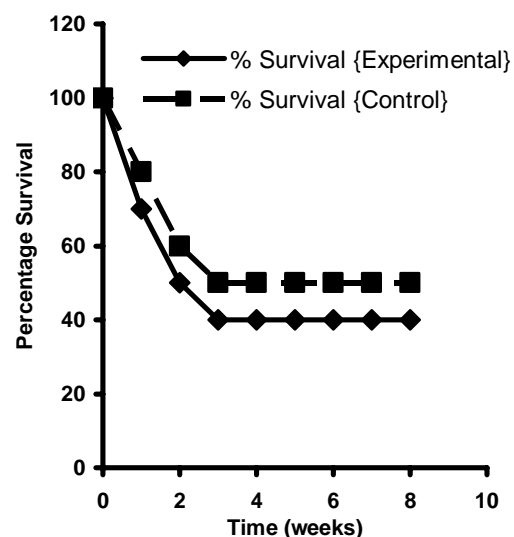


Figure 1: Percentage survival of *Bulinus globosus* 8 weeks after exposure to 150 mg/l of permethrin

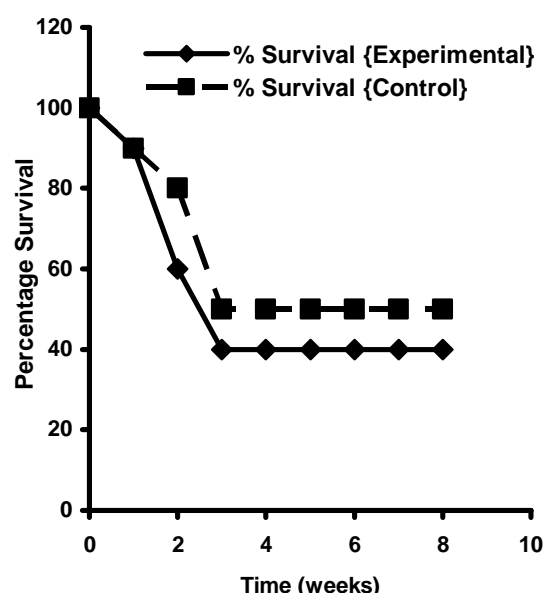


Figure 2: Percentage survival of *Bulinus truncatus* 8 weeks after exposure to 150 mg/l of permethrin

Effect on Longevity: Forty percent of treated molluscs survived to week 8 and beyond versus 50 percent for the controls in both species, suggesting that there were no real differences in post exposure longevity between the different treatments (Figures 1 and 2).

Effect on Fecundity: ANOVA results of differences between treatment-means differed significantly for both egg mass and embryo fecundity at 5% alpha level in the two species. The results are summarised in Table 1.

Table 1: Effect of different concentrations of permethrin on fecundity of two *Bulinus* species

Concentration (mg/l)	<i>B. globosus</i>	
	Egg mass	Embryo
0 (control)	11.30	165.00
0.09	9.40	96.70
0.90	11.40	136.30
20.00	5.90	54.90
150.0	4.60	45.70
LSD (0.05)	2.81	49.60
	<i>B. truncatus</i>	
	Egg mass	Embryo
0 (control)	12.10	175.70
0.09	13.10	147.70
0.90	10.00	100.00
20.00	6.80	67.80
150.0	4.70	73.80
LSD (0.05)	2.97	55.72

DISCUSSION

The results of the toxicity tests demonstrated that permethrin was not adulticidal on the 2 molluscs species. It is thus concluded that the substance does not possess molluscicidal activity and cannot be considered a candidate for mollusc control. Therefore, use of this pesticide to control simuliid larvae as in the Onchocerciasis Control Programme of West Africa (Calamari *et al.*, 1998) does not seem to have posed any direct threat to freshwater gastropods. The absence of a significant difference in the rate of survival of the snails treated to different concentrations of the pesticide suggests that the pyrethroid did not influence the mortality pattern of the snails. Fecundity of the molluscs decreased significantly at permethrin concentrations from 20mg/l and above. This is evident from Table 1. This suggests that though the substance might be superficially non-toxic to the snails, it could depress egg output significantly as to bring about variations in mollusc populations. Such changes in vector population densities could achieve a certain level of transmission control. Okafor (1990) reported that sublethal doses of ivermectin had similar effects on *B. globosus*, *Biomphalaria pfeifferi* and *Lymnaea natalensis*. Impairment of fecundity by sublethal doses of toxicants has been attributed to changes in the gonads of molluscs. Rondelaud and Dreyfuss (1996), demonstrated necrosis in gonad epithelia with sublethal doses of niclosamide; Haroun *et al.* (1996), demonstrated atrophy of the hermaphrodite gland with x-irradiation, which resulted in the suppression of gametogenesis; Motta and Melo (1997).

Conclusion: The findings of this study indicate that the dust formulation of permethrin (Rambo 0.6 a.i.), did not influence overall survival and

longevity adversely. However, it depressed egg output significantly.

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THE PHYSICO-CHEMICAL PARAMETERS OF AN AFRICAN ARID ZONE MAN MADE LAKE

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ABSTRACT

Physico-chemical studies were conducted in lake Alau, a large reservoir in the northeast arid zone of Nigeria, between October, 2001 and September, 2002. Five stations were selected to determine the physico-chemical characteristics. The results showed that water temperature values ranged from 23 °C to 27 °C, depth varied from 2.85 m to 7.23 m, water current was between 19.62 cm/sec and 26.71 cm/sec, Secchi disc transparency ranged from 0.26 m to 0.42 m, pH varied from 6.59 to 7.29, conductivity was between 118.41 $\mu\text{mhos/cm}$ and 131.45 $\mu\text{mhos/cm}$, free CO_2 ranged from 2.55 mg/l to 3.06 mg/l, Biochemical oxygen demand (BOD) was between 4.30 mg/l and 5.31 mg/l and nitrate-nitrogen concentration was between 30.30 mg/l and 47.0 mg/l. There were significant differences ($P < 0.05$) between these parameters in relation to stations. Generally, the physico-chemical characteristics of lake Alau fall within the productive values for aquatic systems, and strongly indicate that the lake is unpolluted.

Keywords: Arid zone, Physico-chemical, Aquatic systems, Lake Alau, Transparency

INTRODUCTION

In Nigeria, man made lakes have been put to many uses. They have been used as sources of drinking water, as a means to control river flood, to generate electricity, to help in irrigation and for recreational purposes. All of the large lakes in Nigeria have been created within the last 40 years by construction of dams within river valleys, such as the Kainji and Jebba lakes in the Niger river valley, Kiri lake in the Gongola river valley, Asejire lake in Oshun river valley. Asa lake and Alau lake in Asa and Ngadda river valleys respectively (CBDA, 1986; Adams and Hollis, 1987; Adeniji, 1989). The ecologic values and economic importance of these multipurpose impoundments are directly related to the hydrologic characteristics of the systems. Okafor *et al.* (1991) noted that water is a scarce commodity in the northeastern arid zone of Nigeria, with hardly any perennial rivers. He observed that the underground waters as well as lakes have been maximally taxed to service various human activities in the zone. Among the measures to revive agriculture and fishery production was the creation of multiple River Basin Authorities with the up-surge in dam building and agricultural development. Because of the rush involved in the establishment of these agencies, many vital environmental

factors were over looked, and most of these systems have thus ended up with mismanagement-related activities (Alaku, 1991).

Many limnological studies of a comprehensive nature have been undertaken in Nigerian lakes (Holden and Green, 1960; Imevbore, 1967; Korlman, 1973; Egborge, 1971, 1972, 1974; Hassan, 1974; Adebisi, 1981, 1989, Mbagwu, 1993).

