Animal Research International®

Animal Research International is an online Journal inaugurated in University of Nigeria to meet the growing need for an indigenous and authoritative organ for the dissemination of the results of scientific research into the fauna of Africa and the world at large. Concise contributions on investigations on faunistics, zoogeography, wildlife management, genetics, animal breeding, entomology, parasitology, pest control, ecology, malacology, phytonematology, physiology, histopathology, biochemistry, bioinformatics, microbiology, pharmacy, veterinary medicine, aquaculture, hydrobiology, fisheries biology, nutrition, immunology, pathology, anatomy, morphometrics, biometrics and any other research involving the use of animals are invited for publication. While the main objective is to provide a forum for papers describing the results of original research, review articles are also welcomed. Articles submitted to the journal is peer reviewed to ensure a high standard.

Subscription Information
Animal Research International is published in April, August and December. One volume is issued each year. Subscription cost is US $200.00 a year (₦1, 400.00) including postage, packing and handling. Each issue of the journal is sent by surface delivery to all countries. Airmail rates are available upon request. Subscription orders are entered by calendar year only (January - December) and should be sent to The Editor, Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka. All questions especially those relating to proofs, publication and reprints should be directed to The Editor, Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka

Change of address
Subscribers should notify The Editor of any change in address, 90 days in advance.

Advertisements
Apply to Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka.

Animal Research International®

Notice to Contributors

Original research papers, short communications and review articles are published. Original papers should not normally exceed 15 double spaced typewritten pages including tables and figures. Short communications should not normally exceed six double spaced typewritten pages including tables and figures. Manuscript in English should be submitted in triplicate including all illustrations to The Editor/Associate Editor Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka. Submission of research manuscript to Animal Research International is understood to imply that it is not considered for publication elsewhere. Animal Research International as a policy will immediately acknowledge receipt and process the manuscript for peer review. The act of submitting a manuscript to Animal Research International carries with it the right to publish the paper. A handling charge of US $ 20.00 or ₦500.00 per manuscript should be sent along with the manuscript to the Editor, Animal Research International. Publication will be facilitated if the following suggestions are carefully observed:

1. Manuscript should be typewritten in double spacing on A4 paper using Microsoft Word. An electronic copy [1.44 MB floppy] should be enclosed, or submit online at divinelovejoe@yahoo.com.
2. The title page should include title of the paper, the name(s) of the author(s) and correspondence address (es).
3. Key words of not more than 8 words should be supplied.
4. An abstract of not more than 5% of the length of the article should be provided.
5. Tables and figures should be kept to a minimum. Tables should be comprehensible without reference to the text and numbered serially in Arabic numerals.
6. Figures (graphs in Microsoft excel format, map in corel draw 10 format and pictures in photo shop format) should be comprehensible without reference to the text and numbered serially in Arabic numerals.

7. Symbols and common abbreviations should be used freely and should conform to the Style Manual for Biological Journals; others should be kept to a minimum and be limited to the tables where they can be explained in footnotes. The inventing of abbreviations is not encouraged- if they are thought essential, their meaning should be spelt out at first use.
8. References: Text references should give the author's name with the year of publication in parentheses. If there are two authors, within the test use 'and'. Do not use the ampersand '&'. When references are made to a work by three or more authors, the first name followed by *et al*. should always be used. If several papers by the same author and from the same year are cited, a, b, c, etc., should be inserted after the year publication. Within parentheses, groups of references should be cited in chronological order. Name/Title of all Journal and Proceeding should be written in full. Reference should be listed in alphabetical order at the end of the paper in the following form:

EYO, J. E. (1997). Effects of *in vivo* Crude Human Chorionic Gonadotropin (cHCG) on Ovulation and Spawning of the African Catfish, *Clarias gariepinus* Burchell, 1822. *Journal of Applied Ichthyology, 13:* 45-46.

EYO, J. E. and MGBENKA, B. O. (1997). Methods of Fish Preservation in Rural Communities and Beyond. *Pages 16-62. In*: Ezenwaji, H.M.G., Inyang, N.M. and Mgbenka B. O. (Eds.). *Women in Fish Handling, Processing, Preservation, Storage and Marketing*. Inoma from January 13 -17, 1997.

WILLIAM, W. D. (1983) *Life inland waters.* Blackwell Science, Melbourne

Manuscripts are copy edited for clarity, conciseness, and for conformity to journal style.

Proof

A marked copy of the proof will be sent to the author who must return the corrected proof to the Editor with minimum delay. Major alterations to the text cannot be accepted.

Page charges

A subvention of US $600.00 (₦ 5,000.00) is requested per published article. The corresponding author will receive five off-prints and a copy of the journal upon payment of the page charges.

Address

Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka

Phone: 042-308030, 08043123344, 08054563188

Website: www. zoo-unn.org

Email: divinelovejoe@yahoo.com

ANNUAL SUBSCRIPTION RATE
THREE NUMBERS PER VOLUME

CATEGORY	DEVELOP-ING COUNTRY	DEVELOP-ED COUNTRY	NIGERIA
STUDENT	$ 200.00	$ 300.00	N 1,400.00
INDIVIDUALS	$ 300.00	$ 350.00	N 2,000.00
INSTITUTION/ LIBRARY	$ 500.00	$ 600.00	N 5,000.00
COMPANIES	$ 600.00	$ 750.00	N 10,000.00

Pay with bank draft from any of the following banks **only**. **(a)** Afribank **(b)** Citizens Bank **(c)** Intercontinental Bank **(d)** Standard Trust Bank **(e)** United Bank for Africa **(f)** Union Bank **(g)** Zenith Bank **(h)** First Bank Nig. PLC **(i)** Western Union Money Transfer.

Addressed to **The Editor/Associate Editor,** Animal Research International, Department of Zoology, P. O. Box 3146, University of Nigeria, Nsukka.

Alternatively, you may wish to send the bank draft or pay cash directly to The **Editor/Associate Editor** at Animal Research International Editorial Suite, 326 Jimbaz Building, University of Nigeria, Nsukka.

For more details contact, The Secretary, Animal Research International, Department of Zoology, Editorial Suite Room 326, Faculty of Biological Sciences Building (Jimbaz), University of Nigeria, Nsukka. Enugu State, Nigeria.

INFLUENCE OF DIETARY PROTEIN CONTENT ON GROSS EFFICIENCY OF FOOD CONVERSION AND NET PROTEIN UTILIZATION OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822) FRY

[1]MGBENKA, Bernard Obialo, [2]ASOGWA, Maureen Obioma and [2]UGWU, Lawrence Linus Chukwuma

[1]Department of Zoology, Fish Nutrition and Aquaculture Unit, University of Nigeria, Nsukka, Nigeria.
[2]Department of Animal Production Technology and Fisheries Management, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.

Correspondence Author: Dr. MGBENKA, B. O**.** Department of Zoology, Fish Nutrition and Aquaculture Unit, University of Nigeria, Nsukka, Nigeria. E-mail: bo_mgbenka@yahoo.co.uk

ABSTRACT

The influence of dietary protein content on some nutritional parameters of Clarias gariepinus fry was studied. Seven diets were formulated to yield 28, 31, 34, 37, 40, 43, and 46% crude protein (CP) while the 8th 48.8% CP diet was prepared from microencapsulated whole egg and preserved in a refrigerator at 7° C. The diets were fed to advanced fry of C. gariepinus (mean initial weight, 1.6 ± 0.24 g) in triplicate 25 L plastic baths per treatment at 5% body weight per day in three portions for 56 days. The mean weight gain (MWG), daily rate of growth (DRG) increased as the dietary protein level increased up to 40% but gradually declined as CP level increased while increase in CP did not significantly affect daily rate of feeding (P > 0.05). The best response of fish to gross efficiency of food conversion was within 37 - 40% dietary CP and thus reflected the best mean weight gain (0.86 - 1.93g), DRG (0.17 – 20 g), and nitrogen metabolism (12.62 - 13.43 mg/100g) respectively. There was a relatively high metabolizable energy:protein ratio for the 48.80% CP diet (9.88 KJ/mg) compared to 40% CP diet (7.60 mg/kg). Similarly, the low net protein utilization (NPU) value recorded with diets of between 31% to 40% CP compared to the NPU value of higher CP level (43% to 48.8%) suggests that despite the apparently better utility of protein by fish fed the higher CP diets, much of the ingested protein might have been affected by endogenous nitrogen losses resulting in its unavailability for productive use by the fish.

Keywords: *Clarias gariepinus,* Dietary protein, Growth, Feeding rates, Net protein utilization.

INTRODUCTION

The effect of feeding is one significant factor among the setbacks to the rapid growth of the African catfish (*Clarias gariepinus* Burchell, 1822) under culture in Nigeria. Nutritionists have tried to study as many growth and nutritional parameters as possible in order to enhance better understanding of production process both in nature (Gerking, 1972) and in fish culture (Brett, 1976). The importance of protein in fish diet is mainly associated to its role as the source of building material for growth and the production of enzymes (Steffens, 1989). Protein is the basic component of animal tissue and is an essential nutrient for maintenance and growth. Kaushik *et al.* (1995) reported that protein in the diet has an obligatory role of replacing lost body proteins (skin, digestive track) as well as the losses due to amino acid oxidation and utilization for purposes other than synthesis and protein turn-over.

Various researchers have carried out several nutrition studies on the protein requirement of warm water fishes. Faturoti *et al.* (1986) reported that 40 % crude protein diet was optimum for growth and utilization of *C. lazera* fry while no significant difference (P > 0.05) was observed between the protein values of the carcasses of the fish fed 37 % and 40 % protein diets. Although no significant correlation (P > 0.05) was established between nitrogen metabolism and specific growth rates of *C. gariepinus* fry (Ugwu *et al.,* 2001), the workers reported that 28 - 56 day-old fry of the African catfish, metabolized less nitrogen than 56 - 84 days old fry. The responses of fish to dietary protein levels could vary among warm water fish species. For instance, while Ogunji and Wirth (2001) reported increased growth rates and body protein of tilapia (*Oreochromis niloticus*) fingerlings as the dietary protein increased, the specific growth rates of *C. gariepinus* fry were not significantly different (P > 0.05) when fed diets ranging between 31 % and 40 % protein (Ugwu *et al.,* 2001). Dabrowski (1977) earlier indicated that the determination of optimal protein diet for fish was complicated because protein level is considerably affected by the components of the

diet such as the type of dietary protein and the experimental condition. This makes it difficult to compare studies. The need to relate all reports about the dietary requirements and the nutritional response studies of fish has been suggested by Ogunji and Wirth (2001).

Other studies (Jauncey, 1982; Wang et al., 1985; and Ogunji and Wirth, 2000) have also identified the protein requirements of different fish species with varied results on the effect of protein on growth rate, food conversion and body composition. Variability in protein requirements could possibly be due to the different effects of the free amino acids supplied by the dietary proteins and the consequent catabolism of tissue proteins. It has been reported that α-keto acids resulting from the catabolism of free amino acids are used as a source of energy or carbon for fat synthesis or for glycogenesis (Kim *et al*., 1992). The workers also maintained that amino acid oxidation is principally influenced by the level of protein (or amino acid) or other energy sources in the diets. In addition, in the circulatory fluid of animals including fish, most lipids form complexes with protein (Gotto *et al.,* 1986) in the form of lipoproteins, which are the major carriers of lipids and other hydrophobic compounds (Ando and Mori, 1993). It is therefore possible that a lack of adequate protein may result in loss of serum lipoprotein thereby affecting the transport and storage of lipids in *C. gariepinus.* Therefore, a deficiency of protein may impede physiological functions and further reiterates the need for optimal dietary protein for effective fish rearing. This study investigated the influence of dietary protein content on gross efficiency of food conversion and protein utilization of African catfish (*C. gariepinus*) fry and also studied other growth and nutritional parameters of the species that are affected by dietary protein intake.