At present in the northeast arid zone of Nigeria, especially in lake Alau, Maiduguri, there is no detailed physico-chemical study. This paper presents detailed physico-chemical data collected from lake Alau during the study period as base line information required for management of the reservoirs.

MATERIALS AND METHODS

The Study Area: Lake Alau is located in the northeast arid zone of Nigeria along Maiduguri - Bama road, in Borno State, about 29 km south of the Maiduguri metropolitan area, on 11° – 13° E West longitude and 13° – 14° E North latitude. The lake, created in 1986 by construction of flat dam on river Ngadda, was the first in a series of four impoundments in the zone, and it lies entirely within the Nigerian savannah. The principal morphometric characteristics of the

lake basin show that it has a total surface area of 56 km² and a maximum depth of 10 m with an effective storage capacity of 54,000 ha. (CBDA, 1986). The hydrological characteristics show that about 25 % of the study area is not favoured with adequate rainfall, receiving less than 250 mm of rain in a year. The vegetation in the lake is extremely variable depending on the prevailing climatic condition. There is a delicate balance between available moisture and vegetation cover (Thomas *et al.*, 1991).

The study was carried out over a 12 month period, namely from October, 2001 to September, 2002. Five sampling stations were chosen based on accessibility and the various activities taking place in and around the lake. The five sampling stations were marked at intervals of 2.5 to 9 km from the head region.

Station 1 is near the dam site, where the spill way and outlet area are situated. The reservoir at this station is used by the Borno state water Board as the main pumping station; it also serves as drinking spot for herds and cattle. The width of the water in this station is approximately 255 m.

Station 2 is adjacent to the School of Fisheries, and is surrounded by thick vegetation consisting of both submerged and emergent macrophytes. The runoff from a canal in the school empties into this station. The approximate width of the water in station 2 is about 168m.

Station 3 is located around the fishing village called Alau Ngaufe. The water body is about 102 m wide, and is supported by a big dyke surrounded by heavy stones which act as pathway for farmers and villagers around the area. The water is used for domestic activities such as washing and bathing and as drinking spots for cattle's as well as for irrigation of the surrounding farm lands.

Station 4 is in Abari village, which is a major fishing camp for Maiduguri Metropolis and its environs. The water body and the beds widen considerably. The width of the water body is about 2 km. This station is a major fish landing site, it is the biggest among the five stations.

Station 5 is behind Bamari village, adjacent to station 4. This is the only water way (for navigation) that leads to station 4. It is a prominent centre for canoe paddlers, artisanal fishermen and women crossing over to station 4. The littoral areas contain burrow pits where sand for road construction and building purposes are dug out.

Determination of Physico-chemical Parameters:

Water samples were collected fortnightly at the five stations on Lake Alau from October, 2001 to September, 2002. Samples from the surface were collected at each site, using fabricated water sampler with attached 2 litre plastic bottle. Water temperature was measured with a mercury-in-glass thermometer (0-50°C). The water current was measured on the site using a buoyant object, and the distance it moved in relation to time was recorded. Depth was measured by using a graduated stick at each site; transparency was determined by using a 20 cm diameter Secchi disc, suspended by a graduated cable.

Hydrogen ion concentration (pH) was measured by using pH meter, model Py-7; conductivity was determined with battery operated conductivity meter, model MC-1; dissolved oxygen was determined by first fixing the water sample in the field with Winkler solutions A and B. This was later analyzed by azide modification of the Winkler method as described by APHA (1976). All analysis for biochemical oxygen demand (BOD), total alkalinity, free carbon dioxide (free CO₂), phosphate – phosphorus (P-PO₄) and Nitrate nitrogen (NO₃-N) were done by using the standard methods described in APHA (1976, 1979), Lind (1979) and Boyd (1979). All statistical analysis was performed using SPSS software. Water parameters were initially subjected to a t-test. Comparisons were made using one-way ANOVA and t-test on log transformed values. Where there are significant differences at P = 0.05 the test was subjected to Fishers Protected LSD to determine the differences between the means.

RESULTS AND DISCUSSION

The physico-chemical parameters of the five sampling stations are summarized on table I. The mean surface water temperature ranged between 25.05 ± 0.14 °C in station 2 to 27.24 ± 0.12 °C in station 4. There was no significant difference (P > 0.05) between the mean values recorded for stations 1, 2, 3 and 5, but they were significantly different (P < 0.05) from station 4. The mean temperature value recorded in lake Alau was higher than that reported for some other lakes: Suka lake 23.47 °C (Kolo and Yisa, 2000), Shen reservoir 19 °C (Azionu, 1983) and Asejire lake 24 °C (Egborge, 1972, 1974). The varied temperature range may be due to the fact that waters in higher latitudes are subjected to temperature extremes.

Table 1: Physico-chemical Parameters is Relation to Stations in Lake Alau

Parameters	S T A T I O N S				
	1	2	3	4	5
Temperature °C	25.25±0.18 ^b	25.05±0.14 ^b	25.05±0.19 ^b	27.24±0.12 ^a	25.13±0.00 ^b
Depth (m)	2.85±0.18 ^c	3.64±0.03 ^b	3.92±0.25 ^b	7.23±0.13 ^a	3.18±0.06 ^b
Current (Cm/sec)	26.71±0.30 ^b	25.46±0.27 ^b	25.08±0.36 ^b	25.10±0.28 ^b	19.62±0.22 ^a
Transparency (m)	0.36±0.01 ^b	0.33±0.02 ^b	0.35±0.01 ^b	0.42±0.03 ^a	0.26±0.01 ^c
pH	6.79±0.05 ^b	6.97±0.03 ^b	6.83±0.02 ^b	7.29±0.05 ^b	6.59±0.01 ^b
Dissolved oxygen (Do) (mg/l)	6.15±0.05 ^a	6.35±0.05 ^a	5.18±0.02 ^b	6.32±0.01 ^a	5.15±0.03 ^b
Conductivity (homs/cm)	31.45±0.75 ^b	128.8±0.52 ^b	119.42±0.83 ^a	115.47±0.75 ^a	118.41±0.16 ^a
Free Co ₂ (mg/l)	2.55±0.05 ^b	2.90±0.01 ^{ab}	2.85±0.02 ^{ab}	3.06±0.04 ^a	2.84±0.04 ^{ab}
Alkalinity (mg/l)	30.30±0.32 ^b	36.85±0.05 ^b	40.67±0.18 ^a	47.00±0.02 ^a	37.25±0.24 ^b
Biochemical oxygen Demand BOD (mg/l)	4.34±0.32 ^a	4.30±0.28 ^a	4.45±0.50 ^a	5.03±0.33 ^a	5.31±0.25 ^a
Nitrate-Nitrogen (No ₃ -N) (mg/l)	4.27±0.18 ^a	5.43±0.19 ^a	5.4 ^o ±0.25 ^a	5.73±0.37 ^a	6.30±0.50 ^a
Phosphate-phosphorus (P-Po ₄) (mg/l)	0.34±0.00 ^b	0.28±0.00 ^b	0.37±0.10 ^a	0.31±0.01 ^b	0.32±0.01 ^b
Total dissolved Solids TDS (mg/l)	63.31±0.30 ^b	67.88±0.28 ^a	59.17±0.42 ^b	65.84±0.62 ^a	60.73±0.33 ^b

The mean values with the same superscript on the same row are not significantly different at $P \leq 0.05$

The mean values recorded for depth showed that station 4 had the highest mean depth of 7.23 ± 0.13 m. The lowest mean value of 2.85 ± 0.18 m was recorded in station 1. Station 4 was significantly deeper than all other stations ($P < 0.05$). There was no significant difference in depth between stations 2, 3 and 5 ($P > 0.05$). These stations were significantly different from station 1 ($P < 0.05$). The depth variations of each station may be attributed to the structure of the Ngadda river valley and the surrounding land prior to impoundment. The water current varied from 19.62 ± 0.22 cm/sec in station 5, to 26.71 ± 0.30 cm/sec in station 1. Station 5 was significantly different from all the other stations ($P > 0.05$). The highest mean water current observed in station 1 may be due to regulation from the dam site, and the release from this station resulting in a constant flow of water. A factor of potential limnological significance is water movement within the lake produced by discharge from the upstream reservoir and the release down stream (station 1). The actual effects of the current on the chemistry and the biota of the lake are not known, but one may speculate that significant water movement through the surface of the lake may prevent development of anoxic conditions in an otherwise stagnant environment and thereby preclude release of sediment bound nutrients. The values recorded for lake Alau were low compared with 32.80 cm/sec recorded in lake Chad (Nwoko, 1991). The reason may be due to large volume of water in lake Chad compared with lake Alau.