MATERIAL AND METHODS

Experimental Procedure: Four hundred and eighty (480) advanced fry (mean initial weight, 1.60 ± 0.24 g) of *C. gariepinus* were randomly allotted to 8 triplicate 25 L plastic baths at 20 fry per bath, replicated three times and allowed to acclimatize for 14 days in the Research Laboratory of Ebonyi State University, Abakaliki, Nigeria. The fish were fed for 56 days with eight diets, seven of which were formulated to yield 28, 31, 34, 37, 40, 43 and 46% crude protein content while the 8th diet comprised a 48.8% crude protein microencapsulated whole egg diet (M). Gross components of diets calculated with Pearson's square method (De Silva and Anderson, 1995) is shown in Table 1. Temperature readings of water were taken thrice daily with a maximum and minimum thermometer while the pH was recorded with a pH meter (model PH J – 201 L). The water conductivity was measured with a conductivity meter and dissolved oxygen was measured with Hach test kit FF3.

The fish were fed at four-hourly interval starting from 0800 h, at the rate of 5% (live weight basis) of their total biomass per day in three portions. Weekly weighing of the fish was carried out with the aid of a Mettler balance (model P 1210) and the feed administered was adjusted in accordance with the body weight of fish. Owing to the fouling of water by faeces and other feed debris, the plastic baths were cleaned on weekly (7 days) basis and replenished with clean tap water.

Analytical Procedure: The proximate compositions of both the experimental diets and fish were analysed by methods described by Windham (1996). Crude protein was determined by micro kjeldahl method, fat by soxhlet extraction method, fibre by the ceramic fibre filter method and ash by combusting in muffle furnace at 600º C for 2 h. The digestible carbohydrate content was computed by obtaining the difference between the % crude protein + % fat + % fibre + % ash contents and 100%. The amino acid concentrations of samples were determined by acid hydrolysis and high performance liquid chromatography (HPLC) method as described by Ogungi and Writh (2001).

Determination of Growth and Nutrient Parameters: The mean weight gain (MWG) of fish was computed following Ishwata (1969) method. The daily rate of growth (DRG) was calculated from the relationship between the mean increase in weight per day and the body weight of fish, thus: DRG = (mean increase in weight)/(body weight of fish). The daily rate of feeding (DRF) was obtained from the expression: DRF = (mean ration per day)/(body weight of fish). While the gross efficiency of food conversion (GEFC) was calculated from the relationship between the daily rate of growth (DRG) and daily rate of feeding (DRF): GEF = DRG/DRF.

The nitrogen metabolism (Nm) was derived using the method of Dabrowski (1977), thus:

$$\mathrm{Nm} = \frac{(0.549)(\mathrm{a}+\mathrm{b})\mathrm{h}}{2},$$

where: a = initial weight of fish, b = final weight of fish, h = experimental duration in days.

The net protein utilization (NPU) was estimated according to Miller and Bender (1955) method, thus:

$$\mathrm{NPU} = \frac{\mathrm{b} - \mathrm{No} + \mathrm{Nm}}{1\mathrm{b}},$$

Table 1: Gross composition (% dry matter) of experimental diets fed to *Clarias gariepinus* fry or 56 days

Diet (% crude protein in diet)	Feed ingredient											
	Yellow maize	Groundnut Cake	Fishmeal	Blood meal	Brewers waste	Oyster shell	Ad-vit[1]	Salt	Palm oil	Bone meal	Egg (M)[2]	Total
1 (28.00%)	43.10	28.71	8.61	5.73	5.00	0.50	0.60	0.25	5.00	2.50	-	100
2 (31.00%)	36.70	32.97	9.89	6.59	5.00	0.50	0.60	0.25	5.00	2.50	-	100
3 (34.00%)	30.30	37.23	11.17	7.47	5.00	0.50	0.60	0.25	5.00	2.50	-	100
4 (37.00%)	29.92	41.49	12.45	8.30	5.00	0.50	0.60	0.25	5.00	2.50	-	100
5 (40.00%)	17.54	45.74	13.72	9.12	5.00	0.50	0.60	0.25	5.00	2.50	-	100
6 (43.00%)	11.14	50.03	15.03	9.95	5.00	0.50	0.60	0.25	5.00	2.50`	-	100
7 (46.00%)	4.80	54.26	16.31	10.78	5.00	0.50	0.60	0.25	5.00	2.50	-	100
8 (48.80%)	-	-	-	-	-	-	-	-	-	-	100	100

[1]Advit: Pfizer livestock feeds production supplying the following vitamins and minerals per grain of diet: A, 19823 IU; D_3 , 1965 I.U; B_{12}, 10 g ton^{-1}; Riboflavin, 41 mg; Niacin, 246 mg; Pantothenic acid, 98 mg; Folic acid, 19 mg; Manganese, 241 mg; Zinc, 100 mg; Iodine, 20 mg; and Oxytetracycline hydrochloride, 20 g ton^{-1}. [2]M = Micro encapsulated whole egg diet.

Table 2: Proximate composition of experimental diet for *Clarias gariepinus* fry

% crude protein diets	Proximate composition (% dry matter)									
	Dry matter	Crude protein	Ether extract	Ash	NFE[1]	Crude fibre	ME[2] (KJ/kg)	TDN[3]	[4]SE	ME:Protein ratio (KJ/mg)
28	86.57	27.86	10.24	12.96	40.93	8.01	12.98	80.44	75.36	10.25
31	88.64	30.46	10.36	13.68	38.23	7.27	13.00	80.56	75.52	10.05
34	90.71	33.68	10.72	14.72	33.78	7.10	13.03	80.84	75.61	9.18
37	89.25	36.56	10.86	10.42	33.96	8.20	12.72	80.72	75.74	8.30
40	88.92	38.68	11.24	10.68	32.33	7.07	12.59	80.96	75.60	7.60
43	87.83	41.72	12.43	9.74	28.9	7.20	12.58	81.36	74.45	7.56
46	91.45	43.46	13.85	9.63	26.64	7.02	12.57	81.45	72.92	7.52
48.80 (M)	-	48.80	43.36	-	-	-	89.47	-	-	9.88

[1]Nitrogen Free Extract = 100 – (% Ash + % crude fibre + % Fat +% Protein). [2]Metabolizable energy; [3]Total digestible nutrient. [4]Starch equivalent.

where: No = nitrogen content of fish before the experiment, Nb = nitrogen content of fish after the experiment, Nm = nitrogen metabolism, 1b = nitrogen content of experimental diet.

Statistical Analysis: The analysis of variance to test for statistical difference among treatment means (Steel and Torrie, 1980), and factorial and regression analyses of measured parameters were done with the computer Statistical Package for Social Sciences (Nie *et al*., 1972). Prediction equations for the growth and nutrient parameters were derived to reflect the degree of linear relationships (Y = a + bx) of the sets of parameters.

RESULTS

The results are shown in Tables 2 – 7. Tables 2 and 3 showed the proximate compositions of the experimental diets and the fish respectively. Table 4 shows the selected amino acids composition of the experimental diets. The mean weight gain (MWG) of *C. gariepinus* fry fed the different diets is shown in Table 5. Table 6 shows the correlation matrix of nutrient parameters of the *C. gariepinus* fry. The mean water temperature was 28.0 ± 1.0º C, the mean pH was 6.8 ± 0.1, the mean water conductivity was 1.03 ± 0.10g S/cm and mean dissolved oxygen was 5.55 ± 1.00 mg/L. There were relatively lower qualities of dietary proteins at the lower CP levels (28% and 31%) (Table 2). These produced lower protein deposition in the body of the fish fed the diets (Table 3) compared to the higher protein deposition in fish fed the higher dietary CP levels (37 % and 40 %). However, the fish also deposited relatively lower protein when fed higher CP diets of 43 % and 46% than the 37% and 40% CP diets. Table 4 also shows that there are satisfactory levels of amino acids in the 37% and 40% CP levels compared to lower CP levels. The amino acid levels in 43% and 46% CP diets were however in most cases higher than the lower CP levels.

Table 3: Proximate body composition of *Clarias gariepinus* fry fed different dietary protein levels for 56 days

% crude protein in the diet	Proximate body composition (% dry matter)					
	Moisture	Crude protein	Ether extract	Crude Fibre	Ash	Gross Energy (KJ g^{-1})
Initial	75.82	59.68	26.68	0.70	12.94	23.15
28	73.64	63.25	25.66	0.68	10.41	22.31
31	72.53	64.36	24.56	0.64	10.44	21.96
34	70.35	65.46	25.26	0.60	8.68	22.68
37	75.46	66.38	24.78	0.64	8.20	23.67
40	75.35	66.66	24.12	0.61	8.61	22.82
43	74.80	65.23	26.02	0.60	8.15	23.32
46	76.30	64.5	26.68	0.62	8.20	23.46
48.8	98.66	-	-	-	-	-

Table 4: Selected amino acids composition (% dry matter) of experimental diets fed to *Clarias gariepinus* frys[1]

Name of amino acid	Experimental diet[2]							
	28%	31%	34%	37%	40%	43%	46%	48.8%
Aspartic acid[3]	1.72	1.99	2.08	3.02	3.20	4.21	2.58	4.36
Glutamic acid[3]	3.26	3.56	3.72	3.95	3.48	4.68	3.73	4.78
Serine[3]	1.00	1.15	1.25	1.32	1.60	0.62	0.72	1.58
Histidine[3]	0.38	0.40	0.60	0.50	1.77	0.80	1.67	1.02
Glycine[4]	0.88	0.92	0.98	0.98	1.06	0.97	0.86	1.35
Threonine[3]	0.98	1.00	1.01	1.05	0.68	0.82	0.72	1.00
Arginine[3]	0.97	1.10	1.23	1.31	1.57	1.66	0.98	1.10
Carnosine[4]	0.04	0.06	0.08	0.08	0.11	0.17	0.45	0.13
Taurine[4]	0.01	0.02	0.05	0.04	0.07	0.02	0.16	0.002
Alanine[3]	0.98	1.00	1.17	1.66	1.80	1.75	1.96	2.96
Tyrosine[3]	0.97	1.00	1.15	1.25	1.43	1.52	1.58	1.66
Valine[1]	0.05	0.06	0.07	0.08	0.11	0.12	0.14	0.13
Phenyl alanine	1.00	1.11	1.21	1.25	1.33	1.68	1.73	1.80
Isoleucine	0.60	0.80	1.00	1.06	1.22	1.37	1.31	2.45
Leucine[3]	1.00	1.04	1.07	1.11	0.80	0.79	0.79	0.94
Ornitine[4]	1.36	1.56	1.66	1.80	2.04	2.28	2.13	2.30
Lysine[3]	0.02	0.02	0.03	0.03	0.04	0.05	0.06	0.06

[1]Analysis was carried out using HPLC; 5 mg samples were hydrolyzed at 110º C for 24 h. [2]All values of each amino acid at different levels in a row are significantly different ($p < 0.05$). [3]Essential amino acids; [4]Non-essential amino acids.

Table 5: Growth and nutrient utilization of *Clarias gariepinus* fry fed different dietary protein levels[1]

% Crude protein in the diets	Growth and nutrient parameters							
	Initial weight (g)	Final weight (g)	Weight gain (g)	DRG[2] (g)	DRF[3] (g)	GEFC[4]	Nm[5]	NPU[6] mg IU
28	1.60	2.38	0.28^{c}	0.013^{b}	0.10^{b}	0.70^{b}	4.58^{b}	1.15^{c}
31	1.84	2.22	0.38^{d}	0.016^{b}	0.11^{b}	0.09^{b}	5.99^{b}	1.23^{c}
34	1.46	2.10	0.64^{c}	0.014^{b}	0.10^{b}	-0.02^{c}	5.18^{b}	1.08^{c}
37	1.62	2.48	0.86^{c}	0.017^{b}	0.14^{b}	0.11^{b}	6.46^{b}	0.85^{b}
40	1.63	3.56	1.93^{a}	0.020^{b}	0.11^{b}	0.11^{b}	12.62^{c}	2.29^{d}
43	1.80	2.36	0.56^{b}	0.008^{c}	0.06^{b}	0.04^{a}	13.43^{c}	2.64^{d}
46	1.60	2.16	0.51^{b}	0.007^{c}	0.07^{b}	0.02^{a}	13.68^{c}	2.86^{d}
48.8	1.56	2.07	0.51^{b}	0.009^{c}	0.03^{b}	0.01^{a}	3.00a	0.39^{a}

[1]Values in the same column having the same subscript are not significantly different ($P > 0.05$); [2]Daily rate of growth. [3]Daily rate of feeding. [4]Gross efficiency of food conversion. [5]Nitrogen metabolism; [6]Net protein utilization

In all cases, for each amino acid there was significant difference in amino acid values among the diets ($P < 0.05$). In Table 5, the weight gain tended to increase with the increase in dietary crude protein (CP) up to 40% and declined as the dietary CP increased up to 48.80%. There was a significant effect ($P < 0.05$) of increase in the dietary CP on MWG (Table 5). While MWG was significantly positively correlated ($P < 0.05$) with the daily rate of growth ($r = 0.87$) no significant correlation ($P > 0.05$) was established between MWG and the daily rate of feeding ($r = -0.42$), the

Table 6: Correlation matrix of nutrient parameters of *Clarias gariepinus* fry fed different dietary protein levels[1]

	DRG[2]	DRF[3]	GEFC[4]	NM[5]	MWG[6]
DRG[2]	1.00	-	-	-	-
DRF[3]	-0.43	1.00	-	-	-
GEFC[4]	0.12	0.18	1.00	-	-
NM[5]	-0.13	-0.03	-0.07	1.00	-
MWG[5]	0.87* *	-0.42	0.26	-0.17	1.00

*[1]For statistical significance, ** = significant at 1% (P < 0.01); those figures without * or ** are not significantly correlated at 1% (P < 0.05) or 1% (P < 0.01). [2]Daily growth rate (g), [3]Daily rate of feeding (g), [4]Gross efficiency of food conversion (g)., [5]Nitrogen metabolism, [6]Mean weight gain per week (g).*

Table 7: Prediction equations of nutrient parameters of *Clarias gariepinus* fry fed different dietary protein levels[1]

Dependent Variable y	Independent variable x	Predication equation y = a+ bx	± S.EM	r	r^2	Significant level.
Daily rate of feeding	Daily rate of growth	Y = 0.13 – 0.80x	0.03	0.43	0.27	n.s.
Nitrogen metabolism	Protein intake per week	Y = 0.47 + 13.49x	1.19	0.94	0.89	* *
Gross efficiency of food conversion	Daily rate of growth	Y = 0.02+ 2.26x	0.32	0.12	0.02	n.s.
Mean weight gain per week	Feed per fish per week	Y = 0.12+ 0.08x	0.11	0.19	0.04	n.s.

*[1]For the statistics, n.s. = not significant at 5% probability; ** = significant at 1% probability; r = correlation coefficient; r^2 = coefficient of determination; SEM = standard error of mean.*

gross efficiency of food conversion (r = 0.26), and the nitrogen metabolism (r = 0.17) (Table 6).

Whereas there was significant difference (P < 0.05) in the mean values of DRG of fish (Table 5), the mean values of DRF were not significantly different (P > 0.05) as the dietary CP increased from 28 to 48.80%. For the DRG, the values were significantly the same from 28% to 40% but significantly declined thereafter as the CP level approached 48.80% (P < 0.05). Both the DRF and the DRG were each not significantly correlated with GEFC and Nm (P > 0.05) (Table 6). While DRG was significantly correlated with MWG (P < 0.05), DRF was not significantly correlated (P > 0.05).

Fish responses to the gross efficiency of food conversion (GEFC) showed no definite pattern at the lower dietary CP levels (28 % to 38%) when compared to the responses at the higher CP levels (43% to 48.8 %) where the GEFC decreased with the increase in dietary CP. The best responses of fish to GEFC was recorded between 37 to 40 % CP diet (Table 5) and this was corroborated by the higher estimates of protein contents in the body of fish that were fed the respective diets (Table 3). The effect of the increase in the dietary CP on GEFC was significantly correlated (P > 0.05) with Nm, DRF, and MWG respectively.

Estimates of the nitrogen metabolism (Nm) and the net protein utilization (NPU) of fish showed that both parameters increased as the dietary CP level increased except for the micro encapsulated diet M (48.80 %), where Nm and NPU estimates were relatively low (Table 5). The effect of the dietary increase in CP on Nm and NPU was significant (P < 0.05). The Nm of fish showed non-significant negative correlations with DRG, DRF, GEFC and MWG. The prediction equations for the growth and nutrient parameters are shown in Table 7.

As expected the relatively lower qualities of dietary proteins at the lower CP levels (28 % and 31 %) (Table 2) produced lower protein deposition in the body of the fish fed the diets (Table 3) compared to the higher protein deposition in fish fed the higher dietary CP levels (37% and 40%). However, the fish deposited relatively lower protein when fed diets of between 37 % and 40 % CP levels even when the dietary CP levels of the former were higher than those of the later. The fish responded more positively to weight increase (MWG) and daily growth (DRG) at the 37 % and 40 % dietary CP levels than at the 28 % and 31 % or 43 % and 48.80 % dietary CP levels (Table 5). Nevertheless, the fish did not exhibit any definite pattern of response to the essential amino acids (EAA) profiles of the experimental diets (Table 4) as the dietary CP levels increased.

DISCUSSION

The increasing levels of dietary crude protein (CP) in this study seemed to affect the increase in weight and daily rate of growth of the young *C. gariepinus* up to 40 % CP (Table 5) but declined at higher CP level (48.80%) This result compares favorably with that of Faturoti *et al*. (1986) on juvenile *C. lazera*, in which the optimum dietary

protein content that enhanced growth was 40%, while the micro encapsulated egg diet depressed growth. The 48.80 % CP in the diet used in this study must have been in excess of the CP required by the fish to support efficient utilization of available nutrients as was the case in Faturoti *et al.* (1986). From the present study, the relatively higher metabolizable energy:protein (ME:P) ratio recorded for the 48.80% CP diet (9.88 kJ/mg) as against that of the 40% CP diet (7.6 kJ/mg) also agrees with the report of Cho (1981). The worker stipulated that a higher energy-protein ratio may result in inadequate protein intake and that the loss of most of the ingested nitrogen as ammonia might retard the deposition of protein for tissue formation.

Previous workers on other warm water fishes have provided ME:P ratio that gave optimum growth with specified dietary protein levels. Jauncey (1982) reported an ME:P ratio of 27.81 mg/kg for tilapia (*Sarotherodon mossambius*) fed with 40% CP diet, while Mazid *et al.* (1979) estimated an ME:P ratio of 19.43 mg/kg for *Tilapia zillii* fed with 35% CP diet. Generally, it is obvious from various reports that ME:P ratio varies significantly between fish species and within species depending on the digestibility and amino acid composition of the protein source, water temperature (Hildalgo and Alliot, 1988) and the environmental parameters which affect the portioning of energy (De Silva and Anderson, 1995). The lower values of DRG recorded at the higher CP levels (43% to 48.80%) were in contrast with the high DRG recorded at the lower CP levels (Table 5).

This indicates that despite the non-significant effect ($P > 0.05$) of the daily feeding rate (DRF) of fish among the test diets, the content of the diet, caused by the higher fibre contents of some of the ingredients pronounced at the higher CP levels. This must have also resulted in less protein being consumed for optimum growth. Dilution effect of bulk resulting from fibre has been reported by Lovell (1989). The best response of fish to the gross efficiency of food conversion (GEFC) was within 37% and 40% dietary CP and this reflected the best weight increase (MWG = 0.86 to 1.93 g); daily rate of growth (DRG = 0.17 to 0.20 g) and nitrogen metabolism (Nm = 12.62 to 13.43 g/100 g) obtained within the experimental period. The high Nm and NPU (Table 5) recorded for 37 and 40% CP diets paralleled the relatively high protein contents of the fish that were fed these diets (Table 3). It is hence obvious that the energy content of the diets were optimum within this CP range (37%- 40%) as to spare the protein for tissue formation. The decline in the GEFC of fish fed 43% to 48.80% diets conforms to the earlier The decline in the GEFC of fish fed 43% to 48.80% diets conforms to the earlier deductions made with respect to the diets. However, the net protein utilization values for fish fed the 37 % to 40 % CP diets were less than those fed at higher CP levels. This result varies from the results of previous worker such as Jauncey (1981) for tilapia (*S. mossambicus)*; Ogino and Saito (1970) for common carp (*Cyprinus carpio*), and Mazid *et al.* (1979) for *Tilapia zillii.* These workers reported a decrease in NPU with increasing dietary protein level. It could be that the *C. gariepinus* fry in the present study utilized protein at the higher CP levels for other physiological processes than for protein synthesis and growth. It is therefore inferred that despite the apparently better utility of protein (NPU) by fish fed the higher CP diets, much of the ingested protein have been affected by endogenous nitrogen losses resulting in its unavailability for productive use by the fish.

REFERENCES

ANDO, S. and MORI, Y. (1993). Characteristics of Serum Lipoprotein features associated with Lipids level of muscle and liver from five species of fish. *Nippon Suisan Gakkaishi 59 (9):* 1565 – 1571.

BRETT, J. R. (1976). Scope for metabolism and growth of sockeye salmon, (*Onyhorhynchus nerka*) and some related energetics. *Journal of Fisheries Research Board of Canada 33:* 30 7 – 313.

DABROWSKI, K. (1977). Protein requirement of carp (*Ctenopharyngodon idella* Val) *Aquaculture, 10*: 63 – 73.

CHO, C. Y. (1981) Microencapsulated *egg diet for fish larvae*. FAO, Rome, Italy ADCP/REP/80/11.

DE SILVA, S. S. and ANDERSON, T. A (1995). *Fish nutrient in aquaculture.* Chapman and Hall London. 319 pp.

FATUROTI, E. O. BALOGON., A. M. and UGWU, L. L. C. (1986). Nutrient utilization and growth responses of Clarias, *Clarias lazera* fed different dietary protein levels. *Nigeria Journal of Applied Fisheries and Hydrobiology, 1*: 41 - 45.

GERKING, S. D. 1972. Influence of rate of feeding and body weights upon protein metabolism of bluegill sunfish. *Physiological Zoology, 44*: 1 - 14.

GOTTO, G. O. POWNALL, H. J. and HAVEL, R. J. (1986). *Introduction to Plasma Lipoproteins.* Pages 3 - 41. In: SEGREST, J. P. and ALBERS, J. J. (Eds.). *Plasma lipoproteins.* Academic Press Inc. Orlando, Florida.

HILDALGO, F and ALLIOT, E. (1988). Influence of water temperature on protein requirement and protein utilization in juvenile sea bass

(*Dicentrarchus labra*). *Aquaculture, 64*: 209 - 217.

ISHWATA, N. (1969). Ecological studies on the feeding of fishes VII. Frequency of feeding and growth. *Bulletin of Japanese Society of Scientific Fisheries, 35*: 985 - 989.

JAUNCEY, K, (1982). The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia (*Sarotherodon mossambicus*).*Aquaculture, 27*: 43 - 54.