The highest mean values of Secchi disc transparency recorded was 0.42 ± 0.03 m in station 4, while the lowest mean value was 0.26 ± 0.01 m in station 5. Stations 1, 2 and 3 were not significantly different ($P > 0.05$). These stations were significantly different from stations 4 and 5 ($P < 0.05$). Light penetration in a lake estimated by use of Secchi disc is an indicator of water transparency and turbidity (Olsen, 1975). The mean transparency values from other stations showed signs of good quality except for station 5, which reflect high turbidity during the study period. The high turbidity may be linked to the constant disturbance and agitation of the water body by canoe paddlers, as well as constant washing in of sand material from the burrow pits. The quality may not, however, have adverse effect on the aquatic organisms in the lake. The values of transparency recorded in lake Alau was low compared with the findings of Nwoko (1983) in Lake Chad (0.92m) and Adeniji (1989) in Jebba lake (0.75 m).

The mean pH values recorded for all stations varied between 6.59 ± 0.01 and 7.29 ± 0.05 . There was no significant difference ($P > 0.05$) between the values recorded for all stations. Lake Alau falls between acidic and alkaline Lake. The pH range obtained in the lake suggests a medium carbon dioxide supply and medium productivity. The pH range is comparatively narrow but falls within the recommended range (6.5 – 9) as suitable for aquatic life (Adeniji, 1989). A pH of 5.5 – 10 was recommended as optimum range for

tropical fish production (Bennet, 1973), while the EEC pH standard proposed for Nigerian lakes and rivers is 6 – 8 (Akeredolu, 1972). The pH range of Lake Alau may be due to concentration effect and decay process that enhance acidic condition (Lloyd, 1992; Kolo and Yisa, 2000).

The dissolved oxygen varied between 5.15 ± 0.03 mg/l in station 5 and 6.35 ± 0.03 mg/l in station 2. Stations 5 and 3 were not significantly different at $P = 0.05$, while station 2 was not different ($P > 0.05$) from stations 1 and 4. The values recorded in Lake Alau were higher than those recorded in Suka reservoir (4.2 mg/l) (Kolo and Yisa, 2000), in Jebba lake (4.8 mg/l) (Adeniji, 1989) and in Delimi river (4 mg) (Anadu and Akpan, 1986). The high values recorded may be as a result of the constant agitation of the water mass by very strong northeasterly winds, thereby aerating the water and sending more oxygen into solution. The values recorded were higher than the 5 mg/l Nigerian standard for most uses (Akeredolu, 1972) and for warm water fish species culture (Boyd, 1979). Complete oxygen depletion was not observed in any of the stations because of significant water movement through the lake as a result of water release from the upstream.

The mean conductivity value was highest in station 1 (31.45 ± 0.75 homs/cm) followed by 128.83 ± 0.52 homs/cm in station 2. Station 4 had the lowest mean value of 115.47 ± 0.75 homs/cm. Higher conductivity in stations 1 and 2 may be possibly due to the concentration of the ions as a result of decreased depth; this may suggest higher relative concentration of ions responsible for high electrical conductivity in these stations. This agrees with the findings of Nwoko (1984) in Lake Chad, Welcomme (1985) on flood plain rivers, and Ejelonu (1996) in University of Maiduguri sewage reservoir.

The mean free carbon dioxide value ranged from 2.55 ± 0.05 mg/l in station 1 to 3.06 ± 0.04 mg/l in station 4. There was no significant difference ($P > 0.05$) between the mean values recorded in stations 2, 3 and 5, but they were significantly different ($P < 0.05$) from stations 1 and 4. These two station were however significantly different from each other at ($P=0.05$). A major influence on the free carbon dioxide concentration can be attributed to the phytoplankton and macrophyte community, which require light, and supply of nutrients in order to convert available dissolved carbon dioxide into plant tissue by photosynthesis. The concentration of free CO₂

recorded in this study falls within recommended value of below 6.0 mg (Boyd, 1979) for fishery production. Adeniji (1975) observed that a good fishery is correlated with low free CO₂ content. Also Olsen and Sommer field (1977) observed high free CO₂ content, as high as 80 mg/c, which resulted in low fish survival and absence of fish in the lower layers of central Arizonal lake. The highest total alkalinity was recorded in station 4 (47.00 ± 0.02 mg/l) followed by 40.67 ± 0.18 mg/l in station 3. The lowest mean value of 30.30 ± 0.32 mg/l was recorded in station 1, which was not significantly different from stations 2 and 5. There was a tendency for alkalinity values to be higher in different stations due to differences in their discharge rates as well as soil factors. The value recorded for lake Alau remained fairly low and sharply contrasts with those reported by Adebisi (1981), and Kolo and Yisa (2000) who recorded higher concentrations in Ogun river, Mfangonfong and Suka reservoirs respectively. The total alkalinity range of 30.30 ± 0.32 mg/l to 47.00 ± 0.02 mg/l obtained in this study was lower than recommended value for production in warm waters. Hem (1970) gave a range of 75 mg/l – 200 mg/l as adequate for productive water.

There was no significant variation between the values of biochemical oxygen demand (BOD) recorded for all stations. The mean value varied between 5.31 ± 0.25 mg/l in station 5 and 4.30 ± 0.28 mg/l in station 2. The higher BOD recorded in station 5 could probably be due to organic matter degradation which utilized oxygen within the Lake. According to Umeham (1989) and Kolo and Yisa (2000) organic matter in the form of increased decomposition of domestic sewage can increase the BOD. Station 5 had the highest mean value of nitrate-nitrogen of 6.30 ± 0.50 mg/l, followed by station 4 with 5.73 ± 0.37 mg/l. Station 1 had the lowest mean value of 4.27 ± 0.18 mg/l. However, there was no significant difference ($P > 0.05$) between the values recorded for all stations. The nitrate-nitrogen range (4.27 – 6.30 mg/l) obtained in this study is high when compared to the range (0.6 – 1.92 mg/l) recorded in Shiroro Lake (Kolo, 1996). But the value is lower when compared to the range (9.6 – 49 mg/l) obtained by Beadle (1981) for some African productive lakes. A characteristic feature of most of the tropical waters is a low nutrient status with a high turn over rate which results in rapid utilization of the nutrients as soon as they are released by decomposition, so that very little remains in the water (Chessman, 1995). The mean values of phosphate-

phosphorus ($\text{PO}_4\text{-P}$) recorded varied between 0.28 ± 0.00 mg/l in station 1 to 0.37 ± 0.10 mg/l in station 3. There was no significant difference ($P < 0.05$) between the stations except station 3 which was significantly different ($P > 0.05$) from all other stations (Table 1). The important limiting factor for aquatic productivity is the phosphate, and aquatic systems can be impoverished if it is used up. Adeniji (1975) observed that phosphorus is the most important limiting substance controlling organic production. The phosphate - phosphorus range recorded in this study falls below the observed range of 3.2 – 6.30 mg/l observed by Beadle (1981) in some productive African rivers and lakes. The reason for the decreased value, compared to those aquatic systems, may involve heterotrophic uptake by micro-organisms, sediment adsorption and removal by the currents. The slightly higher value recorded in station 3 may be connected with the domestic activities such as washing, bathing and perhaps effects of fertilizers and agricultural by-products washed directly into the lake.