KAUSHIK, S. J., DOUDET, T., MEDOLE, F., AGUIRRE, P. and BLANK, D. (1995). Protein and energy needs for maintenance and growth of Nile Tilapia *(Oreochromis niloticus). Journal of Applied* Ichthyology*, 11*: 290 - 296.

KIM, K. I., GRENSHOW, T. W, KAYES, T. B. and AMUNSO, C. H. (1992). Effects of fasting or feeding diets containing different levels of protein and amino acid on the activities of the live amino acid-degrading enzymes and amino acid oxidation in rainbow trout (*Onchorynchus mykis*). *Aquaculture, 107*: 89 – 105.

LOVELL, T. (1989). *Nutrition and feeding in fish*. Van Nostrand, New York. 260 pp.

MAZID, M. A., TANKA, Y., KATAYAMA, I., RAHIMAN, M. A., SIMPSON, K. L. and CHI CHESTER, O. C. (1979). Growth responses of *Tilapia zillii* fingerlings fed iso-caloric diets with variable protein levels. *Aquaculture, 18*: 115 -122**.**

MILLER, B. and BENDER, A. (1955). Determination of net utilization of protein by estimated method. *British Journal of Nutrition, 9:* 382 – 388.

NEW, M. B. (1987). *Feed and Feeding of Fish and Shrimp – A Manual on the Preparation and Presentation of Compound Feeds for Shrimp and Fish in Aquaculture*. Aquaculture Development and Coordination Programme ADCP/REP/87/ 26. United Nations Development Programme, FAO Rome. 275 pp.

NIE, M.N., HULL, C. C. M. JENKIMS, J.G. TEIN-BRENNER, K. and BENT, D.H. (1975). *Statistical package for social sciences*: 2nd ed. McGraw-Hill, New York. 155 pp.

OGINO, C. and SAITO, K. (1970). Protein nutrition in fish 1. The utilization of dietary protein by carp. *Bulletin of Japanese Society of Scientific Fisheries, 39:* 250 - 254.

OGUNJI, J. O. and WIRTH, M. (2000). Effect of dietary protein content on growth, food conversion and body composition of tilapia *(Oreochromis niloticus*) fingerlings fed fishmeal diets. *Journal of Aquaculture in the Tropics, 15(4):* 381 - 389.

OGUNJI. J. O. and WIRTH, M. (2001). Alternative protein source as substitutes for fish meal in the diet of young tilapia (*Oreochromis niloticus* Linn.). *Israeli Journal of Aquaculture, Bamidgeh, 43(1):* 34 – 43.

STEFFENS. W. (1989). *Principles of fish nutrition*. Euis Horwood, London. 384 pp.

STEEL, R. G. D. and TORRIE, J. H. (1980). *Principles and procedures of statistics: a biometrical approach.* McGraw-Hill, New York, NY. 633 pp.

UGWU, L. L. C., MGBENKA, B. O. NWAMBA, H. O. and ODO, B. I. (2001). Nitrogen Metabolism and specific growth rate of Africa Catfish fry, *Clarias gariepinus* fed different dietary protein levels. *Journal of Science of Agriculture Food Technology and Environment, 1:* 5 – 8.

WANG, K TAKEUEHI, T. and WATANABE, T. (1985). Effect of dietary protein levels on growth of *Tilapia niloticus. Bulletin of Japanese Society of Scientific Fisheries, 51:* 133 – 140.

WINDHAM, W. R. (1996). Animal feed. Pages 1 - 38 (Chapter 4). *In:* Cunniff, P. (ed.). *Official Methods of Analysis of Association of Official Analytical Chemists International (AOAC), 16th edition Vol I*, Gaithersburg, Maryland, USA.

THE EFFECTS OF SEASON AND DISTANCE ON THE PREVALENCE AND INTENSITY RATES OF URINARY SCHISTOSOMIASIS IN AGULU-LAKE AREA OF ANAMBRA STATE, NIGERIA

EKWUNIFE, Chinyelu Angela
Department of Biology, P.M.B 1734, Nwafor Orizu College of Education Nsugbe, Anambra State, Nigeria

ABSTRACT

A survey of the effects of season, and distance to water source on the prevalence and intensity rates of Schistosoma haematobium infection in Agulu community of Anambra State was conducted using the primary schools in the town, using parasitological screening approach. Prevalence was similar in both the dry and rainy season months. Seven out of the 15 Primary schools surveyed had pupils with infection. Prevalence in schools with infection ranged from 4.1- 55.2 % during the dry season to 3.3-55.6 % during the rainy season. Prevalence and geometric mean egg count were highest in the 10-14 years age group in all the schools and in both dry and rainy seasons. Geometric mean of egg count / 10ml urine (intensity) decreased from 22.5 egg/10 ml in dry season to 10.7 egg/10 ml in wet season. Prevalence rates and intensity showed significant decrease with increase in the distance from the village to the lake. The implications of these are discussed.

Keywords: Urinary schistosomiasis, Season, Distance, Prevalence rate, Intensity, Geometric mean

INTRODUCTION

Quantitative urine examination techniques have in recent years replaced qualitative procedures in community studies of schistosomiasis because of the useful information provided by quantization of egg output. This is because the sensitivity of the method could be tested and the results could be expressed in terms that allow comparism with other studies e.g. geometric mean. The usefulness of quantitative technique is now recognized by national control programmes, which have achieved significant reduction on prevalence from double to single figures in large areas. The epidemiology of S. haematobium in man has been described in term of age - specific prevalence and school children have often been studied. This is because they represent he age groups at greatest risk and with greatest intensity of infections, thus providing convenient baseline data for the whole population (Forsyth, 1969, Wilkins, 1977). Thus quantitative urine examination using school children could help to give useful information on the state of urinary schistosomiasis in Agulu town during both rainy and dry season conditions. This shall help in disease control and shall allow comparison with other studies on urinary schistosomiasis from different regions.

One of the important determinants of a household's choice of water source has been reported to be distance (Blum *et al* 1987). Jones (1973) found the distance beyond which people had negligible contact with lake Volta to be 5km. Appleton and Bruton (1979) also reported that only about 1/4 of the people who live within 5km of lake Sibaya (South Africa) probably depend on it or its adjacent ponds for water and so have frequent contact with these habitats. The remaining 3/4's they reported use streams or pans for domestic purposes although some of those people may have to use the lake or ponds during winter or dry years. There is a need for more studies of that kind to evaluate the relationship between distance to water sources in endemic areas and prevalence of schistosomiasis. Such are deemed to be of great value in planning disease control programmes. The paper reports a study of schistosomiasis infection in Agulu town of Anambra State, Nigeria, where a lake implicated in the transmission of the disease is situated.

MATERIALS AND METHOD

The Study Area: Agulu town (Figure 1) was purposively selected for the study because of previous knowledge of the presence of *S. haematobium* infection in the town (Emejulu 1994, Emejulu *et al,* 1994). Agulu which is in Anaocha Local Government Area is located between latitude $6^{o}06^{/}$N and longitude $7^{0}03^{/}$E. Coming from the South, the land is generally a steep dive towards the lake. It enjoys tropical type of climate.

Urine Collection and Analysis: Parasitological screening of all the primary and attached preprimary school children in the 15 primary schools in the town was carried out between 1999 – 2000 in both dry and rainy seasons. Wide mouthed screw cap containers with numbers for identification were used to collect urine samples from each pupil in the different schools on visitation. Urine collection was made between 10.00 and 14.00h. This is the period of greatest egg out put (Stimmel and Scott, 1956, Bradley, 1963). This was done during the dry season months for 16 weeks (Nov.1999-Feb.2000) and

repeated during the rainy season months for 16 weeks (May-August 2000). A simple centrifugal sedimentation procedure (5min at 5000rpm) of 10ml aliquot urine drawn from each specimen was used (Oliver and Uemura, 1973). *S. haematobium* ova in the sediment poured on a Macmaster slide were counted under X10 microscope eyepiece. Calculation of geometric mean egg count was done using the method in WHO (1987). Distance from the centre of each village to the lake, the crowflies was estimated and recorded.

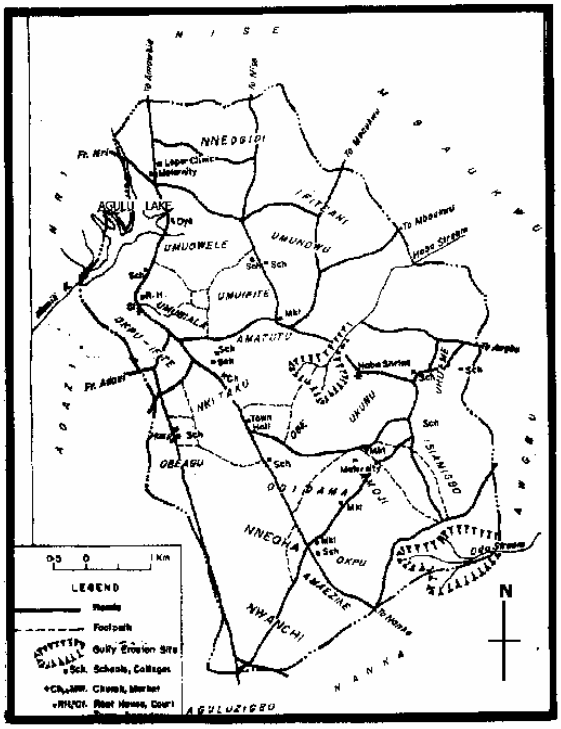

Figure 1: Map of Agulu town

Data Analysis: Dry season age group Infection Rate = Number people Infection among age group in affected school during the dry season/ Total Number of people in the age group X 100.

Rainy season age group Infection Rate = Number people Infection among age group in affected school during the rainy season / Total Number of people in the age group X 100.

Total infection for each season = Total of all infected in all school for that season / Total Number of people in all the school x 100. Age group GM = Geometric mean of all the infected individuals of an age group in all schools together.

Total GM = Geometric mean of all the infected individuals in all the schools together. In all the cases descriptive statistics was employed to ascertain the means and mean differences between season and distance ascertain using t-test.

RESULTS

Seven schools out of fifteen had individuals that excreted *S. haematobium* ova during both the dry and rainy seasons (Table 1). The schools include Umuowelle Primary School, Umuifite Primary School, Umunowu Primary School, Nneogidi Primary School, Practicing Primary School, Ifiteani Primary School and Obeagu Primary School. For dry and rainy seasons, Umuowelle Primary School recorded the highest infection rates of 55.2% and 56.6% respectively followed by Ugwuaba Primary School with infection rates of 43.2% and 43.1% for both seasons. Obeagu Primary School recorded the lowest infection rates of 4.1% and 33.3% for the dry and rainy seasons respectively. Table 2 shows that in Umuowelle Primary School, infection rates of 29.4 %, 61.1 %, 70.1 % and 55.2 % were recorded for 0-4, 5-9, 10-14 and 15-19 age groups respectively during the dry season while infection rates of 34.1 %, 54.8 %, 73.3 % and 52.2 % respectively were recorded for same age groups during the rainy season. Infection rates for the other schools had similar patterns for both season. However, the geometric mean of egg count/10ml urine decreased during the rainy season for the different ages in the different schools. Umuowelle Primary School recorded the geometric mean (GM) of egg output of 11.2, 29.9, 48.4 and 28.5 during the dry season for the age groups 0-4, 5-9, 10-14, 15-19, respectively while during the rainy season the GM were 8.1, 20.2, 36.4 and 29.9 for same school and same age groups. Overall geometric mean of egg count/10ml urine decreased from 22.5 egg/10ml in dry season to 10.7 egg/10ml in wet season (Tables 2 and 3).