The highest mean value for total dissolved solids was 67.88 ± 0.28 mg/l in station 2, while the lowest mean value of 59.17 ± 0.42 mg/l was recorded in station 3. There was no significant difference ($P > 0.05$) between the values in stations 1, 3 and 5, but they were significantly different ($P < 0.05$) from stations 2 and 4. The probable reason for an increase in TDS between stations may be due to differences in bathing and household cleaning in these stations. Dissolved materials have been observed to constitute the ionic or chemical portion of water quality (Meadle, 1989). Naturally the concentration and relative abundance of ions in lakes is highly variable, although there is a tendency for variability in different stations. Weidemman *et al.* (1985) working on Otisco Lake recorded TDS of 37 – 50 mg/l, while Gliwiaz (1986) recorded the range of 74 – 154 mg/l in a tropical reservoir. The TDS values in these reservoirs compare favourably with the values of this study. The differences in TDS values in Lake Alau can also be related to the different activities in and around the lake, the discharge rate as well as bed soil factors.

The results obtained from the study showed that most of the physico-chemical parameters were within the observed range recorded by other researchers, and were found to be within tolerable limits for species richness and high yield of fish production. The limnological features of Lake Alau strongly

suggest that the water body is maintaining a productive status and is far below the pollution level.

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FRESHWATER SNAILS OF NIGER-CEM, NKALAGU EASTERN NIGERIA: OBSERVATIONS ON SOME DEMOGRAPHIC ASPECTS OF THE SCHISTOSOME-TRANSMITTING BULINIDS

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ABSTRACT

The results of snail collections carried out in the freshwater habitats of Niger-Cem in Nkalagu from August to November 2002 are reported. Also reported are findings on abundance, diversity and age structure of the snails. A total of 3491 pulmonate snails were collected, belonging to 3 families: Planorbidae (3133); Lymnaeidae (199) and Ampullariidae (159). Bulinus globosus was most abundant, with mean abundance (MA = 627.66) followed by B. truncatus (MA = 294) and Biomphalaria pfeifferi, the least abundant (MA = 6.33). Analysis of the collected snails gave the following: Shannon's index of diversity, $H = 1.2889$; Simpson's index of dominance, $D = 0.3642$ and the number of snails per man-hour = 174.6. Age structure findings demonstrated a 'lag' phase in the period of peak abundance between B. globosus and B. truncatus. Findings on the reproductive to pre-reproductive (R/P) ratios, suggest similar demographic strategies for the two buliniid mollusc species.

Keywords: Abundance, Diversity, Demographic Strategy, *Bulinus globosus*, *Bulinus truncatus*

INTRODUCTION

Papers on freshwater snails, especially those of medical and veterinary importance in Nkalagu, Eastern Nigeria, are about a decade old: Anya and Okafor (1986), Okafor (1990a,b), Okafor (1991), Okafor and Anya (1991). These studies showed that Niger-Cem was a major focus for transmission of urinary schistosomiasis and that *B. globosus* was the intermediate host snail actively involved in the disease cycle. *Bulinus truncatus* was consistently absent from the previous surveys. Recent observations have recorded the coexistence of this mollusc with *B. globosus* in marshy pools at Niger-Cem.

This is a cause for concern given that this mollusc is currently transmitting the infection in some parts of Eastern Nigeria, notably the Amagunze and Agulu lake environs (Ozumba *et al.*, 1989; Emejulu *et al.*, 1994). This study was, therefore, undertaken to provide: (1) an update on the freshwater snails of medical and veterinary importance and (2), to determine the abundance and demographic strategy of *B. truncatus* vis-à-vis *B. globosus* in the Niger-Cem locality.

MATERIALS AND METHODS

Study Area: The study area is located in Nkalagu lying in the Guinea savannah zone between latitudes $6^{\circ}25'$ to $6^{\circ}35'$ N and longitudes $7^{\circ}45'$ to $7^{\circ}55'$ E (Anya and Okafor, 1986) in the present Ebonyi State in Eastern Nigeria.

Snail Collections: The molluscs were collected from three representative habitats (2 marshy pools, 1 pond and 1 quarry lake). The collection was done, using a plastic kitchen strainer (Ratard and Greer, 1991) of pore size 1.2 x 1.2 mm. Four collections were made, each for the months of August, September, October and November 2002. The snails were identified by shell morphology as in the Danish Bilharziasis Laboratory (DBL) Denmark, reference snails.

Demographic Studies: The monthly collections were summed up to obtain the grand total from which percentage compositions by species were calculated (Kloos *et al.*, 2001). The following parameters were calculated for each species:

Table 1: Fresh water snails from Niger-Cem, Nkalagu with associated population and habitat attributes

Attributes studied	Snail Type							Total
	<i>B. globosus</i>	<i>B. truncatus</i>	<i>B. senegalensis</i>	<i>B. forskalii</i>	<i>Biom. pfeifferi</i>	<i>Lanistes varicus</i>	<i>Lymnaea natalensis</i>	
No. Collected	1883	882	288	61	19	159	199	3491
% Collected	53.93	25.26	8.24	1.74	0.54	4.55	5.70	100
Mean Abundance (MA)	627.66	294	96	20.33	6.33	53	66.33	1163.65
Relative Abundance (RA)	1.0	0.46	0.15	0.03	0.01	0.08	0.10	1.83
Habitat type	Marshy Pools	Marshy Pools	Marshy Pools Pond irrigation canals	Marshy Pools ponds irrigation canals	Quarry Lake	Marshy pools		
Associated plants	<i>Atherna-nthera sessilis</i> <i>Croix lacryma-jobi</i>	<i>Lemna</i> sp	<i>Panisetum</i> Rice Maize <i>Polygonium</i>	<i>Panisetum</i> Rice maize	Algae lichen	Nymphae		
Presence of snail egg masses	Yes	Yes	-	-	-	-	Yes	

Shannon's Index of diversity, $H = 1.2889$ Simpson's Index of dominance, $D = 0.3642$. No. of snails collected per man-hour (3491/20) = 174.6

1. mean abundance (MA = total number of individuals/number of collection sites (Sturrock *et al*, 1994);
2. relative abundance (RA = MA for the given species/ MA for the most abundant mollusc (*B. globosus*);
3. Shannon's diversity index [$H = -\sum (p_i \ln p_i)$]
4. Simpson's index of dominance [$D = \sum (p_i)^2$]

The age structures were determined by sorting the molluscs into 3 size classes corresponding to 3 age groups (older, middle aged and young). The ratio of the reproductive to the pre-reproductive snails was also determined, the older molluscs constituting the reproductive, the middle-aged and young, the pre-reproductive.

RESULTS

Demographic Findings on Snails: Seven freshwater gastropods species were collected, 5 planorbids, 1 lymnaeid and 1 ampullariid. The planorbids include: *Bulinus globosus* (Morelet); *Bulinus truncatus* (Audouin); *Bulinus senegalensis* (Müller); *Bulinus forskalii* (Ehrenberg) and *Biomphalaria pfeifferi* (Krauss).

Table 2: Age structure of two freshwater gastropods from Niger-Cem Nkalagu

Age Group	B. globosus		
	Size class	No. Collected	% Composition
Older snails	11 – 15mm	330	17.52
Middle-aged snails	6 – 10mm	1060	56.29
Young snails	< 6mm	493	26.18
R/P ratio ^a		330/1553 (0.21)	
Age Group	B. truncatus		
	Size class	No. Collected	% Composition
Older snails	12 – 17mm	154	18.73
Middle-aged snails	7 – 11mm	274	33.33
Young snails	< 7mm	394	47.93
R/P ratio ^a		154/668 (0.23)	

^aR/P ratio = ratio of the reproductive to the pre-reproductive snails.

The other 2 were *Lymnaea natalensis* (Krauss) and *Lanistes varicus* (Morelet). Snail collections

were as follows: 342 in August (9.79%); 702 in September (20.1%); 1049 in October (30.04%) and 1398 in November (40.04%). Ova were seen at the sites in August and early September. *B. globosus* was the most abundant (1883 individuals), followed by *B. truncatus* (882 individuals). *Biomphalaria pfeifferi* being the least (19 individuals). It was observed that *Planorbis varicus* coexists with both *B. globosus* and *B. truncatus*. But over 90% of these molluscs were collected from *B. truncatus* microhabitats. *B. truncatus* tended to inhabit more eutrophic parts of the habitats alongside *L. varicus*, whereas *B. globosus* appear to prefer the less eutrophic zones. While the bulinids were most often found immersed and attached to submerged vegetation and decaying organic matter, the lymnaeids seemed to prefer peripheral sites of the water bodies (Table 1). Table 2 shows a pyramidal structure for *B. truncatus*, which is suggestive of an expanding population. That of *B. globosus* is rather urn-shaped, suggestive of a dying population. The older snails are considered to constitute the reproductive while the middle – aged and young snails, the pre-reproductive age classes.

DISCUSSION

Snail collection findings agree fairly well with those of Anya and Okafor (1986), especially as concerns potential schistosome intermediate hosts in this locality. The absence of some molluscs in this study as opposed to the former studies was due to the fact that the present collections were limited in both time and space. Despite this, the present study reports a greater abundance of snails than is suggested by all previous ones (Anya and Okafor, 1986; Okafor, 1990a; Okafor and Anya, 1991). Although 10 sites were sampled in the previous studies and only 3 in the present, there is a remarkable difference in site abundance of 627 (present) and (41 and 45) (calculated) for 1981 and 1982, respectively and during the same month of the year (Okafor, 1990a). This suggests, therefore, that *B. globosus* has undergone a massive increase in population between 1990 and 2002. This situation suggests that neglect of snail control in previous disease control efforts should be redressed. Earlier studies such as those of Noda *et al.* (1990) and Woolhouse and Chandiwana (1989) demonstrated that rainfall has a distinct influence on the densities of *B. globosus*. It would appear that the increase in absolute numbers of the snails found in the present study, can also be explained by the

logic of inter-decadal influence of precipitation and temperature on snail numbers thus corroborating the assertions of Githeko *et al.* (2000). Hence, the simulation model of Woolhouse and Chandiwana (1990) which predicated fluctuations in *B. globosus* numbers of over 2 orders of magnitude over time-scales of 10 years or more, fits the present findings. *B. truncatus* was also isolated but not as abundant as *B. globosus*.

The occurrence of the *Bulinus forskalii*-group snails *B. forskalii* and *B. senegalensis* in a pond at Ezilo and also at Niger-Cem confirms earlier reports of Anya and Okafor (1986) and Okafor (1990a), that these two were often found associated with one other. Greer *et al.* (1990) reported same finding in Cameroon. Both snails had earlier been shown not to be transmitting the infection in this locality (Anya and Okafor, 1986).

There is a high potential for intensification of transmission of schistosomiasis in this area in the future given the occurrence of *B. truncatus* as well. This follows from the likelihood of a chanced introduction of parasite strains compatible with the snails. The increase in snail density only goes to further enhance such a chance encounter. Such is believed to be the case in the Middle Valley of the Senegal River Basin (SRB), where recent data have demonstrated that the occurrence of *S. haematobium* larvae correlates with snail abundance. Also in the Cameroon's Ratard and Greer (1991) reported a new focus of *S. haematobium* / *S. intercalatum* hybrid in Kinding Njabi, arising as a chanced introduction of the hybrid schistosome from Loum town. Therefore, even if the Niger-Cem *B. truncatus* is not transmitting currently, given their abundance, chanced introduction of the compatible parasite strains possibly from Amagunze or Agulu or from both communities could at some future time be of epidemiological significance.

The age structure findings agree generally with those of Okafor and Anya (1991) on *B. globosus*. They equally apply to *B. truncatus*, broadly speaking. However, there are slight variations as can be deduced from Table 2. For instance, the similarity in percentage composition of the older snails (17.52 %) for *B. globosus* and (18.73 %) for *B. truncatus* as well as the R/P ratios 0.21 and 0.23, respectively for *B. globosus* and *B. truncatus* are pointers to the fact that both species have similar demographic patterns. The variations arise when the percentage

compositions of the middle-aged and young snails are compared between the species (Table 2) being 56.29 as against 33.33 respectively for *B. globosus* and *B. truncatus* with respect to the middle-aged age group at the same time of the year. This indicates that at any point in time in the course of this study, a higher percentage of young snails were graduating from that age-group into the middle-aged age group. And the finding that only 26.18% of the young snails were present in the age structure for *B. globosus* as opposed to 47.93 % for *B. truncatus* suggests that reproductive activity in the latter species started later than in the former. It therefore appears that there is a 'lag phase' between the period of peak reproduction in *B. globosus* and that of *B. truncatus* in these habitats, the *B. truncatus* peaks coinciding somewhat with the *B. globosus* depressions. By the same token, peak abundance of *B. truncatus* is expected to lag behind that of *B. globosus*. This appears to have been borne out in the field where the October and November collections of *B. truncatus* were markedly heavier than those of August and September, contrary to *B. globosus* where the differences were not so obvious.

Okafor (1990a), has proposed that snail control be included as part of the overall plan of schistosomiasis control and that for these stagnant water habitats, mollusciciding against *B. globosus* be timed to begin in the middle of the rainy season (June – August) apparently to coincide with the July peak abundance of this species. Given the findings in this study it is suggested that the period of peak abundance of *B. truncatus* may occur later than for *B. globosus*. Thus there is need to investigate the field age structure of *B. truncatus* as has been done for *B. globosus*. The resultant information would enable proper timing of mollusciciding in this locality with a view to obtaining effective control of both species of snails.

Conclusion: The findings as reported indicate that there has been an emergence of *Bulinus truncatus* and a massive increase in the population of *Bulinus globosus* in the Niger-Cem locality in recent years.

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THE HAEMATOLOGICAL PROFILE OF THE SPRAGUE-DAWLEY OUTBRED ALBINO RAT IN NSUKKA, NIGERIA

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ABSTRACT

This study determined the haematological profiles of Sprague-Dawley (SD) outbred albino rats of both sexes and different age sets bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka, Nigeria. Erythrocyte counts (EC), packed cell volume (PCV), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), total leukocyte counts (TLC), and differential leukocyte counts (DLC), were carried out following standard procedures on blood samples collected from 543 rats (267 males and 276 unbred females) during a 14-month study period. Results of the determinations for each of the haematological characteristics were compared with standard reference values generated in temperate countries for specific age sets and sexes of the rats. Findings from our study showed that there were significant differences in the normal values of some of the indices between the sexes and age sets of rats studied; also there were significant differences for some indices in some age sets and sexes between the results obtained in Nsukka Nigeria and the ones generated in temperate climatic conditions - means of the PCV, Hb, mean corpuscular volume, mean corpuscular haemoglobin and absolute numbers of the different leukocytic cellular elements of the rats studied were found to significantly differ from comparable standard reference values generated in temperate locations for specific age sets and sexes, but the means of the EC and TLC were not found to significantly differ from the temperate values. The results of the study were discussed in relation to climatic and geographical locational factors (especially temperature) as they affect the normal reference haematological values, and the relevance of haematology in model animal experimentation and biomedical research.