The prevalence and intensity rate among the schools in Agulu with respect to distance is shown in Table 4. Agulu lake is situated at the northwest of the town. The infection rate increased as one moved from south to north and from east to west. The number of positive cases of urinary schistosomiasis decreased as one moved away from the lake. Umuowelle Primary School which is closest to the lake (200m) had the highest prevalence rate of 55.2% followed by Umunifte Primary School at a distance of 300m which had a prevalence of 43.2%, while Obeagu Primary School which is up to 2km had the lowest prevalence 4.1%. Other schools which are at a distance of 2.5km or more had 0%. Correlation analysis confirm this to be significant (t=2.57, df=5) at 5% level. The intensity of infection also decreased as one moved away from the lake. Umuowelle Primary School nearest to the lake (200m) had 31.1 intensity while Obeagu Primary School which is about 2km from the lake had an intensity of 8.9. The decrease in intensity of infection with increase in distance from the lake is significantly correlated at 5% level (t=2.57,df=5).

DISCUSSION

Generally, Prevalence and egg output go together. The most significant association was with age. Both egg output and prevalence rose rapidly in the early years to a peak level in the 10-14 yrs age

Table 1: Prevalence rates of urinary schistosomiasis in primary schools (P/S) in Agulu by season

School	Village located	Dry season			Rainy season		
		No. Exam.	No. Infection	% Infection	No. Exam.	No. Infection	% Infection
Agunkwo P/S	Amaorji	70	0	0	42	0	0
Central ,,	Odidama, Obe	200	0	0	192	0	0
Chukwuka ,,	Uhueme,Ukunu	241	0	0	243	0	0
Community ,,	Umunowu	219	76	34.7	216	74	34.3
Ezeanyanwu ,,	Odidama, Okpu	233	0	0	220	0	0
Nwanchi ,,	Nwanchi,Nneoha Amaezike	110	0	0	90	0	0
Obe ,,	Obe	233	0	219	0	0	0
Obeagu ,,	Obeagu	169	7	4.1	151	5	3.3
Onike ,,	Okpu	140	0	0	112	0	0
Practicing ,,	Nkitaku, Umubiala, Okpuifite, Amatutu	532	128	24.1	506	119	23.5
Udoka ,,	Ukunu, Isimaigbo	189	0	0	180	0	0
Ugwuaba ,,	Umuifite	185	80	43.2	174	75	43.1
Umuowelle ,,	Umuowelle	201	111	55.2	189	105	55.6
Ifiteani ,,	Ifiteani	141	33	23.4	101	22	21.9
Nneogidi ,,	Nneogidi	186	55	29.6	172	50	29.1
Total		3029	450	16.2	2807	450	16.1

Table 2: Infection rate (%) and geometric mean (GM) egg count during dry and rainy season in the endemic villages among age groups

School	Dry season								Rainy season							
	0 – 4		5 – 9		10 – 14		5 – 19		0 – 4		5 – 9		10 – 14		5 – 19	
	%	GM	%	GM	%	GM	%	GM	%	GM	%	GM	%	GM	%	GM
1.	29.4	11.2	61.1	29.9	70.1	48.4	55.2	28.5	34.1	8.0	54.8	20.2	73.3	36.4	52.2	24.9
2	0	0	33.8	19.4	51.2	39.9	42.4	24.5	0	0	36.5	14.0	52.5	30.3	32.3	20.2
3	10.0	11.5	32.4	16.9	50.0	36.7	27.5	23.5	16.7	8.2	30.0	12.4	48.7	22.5	27.5	15.5
4	0	0	22.9	14.7	47.1	31.5	18.8	20.2	0	0	19.1	11.2	47.0	24.2	20.0	13.7
5	0.7	5.0	24.1	10.7	41.3	28.0	32.0	20.0	0	0	22.4	8.2	41.6	21.7	31.9	12.9
6	0	0	14.3	14.7	33.3	19.4	22.2	11.0	0	0	13.3	11.0	33.3	5.2	14.3	7.0
7	0	0	1.9	8.3	8.9	14.8	5.3	14.0	0	0	4.2	8.0	5.9	12.2	0	0

1. Umuowelle Primary School 2. Ugwuaba Primary School 3. Community Primary School 4.Nneogidi Primary School 5. Practicing Primary School 6. Ifiteani Primary School 7. Obeagu Primary School

group and then declined. The infection rates among the various age groups in the different schools were close for both dry and wet season. This could be due to the long life span of the worm (3 – 5 years), (Wilkins *et al* 1984, Fulford *et al* 1995), thus same infected individuals remain infected during both seasons. However, the Geometric mean of egg output declined remarkably in the rainy season. Though the people remain infected in the rainy season, the low egg count during that period could be due to a break in the transmission of disease during the wet season occasioned by non visit/reduced contact with transmission sites at such times since rain water can be collected from the home. This would reduce re-infection as well as accumulated worm load. Further, some worms may have died in the infected individuals and because there would be a reduction in rate of contact with the transmission site, re-infection would not occur. Thus Blum *et al* (1987) in the study of the effects of distance and season on the use of boreholes in northeastern Imo State, Nigeria reported that in wet season when the availability of water sources was much greater, rain water was the main sources of 64% of households since it was collected directly at home. In contrast however, McCullough and Bradley (1973) showed that egg output was stable in individuals for long period of time. But then, their study was conducted in Tanzania during the dry season months of 3 different years. The egg out put in their study population could have dropped during wet season and risen again during the dry season as a result

Table 3: Total seasonal infection rate and GM by age

Age group	Dry season Infection rate (%)	GM	Rainy season Infection rate (%)	GM
0 – 4	6.4	6.7	8.3	2.3
5 – 9	26.7	16.8	26.2	10.1
10 – 14	44.0	31.2	44.5	21.2
15 – 19	30.9	20.3	28.0	12.3
Total	30.0	22.5	29.6	10.7

Table 4: The relationship between prevalence and intensity rates and distance of schools from Agulu lake

Geogra-phical location	Villages	Distance to lake (m and km)	School they attend	% Prevalence	Intensity (GM)
West	Umuowelle	≤200m	Umuowelle P/S	55.2	31
West	Umunifite	.3km(300m)	Ugwuaba ,,	43.2	20.9
North	Umunowu	.45km(450m)	Community ,,	34.7	20.0
North	Nneogidi	.70km(700m)	Nneogidi ,,	29.6	16.9
West	Umubiala	500m	Practicing ,,		
West	Amatutu	700m	Practicing ,,		
West	Okpuifite	1km(.93km)	Practicing ,,	24.1	14.7
West	Nkitaku	1.5km	Practicing ,,		
North	Ifiteani	1.2km	Ifiteani ,,	23.4	11.6
South	Obeagu	2km	Obeagu ,,	4.1	8.9
South	Obe	2.5km	Obe & Central ,	0	0
South	Odidama	2.7km	Central ,,	0	0
East	Ukunu	2.9km	Udoka	0	0
East	Isiamaigbo	3.0km	Chukwuka ,,	0	0
East	Amaorji	3.2km	Agunkwo ,,	0	0
East	Uhueme	3.4km	Agunkwo ,,	0	0
South	Nneoha	3.7km	Ezeanyanwu ,,	0	0
South	Okpu	4.0km	Ezeanyanwu ,,	0	0
South	Amaezike	4.2km	Onike ,,	0	0
South	Nwanchi	4,5km	Nwanchi ,,	0	0

of re-infection. The high Geometric mean egg output recorded by Scott *et al* (1982) in lake Volta Ghana could also be as a result of dry season, only one small water contact site was recognized at Agulu lake during the rainy season. Usual sites on the different arms were over grown by weeds and were very bushy and lacked human activity during the rainy season.

Prevalence and intensity of infections showed a significance decrease with increasing distance to the lake, which is the focus infection. This finding is a pointer to the important role of distance from focus of infection in the prevalence of schistosomiasis in a location, which is portrayed by the fact that children who lived at a considerable distance from the lake had no infection. It is also an indication of the relationship between distance of water bodies from communities and the extent of usage of each water body by the communities, pointing to the fact that communities rely on water sources which are close to their location. Since frequency of contact with focus of infection diminishes with increase in distance, prevalence of water borne disease such as schistosomiasis would be expected to decline with increase in distance from such foci of infection. Similarly, since the population relies on harvested rain water during the rainy season, the number of new cases or re-infected individuals would be expected to drop during the rainy season. The findings of the present study conform to these expectations, and lend support to the observation by Emejulu (1994) that most households use water sources which are very close to them and that very few use sources up to 2km away from home. Distance determines the time spent in collecting water and so affects travel times. Saving of time for other chores could be one of the reasons why the villagers resort to nearest water source. In this study however, few people, who live at a distance of 1.2 km to 2 km or more were found to be infected. This is probably because Agulu lake inspite of its distance from some villages holds sufficient attraction for children from such villages, and such children from far distance would still visit it for recreational purposes. Further, high temperatures in tropical Africa especially in the afternoon compel inhabitant of such tropical areas to look for a place to cool off and the people find such a place of comfort in the lake.

The seasonal decrease on geometric mean egg output of individuals is very important in the context of control measures for the disease. Since

people do not go to the lake during the rainy season, mass treatment of villagers around the schools identified with *S. haematobium* during the rainy season would be beneficial in curtailing incidence of schistosomiasis. Such an approach will have the effect of reducing the parasite load of infected numbers of the affected communities and thus reducing the chances of re-infecting the snails in the water during the dry season. It is also possible that some infected snails would die during the lengthy period of the rainy season because Bayne and Loker (1987) reported that infection significantly reduced snail host survival a happenstance that would further weaken the transmission cycle and make the control measure more effective.

ACKNOWLEDGEMENT

The author wishes to thank Mr. John Emekwulu who served as guide by taking me around both to various Primary schools and during the estimation of lake distance to the villages.

REFERENCES

APPLETON, C. C. and BRUTON, M. N. (1979) Epidemiology of schistosmiasis in the Vicinity of Lake Sibaya, with a Note on other Areas of Tonga land (Natal, South Africa). *Annals of Tropical Medicine and Parasitology, 73 (6):* 316 – 516.

BAYNE, C. J. and LOKER, E. S. (1987). Survival within the Snail Host. Pages 321 – 346. *In:* ROLLINSON, D. and SIMPSON, J. A. (Eds). *The Biology of Schistosomes from Gene to Latrines.* Academic Press Ltd. London.

BLUM, D., FEACHEM, R. G., HUTTY, S. R. A., KIRKWOOD, B. R., and EMEH, R. N. (1987). The Effects of distance and season on the use of boreholes in northeastern Imo State, Nigeria. *Journal of Tropical Medicine and Hygiene, 99:* 45 – 48.

BRADLEY D. J. (1963). A Quantitative approach to Bilharza. *East African Medicine Journal, 49 (5):* 240 – 249.

EMEJULU, C. A. (1994). *Epidemiology of urinary schistosomiasis in Agulu lake area of Anambra State, Nigeria*. MSc Thesis University of Nigeria, Nsukka. 96 pp.

FORSYTH, D. M. A. (1969). A longitudinal Study of Endemic Urinary Schistosomiasis in a Small East African Community. *Bulletin of World Health Organization, 40:* 711 – 783.

FULFORD, A. J., BUTTERWORTH, A. E., OUMA, J. H., STURROCK, R. F. (1995). A statistical approach to schistosome population dynamics and estimation of the life-span of *Schistosoma mansoni in man. Parasitology, 110:* 307 – 316.

JONES, C. R. (1973). Health component in the Volta lake research project - Report on project result, conclusion and recommendations. *WHO unpublished mimeograph Document,* AFR/SCHIST/27

McCLUCCOUGH, F. S. and BRADLEY, D. J. (1973). Egg Output Stability and the Epidemiology of *Schistosoma heamotobuim.* Part I Variation and Stability in Egg Counts. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 67(4):* 475 – 500.