Keywords: Haematology, Rat, Sprague-Dawley strain, Nsukka, Nigeria.

INTRODUCTION

Laboratory or model animals are recognised as important tools for investigating and understanding human and animal diseases and other biological processes (NIH, 1999a; MI, 2002). Experimentation with laboratory animals forms the backbone of biomedical, bio-agricultural and bio-industrial researches. It enables the development and selection of new medical and veterinary pharmaceuticals, their toxicity testing, development and improvement of surgical materials and procedures, investigation of experimental diseases and

pathology, development and production of antisera and vaccines, and development of medical and veterinary diagnostic techniques amongst other applications (Uzoukwu, 1981; NIH, 1999a; MI, 2002; Gallagher, 2003). Worldwide, rodents are the major and most commonly used animals for biomedical research; the use of other animals is small in comparison (MI, 2002). The rat is the most widely studied experimental animal as demonstrated by the number of publications on studies using rats in the last decade – nearly 500,000 PubMed publications (NIH, 1999b). The rat model with its enormous strengths and

versatility of application has been found to be the most appropriate experimental model for the study of human diseases (NIH, 1999b), and it remains the dominant animal model for risk assessment of virtually all forms of therapeutics and chemical toxicities (Jelovsek *et al.*, 1989; Berkowitz and Katzung, 2001). The Sprague-Dawley (SD) rat, an albino strain of the Norwegian rat (*Rattus norvegicus*), is a widely accepted and dependable general purpose research model used in virtually all disciplines of biomedical research; it is the commonly available rat for experimental studies worldwide (TTL, 1998a), and in Nsukka, Nigeria.

Haematological studies are important in animals and humans because the blood is the major transport system of the body and both the input and output substances of almost all the body's metabolic processes and any deviations from normal are detectable in the blood profile. An evaluation of the haematological profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress. Such an evaluation is indispensably important in arriving at a diagnosis, making a prognosis, assessment of the efficacy of therapy and toxicity of drugs and chemical substances. The erythrocytic and leukocytic profiles of greatest importance include erythrocyte counts, packed cell volume (haematocrit), haemoglobin concentration, erythrocyte sedimentation rate, mean corpuscular values, total leukocyte counts and differential leukocyte counts (Schalm *et al.*, 1975).

The standard reference values for the erythrocytic and leukocytic profiles are influenced by certain physiological factors such as age, sex, breed, species and physiological activity status, and climatic/geographical locational factors such as temperature, humidity, altitude and day length. This implies that for an animal to be used for study in any specific location standard reference values for these haematological indices must be established for that geographical location (Schalm *et al.*, 1975; Coles, 1986). Till date, standard reference haematological values of the SD albino rat, which is commonly used for research and experimentation in the University of Nigeria's Life Sciences and Biomedical departments and other biomedical research centres located in Nsukka, has not been determined. Rather, published standard reference values established for the temperate regions of the world are often used by our researchers as the only available alternative.

The present study reports the results of determinations of the haematological profile (erythrocytic and leukocytic indices) of the SD albino rat bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka, Nigeria.

MATERIALS AND METHODS

Study Area: Nsukka is situated within the derived savannah belt of Eastern Nigeria between latitudes 5°50' and 7°00' north and longitude 6°52' and 7°54' east, at an average elevation of approximately 500 metres above sea level (FMANR, 1999). It is an area of fairly high temperature with a yearly minimum and maximum of 21.17 °C and 29.67 °C with a mean of 25.42 °C (FMANR, 1999). The angle of the sun's rays over Nsukka is near vertical; the difference between the longest and shortest days in the year is only 48 minutes (FMANR, 1999). There is rainy season from March to October and dry season from November to February with a yearly average rainfall of 119.5 mm; the relative humidity in Nsukka is about 70 % during rainy season and falls to about 20 % during the dry season (FMANR, 1999). These climatic factors are capable of influencing the haematological profiles of SD rats bred and currently used in Nsukka research laboratories.

The Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka is the principal source of laboratory animals for the university community's biomedical researchers, independent research centres in the Nsukka and Enugu town and other universities and research centres in Eastern Nigeria.

Rats: The rats used for the study were 543 conventional grade Sprague-Dawley outbred rats bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka between February 2002 and April 2003. The 543 rats comprised of 267 males and 276 unbred females of age range varying from 3 to 72 weeks. The rats were kept in groups according to their ages and sexes in clean cages in a screened animal house. They were fed on standard rat diet composed of 16 % crude protein, which was formulated to meet their nutritional requirements (NAS, 1972). They were also provided with clean drinking water *ad libitum*.

Blood Sample Collection: The blood samples for the haematological study were collected

between the hours of 8.00am and 10.00am each day of the study from the ophthalmic venous plexus located in the orbital sinus of the rats using a micro-capillary pipette (Stone, 1954), as modified by Riley (1960). About 1ml of blood was collected from each rat into a labelled clean sample bottle containing 1 mg of Na-EDTA powder as anticoagulant. Blood was collected from each rat only once. For each age set and specific sex, blood samples were collected from at least 30 rats made up of three batches of rats bred at various times within the experimental period. The relevant haematological determinations were carried out on the blood samples immediately upon collection.

Haematological Procedures: Standard procedures were followed in all the haematological determinations – erythrocyte counts and total leukocyte counts were carried out by the haemocytometer method using an improved Neubauer counting chamber (Hawksley, England); packed cell volume was determined by the microhaematocrit method; the haemoglobin concentration was determined using a standard haemometer (Marienfeld, Germany); and the erythrocyte sedimentation rate was determined by the Wintrobe method (Schalm *et al.*, 1975; Cole, 1986). The mean corpuscular values were computed using the standard formulae. Smears for differential leukocyte counts were prepared and stained by the Leishman technique and the different cells of the leukocytic series were enumerated by the longitudinal counting method (Coles, 1986).

Statistical Analysis: Results generated were collated and presented as means with standard deviation of the specific haematological values for the different age sets and sexes of the rats and tested for significance using ANOVA and Student's *t* test as appropriate; difference was accepted at the probability level of $p < 0.05$. Results of the present study were further compared with the most widely used comprehensive haematological profile of the SD rat compiled by Schalm *et al.* (1975) using a Student's *t*-test. Other published haematological profiles of the SD rat (TCRBL, 1973; TTL, 1998b; Saito *et al.*, 2003) were not used for comparison because these studies did not comprehensively detail the variations usually associated with the different age sets and sexes.