OLIVER, I. J, and UEMURA, K. (1973). Techniques Statistical Methods and Recording Forms. Pages 620 – 748. *In:* ANSARI, N. (Ed). Epidemiology and control of schistosomasis. Karger Basel and University Park Press, Baltimore.

SCOTT, D., SENKER, K. and ENGLAND, E. C. (1982). Epidemiology of human *Schistosoma haematobium* infection around Volta lake, Ghana (1973 – 75). *Bulletin of World Health Organization, 60 (1):* 89 – 100.

STIMMEL, C. M. and SCOTT, J. A. (1956). The regularity of egg out put of *Schistosoma heamatobuim. Texas Reports in Biology and Medicine, 14:* 440 – 458.

WILKINS, H. A. (1977). *Schistosoma haematobium* in a Gambian community I, The intensity and prevalence of infection. *Annals of Tropical Medicine and Parasitology, 71:* 53 – 88.

WILKINS, H. A., GOLL, P. H., Marshall, T. F. and Moore, P. J. (1984). Dynamics of *S. haematobium* Infection in a Gambian community III, Acquisition and loss of infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 78:* 227 – 232.

WHO (1987). Basic Aspects of the Epidemiology of Human African Schistosomiasis. *In:* CHRISTENSEN. N. and FURU, P. (Eds). World Health Organization, Collaborating Centre, Danish.

THE EFFECT OF TRANSPORTATION STRESS ON HAEMATOCRIT LEVEL OF *Oreochromis niloticus* LINNAEUS

ORJI, Raphael Christopher Agamadodaigwe
Department of Fisheries, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

ABSTRACT

Transportation stress was investigated in Oreochromis niloticus (Linnaeus) by transporting samples in open rectangular iron tanks from Panyam fish farm, Plateau State of Nigeria to University of Jos, Nigeria. All fish appeared more stressed with higher densities and increasing media salt employed in transportation. There was a significant difference between mean haematocrit values of control (before transportation) and those of low, medium and high densities ($p < 0.05$). Transportation under different saline concentrations showed significant difference between means haematocrit value of control and varying media saline levels ($p < 0.05$) except 1% saline. There were immediate and delayed mortalities, lasting up to three days after transportation except in aerated samples.

Keywords: Transportation stress, Haematocrit, *Oreochromis niloticus*, Salinity, Oxygen.

INTRODUCTION

Prior studies on problems of handling and transportation of fresh water fishes indicated that severe physiological disturbances accompany capture and transportation. The transportation of live fish from hatchery to the waters in which they are to be stocked is an extremely important aspect of fish culture and fish farm management, (Mc-Craren, 1978; Beraka, 1986). Transportation usually involves handling large numbers of fish in a small amount of water, which, depending on the time involved can result in considerable deterioration of water quality. Sometimes, fish arrive at the stocking site in poor condition due to handling and transportation stress and some may die at the stocking site or shortly there after stocking (shreck and Lorz 1978).

Conditions that alleviate harmful handling and transportation stress have become the focus of research in recent times. Carmichael (1984) observed that when fish were transported at higher densities, the level of cortisol and glucose in the plasma increased. Orji (1998) pointed out that transportation of *O. niloticus* led to decreased hepatic glycogen. Sharp et al (1998), working on effects of routine handling and tagging procedures on the physiological stress response to juvenile Chinook salmon, observed that cortisol increased from resting level of 2 mg/ml by 1 hour post stress and returned to near resting level about 8 hour post stress. Also, Orji (2003), observed that transportation of *O. niloticus* led to increase in interrenal cell nuclear height. Similarly, Specker and Shreck (1979), on stress induced by transportation of Coho Salmon – *Oncorhynchus kitsutch* reported thus: - transportation caused an increase in glucose and cortisol circulation. Acclimated coho salmon are more likely to survive a second stress than unacclimated coho salmon. The stress of transportation occurs during loading and first few hours enroute.

The aim of this paper was to investigate the level of fish mortality as well as some stress reactions of transported fish from a fry production centre – Panyam Fish Farm, Plateau state Nigeria, to the stocking site, - Fisheries Laboratory experimental pond, Zoology department, University of Jos, Nigeria. The transportation covered a measured distance of about 80 kilometres. It also attempted to identify conditions that could heighten or alleviate transportation and handling stress in fish.

MATERIALS AND METHODS

Fish were caught with fish net and kept in acclimation tank for one week after which they were transported in open rectangular iron tanks, measuring (98 x 76 cm), coated with aluminium paint inside. After transportation, they were stocked in three experimental holding ponds each measuring (31.6 x 25.5 cm). Each pond was partitioned into three, so that fish with similar transportation treatments were stocked in the same apartment. Sampling of fish was carried out before and immediately after transportation. Sampling of fish involved taking total length measurements, caudal severance and collection of blood into heparinized capillary micro tubes and sealing them with plasticine. Blood samples collected before transportation served as controls.

Table 1: Mean haematocrit values of *O. niloticus* transported under different densities

Treatment	Mean total length (cm)	Mean heamatocrit value (%)
Control	12.00 ± 1.2	32.82 ± 1.5
Low Density (40 fry/96)	11.35 ± 2.44	22.00 ± 2.69
Medium density (40 fry/72)	11.22 ± 0.88	19.97 ± 2.84
High Density (40 fry/48)	11.30 ± 2.70	15.45 ± 2.97

Table 2: Mean haematocrit values of *O. niloticus* transported under different saline concentrations, with and without aeration

Treatment	Mean total length (cm)	Mean heamatocrit value (%)
Control	12.29 ± 0.77	32.80 ± 1.20
0% Saline + 0_2	12.28 ± 1.43	28.90 ± 2.34
0.Saline + 0_2	13.17 ± 1.56	28.90 ± 2.91
1% Saline + 0^2	13.10 ± 1.46	31.90 ± 1.90
0% Saline - 0_2	12.81 ± 1.80	24.81 ± 1.90
0.6% Saline + 0_2	15.10 ± 1.56	25.50 ± 2.10
1% saline - 0_2	12.20 ± 1.21	26.20 ± 3.02

Table 3: Instant and delayed mortalities of *O. niloticus* transported under different saline concentrations with and without aerations

Date	No	% mortality	Treatments
1st Day	8	8.8	0 Saline without 0_2
"	5	5.5	1 Saline without 0_2
"	3	3.3	0.6 Saline without 0_2
2nd Day	17	18.8	1 Saline without 0_2
"	19	21.1	0 Saline without 0_2
"	7	7.7	0.6 Saline without 0_2
3rd Day	2	2.2	0 Saline without 0_2
"	1	1.1	0.6 Saline without 0_2
"	1	1.1	1 Saline without 0_2

Transportation under Different Densities: The following densities of Tilapia fry were maintained per group (A-C).
A – 40 fry per 48L, for high density; B – 40 fry per 72L, for medium density and C – 40 fry per 96L for low density, each group was replicated thrice.

Transportation under Different Saline Conditions: Sodium chloride (NaCl) levels of 0.6% (25.29g/72L) 1% (113.22g/72L) and 0% (water without addition of saline) were obtained by preparing slurry of the appropriate weights of salt in 200 mls of water thus,
A – 40 fry in 0% saline (water without saline); B – 40 fry in 0.6% saline and C – 40 fry in 1% saline.

Blood Haematocrit Value: Blood was collected into heparinized micro haematocrit tubes from a severed caudal peduncle vessel, centrifuged under standard conditions at 2500 rpm for five minutes. The packed red blood cell volume was measured directly and expressed as percentage of the total blood volume with a microhaematocrit meter according to Wedemeyer and Yasutake (1977).

Determination of Dissolved Oxygen: Water samples were collected with sampling bottles and analysed for dissolved oxygen (DO), using Hach Fish Farmer's water quality test kit (Model FFIA). Two way analysis of variance (ANOVA) was employed to test the significant levels of deviation means from control values. The analysis of variance was extended by use of LSD Test, for evaluating treatment means.

RESULTS

Transportation of *O. niloticus* under different densities (Table 1) showed that there was significant differences between the control mean and those of low, medium, and high densities ($P<0.05$) There were decreases in the haematocrit value from 32.82± 1.5% in control to 22.0± 2.69% in low, 19.97± 2.84% in medium and 15.45± 2.97% in the high densities respectively. This implies that increase in density accentuates stress in *O. niloticus.*

Transportation under different saline concentrations (Table 2) showed significant difference between the mean haematocrit value of control and varying media saline levels ($P<0.05$), except 1% saline. However, there was sequential trend of increased values correlated with increase in salinity. This phenomenon could be ascribed to haemoconcentration as opposed to haemodilution, which characterized the previous result. With the application of aeration into samples transported in various saline concentrations there was also evidence of stress in all the transported samples from the results of the haematocrit values, again repeating the effect of haemoconcentration. The haematocrit values increased towards increasing saline concentrations. However, aerated samples had higher haematocrit values than the non-aerated.

Mortality was high during the first day, increasing on the second day and decreased on the third day, after transportation (Table 3). During pilot transportations, there was drastic reduction of dissolved oxygen content of the water samples from 10.99mls per litre to 2.4 mls per litre and from 7.62 mls per litre to 2.69 mls per litre respectively on two transportations. This drastic reduction in dissolved oxygen (DO) necessitated the use of aerator in subsequent transportations, which yielded better results in terms of higher haematocrit value and lower mortality.

DISCUSSION

The overall results obtained so far on the physiological indices of stress reveal that transportation of *Oreochromis niloticus* let to decreased haematocrit. This decrease in the value of haematocrit caused by stress conforms to results obtained by (Soivio and Oikari 1976, Madden 1977, Hattingh 1976, Nomura and Kawatsum 1977 and Sikoki, *et al* 1989), but differed from that of (Casillas and Smith 1977). The later observed an increase in haematocrit value of fish when stressed. A possible explanation of this variation could be that haematocrit value increases within the first 20-30 minutes after stress inducement and later starts to decrease. This proposition is based on the fact that Casillas and Smith sampled their fish blood within 20 minutes after stress inducement. Alternatively, it could be argued that Casillas and Smith sampled their fish blood in an aerobic environment. Since (Soivio and Nybols 1973) stated that haematocrit of Rainbow trout could be changed invitro by placing the blood in an aerobic or anaerobic environments. An anaerobic environment could cause a decline of greater than 10% of the original haematocrit value, whereas an aerobic condition could cause an increase of10-30% and this was the range recorded by Casillas and Smith. Be that as it may, it will be useful to carry out a time-course study of the pattern of the haematocrit stress response under varying media conditions in fish.

REFERENCES

BERKA, R. (1986). The transportation of live fish, a review, Fisheries Research Institute, Scientific information centre. *EIFAC Technical Paper, 48:* 1 – 48.

CARMICHAEL, G. J. (1984). Long distant truck transport of intensively reared large month bass. *Progressive Fish Culturist, 46(2):* 111 – 115.

CASILLAS, E. and SMITH, A. (1977). Effects of stress on blood coagulation and haematology in rainbow trout, *Salmo gairdneri. Journal of Fish Biology, 10:* 481 – 487.

HATTINGH, J. (1976). Blood sugar as an indicator of stress in fresh water Fish - *Labeo capensis. Journal of Fish Biology, 10:* 191 – 195.

MADDEN, J. A. (1976). Use of electroanaesthesia with fresh water teleosts: some physiological consequences in *Salmo gairdneri. Journal of Fish Biology, 9:* 451 – 467.

MC-CRAREN, J. P. (1978). *Manual of fish culture – fish transportation*. United State Fish and Wild Life Services. pp 12 – 15.

NOMURA, J. and KAWATSU, N. (1977). Variation of haematocrit value of rainbow trout blood samples incubated under different conditions. *Aquatic Society and Fish Abstract, 43(3):* 301 – 306.

ORJI, R. C .A. (1998). Effect of transportation stress on hepatic glycogen of *Oreochromis niloticus. Naga ICLARM, 21(3):* 20 – 22.

ORJI, R. C. A. (2003). Effect of transportation stress on interregnal cell nuclear size of Oreochromis niloticus, *Journal of Innovation of Life Science, 7:* 32 – 36.

SHARP, C. S., THOMSON, D. A., BLANKERSHIP, H. L. and SHRECK, C. B. (1998). Effect of routine tagging and handling procedures on physiological stress responses in juvenile Chinook salmon. *Progressive Fish Culturist, 60(2):* 81 – 87.

SHRECK, C. B. and LORZ, N. W. (1978). Stress response of coho salmon elicited by Cadmium and Copper and potential use of cortisol as an indicator of stress. *Journal of Fishery Research Board Canada, 35:* 1124 – 1129.

SOIVIO, A. and NYHLOS, K. (1973). Notes on rainbow trout – *Salmo gaidneri. Aquaculture, 2:* 31 – 35.

SOIVIO, A. and OIKARI, A. (1976). Haematological effects of handling stress *on Esox lucius. Journal of Fish Biology, 8:* 397 – 411.

SIKOKI, F. D., CIROMA, A. I. and EJIKE, C. (1989). Haematological changes in *Clarias gariepinus,* following exposure to sub lethal concentrations of Zinc, lead and Cadmium. Pages xx - zz. *In:* ONYIA, A. D and ASALA, G. N. (ed). Proceedings of the 7th Annual Conference of Fisheries Society of Nigeria, FISON, Bukuru, Nigeria. Held November 13 – 17, 1989.

SPECKER, J. L. and SHRECK, C. B. (1979). Anaesthetic and cortisol concentration in yearling Chinook salmon. *Journal of Fishery Research Board Canada, 29:* 178 – 183.

WEDEMEYER, G. A. and YASUTAKE, W. T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Pages 11 – 18. *In: Technical Paper of the United States Fish and Wildlife Services,* Washington D. C.

EFFECTS OF PALM OIL ON SOME OXIDATIVE INDICES OF ALLOXAN INDUCED DIABETIC RABBITS

OGUGUA Victor Nwadiogbu and IKEJIAKU Chukwuemeka Afam
Department of Biochemistry, University of Nigeria, Nsukka Nigeria

Corresponding Author: Dr. OGUGUA V. N. Department of Biochemistry, University of Nigeria, Nsukka Nigeria

ABSTRACT

The effects of palm oil on oxidative indices of alloxan induced diabetic rabbits were investigated. The result obtained showed that palm oil significantly decreased (P < 0.05), lipid peroxidation in diabetic treated animals. The vitamin C (antioxidant vitamin) level increased significantly (P < 0.05) in the supplemented group but decreased significantly (P < 0.05) in non supplemented group. The glucose levels in both the diabetic supplemented group and non supplemented group were not significantly different (P > 0.05). The results indicate that palm oil supplementation decreased the level of lipid peroxidation but increased the level of vitamin C, an indication that palm oil can attenuate oxidative stress generated in diabetic condition. This result may suggest that supplementation of palm oil may be effective in the management of diabetes mellitus.

Keywords: Red palm oil, Diabetes mellitus, Antioxidant, Oxidative stress, Lipid peroxidation

INTRODUCTION

The pathogenesis of many diseases involve free radical – mediated lipid peroxidation of biological membrane (Eze, *et al*., 1993; Eze, 1992; Ogugua, 2000) Diabetes mellitus and its complications have been associated with oxidative stress (Okamoto, 1981; Ogugua, 2000). It is reported that the oxidative destruction of the islets of Langerham of the pancreas by alloxan leads to diabetes mellitus and is free radical-mediated (Ofordile, 1987; Aruoma, *et al.,* 1991; Ogugua, 2000; Galluzzo, *et al.,* 1990). Membranes are prone to oxidation by reactive oxygen species (ROS) and because of the devastating complications and health hazard associated with diabetes mellitus, its management has continued to occupy researches in both medicine and related discipline (Eze, 1992; Ogugua, 2000; Aruoma, *et al.,* 1991)

Diabetes mellitus can only be managed or prevented but not cured. The role of antioxidant nutrients for the management of various diseases associated with oxidative stress has been well documented (Halliwell, *et al.,* 1992, Gutheridge, 1994.) Vitamins A, C, E and B carotene have been found useful (Muma, 1994; Ogugua, 1994; Gutheridge, 1994). Thus adequate dietary intake of vitamin E, a major lipid soluble inhibitor of peroxidation may be important in inhibiting the development of disease conditions including diabetes mellitus. Palm oil has been found to contain a lot of vitamin E and other lipid soluble vitamin nutrients (Atroshi, *et al.,* 1992; Choo, *et al.,* 1992; Packer, 1992; Gutheridge, 1994).

Since this vegetable oil is very common, affordable and used by majority of people across the globe especially in the tropics, its use as antidote to prevent some oxidative stress related diseases and complications is advocated. As reported else where (Choo, *et al*., 1992; Atroshi, *et al*., 1992) that palm oil contains mainly vitamin E, a major chain-breaking antioxidant in the membrane, it is the thrust of this work to study the effects of palm oil supplementation on some oxidative indices in alloxan induced diabetic rabbits. The outcome of the work may become useful in the management of human diabetics.

MATERIALS AND METHODS

Palm oil was bought from Nsukka local market and used for the experiment. Twelve albino rabbits weighing (2.5 kg on the average) were bought from Chigbo rabbitary Research Centre Awka Anambra State, Nigeria. The animals were kept for two weeks in laboratory to acclimatize with the environment. They were grouped into three groups of four rabbits each namely group 1 (normal rabbits), group 2 (diabetic rabbits but not treated with palm oil) and group 3 (diabetic rabbits but treated with palm oil). Diabetes was induced by administration of alloxan at 180 mg/kg body weight. The test diabetic animals (group 3) were given 5 ml of palm oil orally twice a day for two weeks. Blood samples were collected from the ear veins of the animals for the assay.

Malondialdehyde (MDA) level was estimated by the method of Albro *et al* (1986) and Das *et al* (1990). The thiobarbituric acid procedure employs the reaction of thiobarbituric acid with malondialdehyde to form a red chromogen which absorbs at 532nm. The concentration is

proportional to the level of peroxidation. Vitamin C level was determined by the method of Tietz (1970). This involves the oxidation of ascorbic acid (vitamin C) in the presence of combined colour reagent. The hydrozone resulting from this procedure dissolves in strong sulfuric acid solution to produce a red complex which absorbs at 500 nm. Glucose level was estimated according to 0-Toluidine method of Cooper & McDaniel (1970) in which the glucose in the sample when heated with O-Toluidine regent gives a blue-green colouration, the intensity of which is proportional to glucose concentration.

The results were analysed using analysis of variance (ANOVA) and expressed as mean ± SD.

RESULTS AND DISCUSSION

Table 1 shows that glucose level increased in diabetic condition (DNT group 2) compared with both the normal rabbits (group 1) and rabbits supplemented with palm oil. It was observed that palm oil slightly decreased glucose level in group 3 (DT).

That glucose levels increase in diabetic condition has been variously reported (Hamme, *et al.,* 1991; Takuncu, *et al.,* 1998; Sharpe, et al., 1998). Also, increase in glucose level has recently been associated with oxidative stress (Atkinson and Maclaren, 1990; Yadar, *et al.,* 1997; Ogugua 2000). Thus, the increased glucose level in group 2 (diabetic not treated group) is a result of intrinsic oxidative stress in diabetic condition. The slight reduction in the glucose level which was not significantly different ($P > 0.05$) is an indication that palm oil contains antioxidant which possibly countered oxidative stress in the animals. Earlier reports (Atroshi, *et al*., 1992; Choo, *et al*., 1992) show that palm oil contains other antioxidant vitamins – A and B carotene besides high level of vitamin E and, these might have acted synergistically, to reduce blood glucose. This observation lay credence to the report of Chung *et al* (1992) that these nutrients played protective roles against oxidative stress in alloxan induced diabetic rats. The very slight increase in glucose level in the normal group as experiment progressed could be due to increased oxidative stress resulting possibly from repetitive bleeding. These inferences corroborate a stipulation by Rey and Besedovsky (1989) that repetitive bleeding increases oxidative stress and glucose level.

Malondialdehyde (MDA) level increased in diabetic rabbits (group 2) compared with the normal. This increase is significant ($P < 0.05$). Diabetic condition has been linked with oxidative stress (Elhadd, *et al.,* 1999), and increased lipid peroxidation product (Ogugua, 2000), expressed as malondiadehyde level (MDA). Thus, the high level of MDA in group 2 – diabetic not treated rabbits – could be explained on the basis of oxidative stress mediated lipid peroxidation while the reduction in the level in group 3 (diabetic supplemented group) could be a result of the antioxidant property of palm oil. This property is conferred to palm oil by its possession of high level of a-tocopherol and other antioxidant vitamin (Packer, 1992; Atroshi, *et al.,* 1992).

Table 1: Effect of red palm oil on blood glucose, lipid peroxidation and vitamin C levels during the experiment

Group	Glucose level mmol/L
1	8.10 ± 0.30 6.40 ± 0.40[a] ,7.66 ± 0.46[b]
2	13.68 ± 0.48* 6.48 ± 0.28[a], 12.50 ± 0.35[b]
3	12.56 ± 0.36* 6.65 ± 0.52[a], 14.20 ± 0.60[b]
	Lipid peroxidation level mmol/ml plasma
1	7.90 ± 0.22
2	13.73 ± 0.41**
3	9.95 ± 0.20**
	Vitamin C level mg/100m
1	0.83 ± 0.18
2	0.62 ± 0.11**
3	0.69 ± 0.13**

*Group 1 is the normal rabbits (non diabetic), Group 2 is diabetic rabbits not supplemented with palm oil, Group 3 is the diabetic rabbits supplemented with palm oil, [a] indicates basal blood glucose level [b] indicates blood glucose before treatment, *p> 0.05, **p<0.05, Rabbits with blood glucose 10mmol/l and above were considered diabetic*

Vitamin E supplementation has been reported to be efficacious in reducing oxidation in diabetics (Bethesda, 1991). It is evidenced in the present study that this antioxidant nutrient in palm oil scavenged free radicals and hence reduced oxidative stress in diabetic treated rabbits. This corroborate a report that palmvitee capsule – a vitamin E capsule from palm oil – reduced serum cholesterol level in oxidative condition (Jacobson, *et al.,* 1990).

The low vitamin C level in group 2 is expected as oxidative stress operates in diabetic condition (Ogugua, 2000). Vitamin C is always the first antioxidant nutrients that counters oxidation at cytosol level and are thus depleted (Frei, 1991, Das and Thurhnam, 1992). The increase in vitamin C level in group 3 again suggests that palm oil contains some antioxidants that helped to scavenge free radicals in the system. This action probably spared the available vitamin C in the

system. It could be that vitamin E being helped by other antioxidant components in the palm oil prevented the generation of free radicals and subsequent oxidation. Similar synergistic action of antioxidant nutrient has been reported (Chiu, *et al.,* 1982).

The work in essence shows that palm oil contains some nutrients possibly antioxidants capable of suppressing oxidative stress. Research elsewhere suggests that vitamin E is a major ingredient. The work thus suggests that palm oil may be a good antioxidative nutrient as its supplementation decreased lipid peroxidation but increased vitamin C level. The effect on glucose level was not pronounced, suggesting that the effect could be more on the complications of diabetes.

In conclusion palm oil may help to attenuate diabetes mellitus and possibly diabetes complications. Its use may then be suggested in the management of diabetic condition and in diseases associated with oxidative stress.