RESULTS AND DISCUSSION

Erythrocytic indices such as erythrocyte counts (EC), packed cell volume (PCV) and haemoglobin concentration (Hb) are important indicators of the functional state of the erythron (Schalm *et al.*, 1975). Erythrocyte counts reflect the total number of red blood cells per unit volume of circulating blood while Hb determinations indicate the oxygen carrying capacity of blood, and PCV determinations show the proportion of blood that is made up of cellular elements and the proportion that is plasma (Coles, 1986). Results of the EC, PCV and Hb determinations for both sexes (Table 1) showed that for both males and females these indices were lowest at weaning (3 - 4 weeks of age) and increased successively with age up till maturity and then declined at old age (60 - 72 weeks of age). The EC obtained ranged from a mean of 4.99 and 5.03 million cells per microlitre of blood in males and females respectively at 3 - 4 weeks of age to as high as 7.87 million cells in 8 - 22 week old males and 7.61 million cells in 15-16 week old females (Table 1). This trend of EC results compared favourably with and was in agreement with the findings of Schalm *et al.* (1975) who reported that mean erythrocyte numbers of SD rats increased with age from an average of 5.25 million cells per microlitre of blood during the first month of life in both sexes to peak of 8.5 million cells in males and 7.5 million cells in females at 6 months of age and older. Results of the PCV determinations showed no significant differences between males and females for the specific age sets; the age trend was an increase in the mean PCV values from 37.60 % in males and 38.00 % in females at 3-4 week of age when it was lowest to its peak of 45.67 % in males and 45.01 % in females at 15 - 16 weeks of age without significant declines as the rats aged (43.80 % and 43.24 % in males and females respectively at 60-72 weeks of age) [Table 1]. This reported trend agrees with the findings of Schalm *et al.* (1975), though after 3-4 weeks of age the mean PCVs reported by Schalm *et al.* (1975) were consistently significantly higher than the one being reported for this study for each age set and sex compared. The significantly higher PCVs reported by Schalm *et al.* (1975) is believed to be due to the relatively colder environmental temperatures of the temperate climates, as studies by Olsen (1973) showed that exposure of animals to cold environmental temperatures lead to increase of about 2 - 5 % of their PCV.

Table 1: The erythrocytic profile of Sprague-Dawley outbred rats of different ages and sexes at Nsukka, Nigeria

Age (weeks)	Erythrocyte counts (10^6 cells/ μ l of blood)		Packed cell volume (%)		Haemoglobin concentration (g/dl)		Mean corpuscular volume (fl)		Mean corpuscular haemoglobin (pg)		Mean corpuscular haemoglobin concentration (g/dl)		Erythrocyte sedimentation rate (mm/hr)		Number of rats sampled	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 – 4	4.99 (0.74)	5.03 (0.84)	37.60 (1.68)	38.00 (1.31)	11.80 ^a (0.39)	12.18 ^b (0.30)	75.35 (4.20)	75.55 (4.56)	23.65 (1.80)	24.21 (2.02)	31.38 (1.28)	32.05 (1.67)	1.24 ^a (0.30)	0.50 ^b (0.11)	30	30
6 – 7	6.38 (0.53)	6.41 (0.18)	41.00 (3.00)	42.00 (2.54)	13.28 ^a (0.43)	12.48 ^b (0.38)	64.26 (5.36)	65.52 (3.80)	20.82 (2.14)	19.47 (1.82)	32.39 ^a (1.08)	29.71 ^b (1.42)	1.14 ^a (0.29)	0.62 ^b (0.17)	30	30
9 – 10	7.06 ^a (0.72)	6.68 ^b (0.38)	43.77 (4.74)	43.90 (1.06)	14.09 ^a (0.58)	13.18 ^b (0.58)	62.00 ^a (4.01)	65.72 ^b (3.96)	19.96 (1.86)	19.73 (1.76)	32.19 ^a (1.12)	30.02 ^b (1.08)	1.04 ^a (0.39)	0.59 ^b (0.25)	45	45
12 – 13	6.71 ^a (0.98)	7.60 ^b (0.76)	43.20 (1.21)	45.01 (2.46)	13.28 ^a (0.08)	13.95 ^b (0.54)	64.38 ^a (3.34)	59.22 ^b (3.68)	19.79 ^a (1.72)	18.36 ^b (2.11)	30.74 (0.96)	30.99 (1.13)	0.84 (0.15)	0.88 (0.23)	30	36
15 – 16	7.61 (0.50)	7.61 (0.49)	45.67 (2.09)	44.70 (2.48)	13.64 ^a (0.22)	13.26 ^b (0.33)	60.01 (3.43)	58.74 (4.07)	17.92 (1.76)	17.42 (1.83)	29.87 (1.24)	29.66 (1.32)	0.93 ^a (0.27)	1.12 ^b (0.22)	36	30
18 – 22	7.87 ^a (0.35)	6.93 ^b (0.64)	44.60 (1.92)	44.30 (2.53)	13.94 ^a (0.08)	13.43 ^b (0.46)	56.67 ^a (3.92)	63.92 ^b (3.74)	17.71 ^a (1.80)	19.38 ^b (1.02)	31.68 ^a (1.32)	30.32 ^b (1.30)	0.76 ^a (0.19)	1.39 ^b (0.40)	30	45
24 – 26	7.72 ^a (0.90)	7.01 ^b (0.72)	44.20 (1.52)	43.96 (2.66)	14.28 ^a (0.62)	13.67 ^b (0.52)	57.25 ^a (3.46)	62.71 ^b (4.01)	18.50 ^a (1.56)	19.50 ^b (1.12)	32.31 ^a (1.40)	31.10 ^b (1.14)	0.80 ^a (0.22)	1.26 ^b (0.31)	30	30
60 – 72	7.27 ^a (0.57)	6.72 ^b (0.63)	43.80 (1.21)	43.24 (2.13)	13.58 (0.33)	13.76 (0.65)	60.24 ^a (3.28)	64.35 ^b (3.43)	18.68 ^a (1.94)	20.48 ^b (1.06)	31.00 ^a (1.23)	31.82 ^b (1.07)	0.56 (0.17)	0.57 (0.15)	36	30

* Results are presented as means with standard deviation in brackets; M = Males, F = Females. ^a^b Different superscripts in an age set row indicate significant differences between the mean values of the parameters between males and females: $ab = p < 0.01$

The Hb recorded in this study was also lowest in rats of 3 - 4 weeks of age (11.80 g/dl for males and 12.18 g/dl in females) with only slight increases as the rats reached maturity, and without any significant declines as the rats aged (Table 1). The mean Hb recorded at 3 - 4 weeks of age for both sexes were slightly higher than that reported by Schalm *et al.* (1975), but from 6 - 7 weeks of age upwards the mean Hb values recorded were significantly lower than that reported by Schalm *et al.* (1975). These significant differences are believed to be temperature related (Olsen, 1973).

The mean corpuscular values [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC)] computed from the EC, PCV and Hb are usually useful in elucidating and classifying anaemia morphologically; they represent an estimation of the alterations in size and haemoglobin content of individual red blood cells (Coles, 1986; TTL, 1998b). Results of this study showed that the MCV and MCH were highest at 3 - 4 weeks of age with an MCV of 75.35 fl for males and 75.55 fl for females, and MCH of 23.65 pg in males and 24.21 pg in females; the MCV and MCH values decreased consistently with age in both sexes without any specified pattern in the variation between the sexes (Table 1). The recorded results compared effectively with that reported by Schalm *et al.* (1975) with significant differences between results of the two studies only at 3 - 4 weeks of age and 60 - 72 weeks of age. The MCHC of both males and females were not found to vary significantly between the age sets (Table 1), and the results reported for this study compared favourably with that of Schalm *et al.*, (1975) and were not found to be significantly different from it.

Erythrocyte sedimentation rate (ESR) is an indicator of the suspension stability of the erythrocyte; changes in the ESR reflect changes in the physicochemical properties of the erythrocyte surface and the plasma (Coles, 1986). The ESR is an important index for evaluating the response of an animal or human body to inflammatory and necrotic processes (Meyer & Harvey, 1998). The ESR results generated from the study showed spectacular age-related trend pattern differences between males and females; the mean ESR of males was highest (1.24 mm/hr) at 3 - 4 weeks of age and progressively decreased with age to 0.56 mm/hr at 60 - 72 weeks of age, in contrast that of females was lowest (0.50 mm/hr) at 3 - 4 weeks of age and progressively increased with

age, reached a peak of 1.39 mm/hr at 18 - 22 weeks of age and then declined to 0.57 mm/hr at 60 - 72 weeks of age (Table 1). There was no published comprehensive ESR results that detailed differences in sex and age that could be used to compare the ESR results recorded in our study. The only report in literature on sex differences in ESR of adult rats by TCRBL (1973) only presented an average ESR of 0.7 mm/hr for adult males and 1.8 mm/hr for adult females.

Total leukocyte counts (TLC) and differential leukocyte counts (DLC) reflect the systemic status of an animal in relation to its response and adjustment to injurious agents, stress and/or deprivation; the indices are of value in confirming or eliminating a tentative diagnosis, in making a prognosis and guiding therapy (Coles, 1986). The TLC and DLC could further provide information on the severity of an injurious agent, the virulence of an infecting organism, the susceptibility of a host, and the nature, severity and duration of a disease process (Meyer & Harvey, 1998). The TLC of the rats studied was found to be lowest at 3 - 4 weeks of age (7.18×10^3 cells per microlitre of blood in males and 7.61×10^3 cells per microlitre of blood in females) and increased significantly with age up until 12 - 13 weeks of age, and then started declining progressively though in the oldest rats (60 - 72 weeks of age) the TLC was found to be at its highest in both sexes (Table 2). This trend did not significantly differ from that reported by Schalm *et al.*, (1975) except in the results of 60 - 72 week old females, which was found to be significantly different (the TLC reported by Schalm *et al.*, (1975) was found to be significantly lower).

Results of the differential leukocyte counts (Table 2) showed that the absolute lymphocyte counts (ALC) was lowest at 3 - 4 weeks of age (4.07×10^3 cells per microlitre of blood in males and 4.76×10^3 cells per microlitre of blood in females) and was found to increase up till maturity and then declined though the values for 60 - 72 week old rats was high. The changes in absolute neutrophil and absolute monocyte counts recorded in the study for both sexes and different ages was not found to follow any definite pattern, though males had higher counts than females for most age sets (Table 2). Absolute eosinophil counts were lowest at 3 - 4 weeks of age for both sexes and increased progressively with age up till old age (60 - 72 weeks of age), with males having a higher eosinophil count than females for all age sets except in 6 - 7 week old rats where absolute eosinophil counts of females was found to be higher than that of males (Table 2).

Table 2: The leukocytic profile of Sprague-Dawley outbred rats of different ages and sexes at Nsukka, Nigeria

Age (weeks)	Total Leukocyte counts		Absolute Lymphocyte counts		Absolute Neutrophil counts		Absolute Monocyte counts		Absolute Eosinophil counts		Absolute Basophil counts		Number of rats sampled	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 – 4	7.18 (1.80)	7.61 (1.36)	4.07 (1.71)	4.76 (1.72)	2.19 ^a (0.56)	1.74 ^c (0.84)	0.76 (0.10)	0.83 (0.21)	0.23 (0.13)	0.21 (0.14)	0.01 (0.03)	0.00	30	30
6 – 7	8.67 ^a (1.87)	10.27 ^b (2.50)	5.89 ^a (1.32)	6.93 ^b (1.22)	1.20 (0.26)	1.06 (0.07)	0.60 ^a (0.35)	1.37 ^b (0.60)	0.10 ^a (0.11)	0.26 ^b (0.16)	0.00	0.01 (0.03)	30	30
9 – 10	12.29 (2.67)	12.52 (2.49)	8.18 ^a (1.45)	9.25 ^b (2.25)	2.17 (0.66)	1.92 (1.04)	1.24 (0.47)	1.13 (0.48)	0.21 ^a (0.11)	0.28 ^b (0.08)	0.00	0.01 (0.03)	45	45
12 – 13	14.00 (2.14)	12.70 (3.28)	9.41 (2.28)	9.40 (2.36)	2.76 ^a (0.83)	2.03 ^b (0.62)	2.14 ^a (0.81)	1.08 ^b (0.44)	0.25 (0.18)	0.24 (0.15)	0.03 (0.06)	0.02 (0.04)	30	36
15 – 16	10.11 (2.99)	10.08 (3.51)	7.48 (2.25)	7.84 (1.76)	1.86 ^a (0.84)	1.37 ^b (0.22)	1.09 ^a (0.10)	0.50 ^b (0.39)	0.36 ^a (0.18)	0.22 ^b (0.13)	0.04 (0.06)	0.03 (0.04)	36	30
18 – 22	10.90 (2.86)	10.03 (2.27)	7.26 (1.75)	7.81 (1.63)	2.21 ^a (0.36)	1.09 ^b (0.56)	1.15 ^a (0.41)	0.77 ^b (0.48)	0.47 (0.36)	0.37 (0.15)	0.00	0.06 (0.03)	30	45
24 – 26	12.78 ^a (2.40)	8.12 ^b (1.84)	7.11 (2.09)	6.32 (1.32)	3.41 ^a (0.91)	0.88 ^b (0.76)	1.82 ^a (0.93)	0.62 ^b (0.39)	0.58 ^a (0.23)	0.30 ^b (0.02)	0.08 (0.07)	0.03 (0.03)	30	30
60 – 72	14.81 ^a (2.25)	16.47 ^c (4.04)	9.30 ^a (2.36)	12.30 ^b (3.71)	3.51 ^a (1.26)	2.44 ^b (0.39)	1.39 (0.21)	1.39 (0.52)	0.59 ^a (0.45)	0.34 ^b (0.27)	0.03 (0.07)	0.00	36	30

* Results are presented as means $\times 10^3$ cells per microlitre of blood with standard deviation in brackets; M = Males, F = Females. ^{a b c} Different superscripts in an age set row indicate significant differences between the mean values of the parameters between males and females: $ab = p < 0.01$; $ac = p < 0.05$

The pattern of variations in absolute basophil counts was not definite for both sexes and different age sets, and in some cases basophils were not found in blood for certain age sets. The inconsistency in absolute basophil numbers and sometimes their total absence in blood of rats is a normal occurrence (Schalm *et al.*, 1975; Coles, 1986). A comparison of the differential leukocyte counts obtained in this study with that reported by Schalm *et al.*, (1975) showed that there were significant differences in the absolute numbers of lymphocytes, neutrophils, monocytes, eosinophils and basophils even when the TLC results of the two studies did not differ significantly; the current study being reported found comparatively lower absolute lymphocyte counts in males mainly from 3-4 weeks of age up till 18 - 22 weeks of age, higher neutrophil counts from 3 - 4 weeks of age up to 12 - 13 weeks of age in both sexes, higher absolute monocyte and eosinophil counts all through the age sets studied and inconsistent variations in absolute basophil counts for both sexes.

Results of this study have shown significant differences in the normal reference values of some haematological indices of the SD albino rat in both sexes and certain age sets when compared with standard reference values generated in temperate regions. The determination and establishment in this study of the erythrocytic and leukocytic profile of the SD albino rat in Nsukka Nigeria is of great significance because of the indispensable relevance of haematological studies in laboratory animal experimentation/research in the University of Nigeria's biomedical research departments and independent life science research centres in the Nsukka and Enugu town and other universities and research centres in Eastern Nigeria. The significance of the study is further buttressed by the fact that the SD albino rat is the most commonly used model animal for experiments and biomedical research worldwide (NIH, 1999b) including in Nsukka, Nigeria.

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