REFERENCES

ALBRO, P. W. CORBETT, J. T. and SCHROEDER, J. L. (1986). Application of the thiobarturic assay to the measurement of lipid peroxidation products in microsomes. *Journal of Biochemistry*. *261:* 4889 – 4894.

ARUOMA, O. I., KAUR, H. and HALLIWELL, B. (1991). Oxygen free radicals and human disease. *Journal of Royal Society Health, 8:* 172 – 177.

ATKINSON, M. A. and MODOREN, N. K. (1990). What causes Diabetes? *Scientific American, July*: 42 – 47.

ATROSHI, F., ANTILA, E., SANKARI, S., TREUTHARAT, J., GAPOR, A., SALONIEMI, H. and WEITER M. T. (1992). Palm oil vitamin effects in hypercholesterolemia. Pages 243 – 254. *In:* ONG, A. S. H. and PACKER, L. (Eds.). *Lipid soluble antioxidants: biochemistry clinical application.* Birkhauser Verg, Boston.

BETHESDA, M. D. (1991). Guidelines for the diagnosis and management of Asthma. *National Institute of Health, US Department of Health and Human Services Publications, 91:* 30 – 31.

CHOO, Y. M., YAP, S. C., OOI, C. K., ONG, A. S. H. and GOLE, S. H. (1992). Production of palm oil carotenoid concentrate and its potential application in nutrition. Pages 243 – 254. *In:* ONG, A. S. H. and PACKER, L. (Eds.). *Lipid soluble antioxidants: biochemistry and clinical applications*. Birkhauser Verg Boston.

CHIU, D., VICHINSKY, E. Y. M., KLEMAN, K. and LUBIN, B. (1982). Peroxidation of vitamin E and sickle cell anaemia. *Annals of New York Academy of Sciences, 239:* 323 – 335.

CHUNG, Y. M. I., CHIU, P. M. M. and WONG, H. L. (1992). Modification of alloxan diabetes in rats by vitamin A status. Pages 265 – 273. *In:* ONG, A. S. H. and PACKER, L. (Eds.). *Lipid soluble antioxidants: biochemistry clinical application.* Birkhauser Verg, Boston.

COOPER, G.R. and McDANIEL, V. (1970). Assay methods. Pages 159 – 170. *In:* McDONALD, R. P. (Ed.). *Standard methods for clinical chemistry*. John Wiley and Sons, New York.

DAS, B. S. THURNHAM, D. I. PATNACK, J. K. DAS, D. B. SATPATHY, R., and BASE, T. K. (1990). Increased plasma lipid peroxidation in riboflavin deficient malaria – infected children. *American Journal Clinical Nutrition*, *51:* 859 – 863.

DAS, B. S. and THURNHAM, D. I. (1992). Plasma lipid peroxidation in *Plasmodium falciparium* malaria. Pages 397 – 405. *In:* ONG, A. S. H. and PACKER, L. (Eds.). *Lipid soluble antioxidants: biochemistry clinical application.* Birkhauser Verg, Boston.

ELHADD, T. A., KHAN, F., KIRK, G., MCLAREN, M. and NEWTON, R. W. (1999). Influence of poverty on endothelial dysfunction and oxidative stress in young patients with type 1 diabetes. *Diabetes Care, 21:* 1990 – 1996.

EZE, M. O. (1991). Production of superoxide by macrophages from Plasmodium chandandi infected mice. *Cytobios, 66:* 93 – 104.

EZE, M. O. (1992). Membrane fluidity, ROS and Cell mediated immunity; implications in nutrition and disease. *Medical Hypotheses, 37:* 220 – 224.

FREI, B. (1991). Ascorbic acid protects lipid in human plasma and low density lipoprotein against oxidative damage *American Journal Clinical Nutrition, 54:* 1113 – 1118.

GALLUZZO, A. GIORDANO, C. and BOMPIANA, G. D. (1990). Cell-mediated immunity in diabetic pregnancy. Pages 273 – 278. *In: Immunology of normal and diabetic pregnancy.* John Wiley and Sons New York.

GUTHERIDGE, J. M. C. (1994). Reactivity of hydroxyl and hydroxyl – like radical discriminated by release of TBARS from deoxysitrose, dudeosides and Beuroate. *Biochemistry, 224:* 761 – 767.

HAMME, H. R. MARTINS, S. FEDERLIN, K., GEISEN, K. and BROWNEE, M. (1991). Aminoguanidine treatment inhibits the development of experimental diabetes retinopathy. *Proceedings of National Academy of Sciences, 88:* 11555 – 11558.

JACOBSON, J. M., MICHAEL, J. R., JAFRI M. H. and CUISTNER, G. H. (1990). Antioxidants and antioxidant enzymes protects against pulmonary oxygen toxicity in the rabbit. *Journal Applied Physiology, 68:* 1252 – 1259.

MUMA, A. F. (1994). *Lipid peroxidation in malaria patients.* MSc Dissertations, Department of Biochemistry University of Nigeria, Nsukka. 145 pp.

OFORDILE, P. M. (1987). *Possible interaction of experimental diabetes with environmental toxins, biochemical and histopattiological evaluations*. MSc Dissertation, University of Nigeria, Nsukka. 108 pp.

OGUGUA, V. N. (1994). *Lipid peroxidation and antioxidant status in sicklers carriers and normal individuals.* MSc Dissertation, Department of Biochemistry, University of Nigeria, Nsukka. 128 pp.

OGUGUA, V. N. (2000). *Assessment of some parameters of oxidative stress in alloxan induced diabetic rabbits.* PhD Thesis, Department of Biochemistry, University of Nigeria, Nsukka. 205 pp.

OKAMOTO, H. (1981). Molecular basis of experimental diabetes degeneration, oncogenesis and regeneration of pancreatic Islets of Langerhams. *Bioassay, 2:* 15 – 21.

PACKER, L. (1992). New horizons in vitamin E research – the vitamin E cycle, biochemistry, and clinical applications. Pages 1 – 16. *In:* ONG, A. S. H. and PACKER, L. (Eds.). *Lipid soluble antioxidants: biochemistry and clinical applications*. Birkhauser Verg, Boston.

REY, A. D. and BESEDORSKY, H. (1989). Antidiabetic effects of interlukin. *Proceedings of National Academy of Sciences, 86:* 5943 – 5947.

SHARPE, P. C., YUE, K. K. M., CATTERWOOD, M. A., McMARTER, D. and TRIBBLE, E. R. (1998). The effects of glucose induced oxidative stress on growth and extracellular matrix gene expression of vascular smooth muscle cells. *Diabetologia, 41:* 1210 – 1219.

TAKUNCU, N. B., BCYRAKTAR, M. and VARLI, K. (1998). Reversal of defective nerve conductions with vitamin E supplementation in type II diabetes. *Diabetes Care, 21:* 1915 – 1918.

TIETZ, N. N. (1970). Carbohydrate. Pages 174 – 176. *In:* Sauders, W. B. (Ed). *Fundamental of Clinical Chemistry*. W. B. Sauders Company, London.

YADAR, P., SARKAR, S. and BHATNAGARD, D. (1997). Action of *Capparis deciduas* against alloxan induced oxidative stress and diabetes in rat tissue. *Pharmacology Research, 36(3):* 221 – 228.

EFFECT OF EXPOSURE TO SUBLETHAL CONCENTRATIONS OF GAMMALIN 20 AND ACTELLIC 25 EC ON THE LIVER AND SERUM LACTATE DEHYDROGENASE ACTIVITY IN THE FISH *Clarias albopunctatus*

OLUAH, Ndubuisi Stanley, EZIGBO, Joseph Chukwura and ANYA, Nnenna Catherine
Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka

Corresponding Author: OLUAH, N. S. Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka

ABSTRACT

One hundred and eighty adult Clarias albopunctatus (mean weight 160±2.7g) were subjected to sublethal concentrations of Gammalin 20 and Actellic 25 EC (0, 0.3;and 1.0 μg/l) in a static bioassay renewal system for 18 days. The changes in the activities of the liver and serum lactate dehydrogenase (LDH) during the period of exposure were studied. The exposure of C. albopunctatus to these pesticides evoked significant increase (P< 0.05) in both the liver and serum LDH activities. There was a progressive increase in both the liver and serum LDH activities following exposure to the pesticides. When compared with the control, the LDH activities were significantly higher (p < 0.05) in the treatment groups. The LDH activities in both the liver and the serum were higher in the fish exposed to Gammalin 20 than in the fish treated with similar concentrations of Actellic 25 EC. The serum LDH activity in the group exposed to a mixture of 0.3μg/l of Gammalin 20 and Actellic 25 EC was significantly higher the activities in the fish exposed the either 0.3 μg/l Gammalin 20 or Actellic 25 EC. These observations suggest that these pesticides affect the energy metabolism of the fish.

Keywords: *Clarias,* Liver, Serum, Lactate dehydrogenase, Gammalin 20, Actellic 25 EC

INTRODUCTION

Actellic 25 EC is an organophosphorous pesticide also known as Primiphos-methyl. It is a broad-spectrum pesticide against stored product pests (Worthing and Hance, 1991). On the other hand, Gammalin 20, a widely applied pesticide in Nigeria, is an organochloride pesticide containing 20 % lindane. The application of pesticides, either for agricultural purposes or for the control of pests of public health interests, contaminates the environment or hence endangers the non-target organisms in both terrestrial and aquatic habitats.

The value of tissue enzyme activities in the diagnosis of the effects of pollutants is one of the emerging areas of interest in aquatic toxicology, monitoring and remediation programmes. Thirugnam and Forgash (1977) studied the anticholinesterase effect of chlorpyrifos to the fish *Fundulus heteroclistis.* Increased glucose-6-phosphatase and glycogen phosphorylase activities were observed in *Cyprinus carpio* exposed to paraquat (Simon *et al.,* 1983). Also, Rashatwar and Ilyas (1983), noted that Basalin, a herbicide affected the activities of lactate dehydrogenase, alkaline phosphatase as well as glutamic pyruvate transaminase in the freshwater fish *Nemachelinus* sp. Increased activities of alanine and aspartate aminotransferases were reported in *Clarais albopunctatus* exposed to copper (Oluah and Amalu, 1998), zinc and mercury (Oluah, 1999). Verma *et al.* (1981) observed that pesticides inhibited alkaline phosphatase and glucose-6-phosphatase activities in the fish *Mytulus vittatus.*

The purpose of this study was to investigate the effect of the pesticides - Actellic 25 EC and Gammalin 20, widely used in Nigeria, on the activities of liver and serum lactate dehydrogenases in the catfish, *Clarias albopunctatus.*

MATERIALS AND METHODS

The one hundred and eighty fish (mean weight 110 ± 2.6 g) used in the study was collected from Anambra River at Otuocha, Anambra East Local Government Area of Nigeria. The fish was transported to our laboratory in a plastic container and was acclimatized for two weeks in plastic aquaria before the commencement of the study.

The fish were divided randomly into six (6) groups (1 – 6) of 30 fish per group. Each group was further divided into three replicate groups of 10 fish per replicate. The fish in group I were exposed to tap water only as the control while the fish in groups 2 and 3 were treated with 0.3 μg/l and 1.0 μg/l of Actellic 25 EC, respectively. Groups 4 and 5 were exposed to 0.3 μg/l and 1.0 μg/l Gammalin 20, respectively. The 6th group was exposed to a mixture of equal concentrations (0.3 μg/l) of Actellic 25 EC and

Gammalin 20.The fish were exposed to these sublethal concentrations of the pesticide in a renewal bioassay system in which the water and the pesticides were changed every two days to maintain the toxicant concentrations. The fish were fed 30 % crude protein diet at 3% body weight daily at 8.00 h. The Experiment lasted for 18 days and the lactate dehydrogenase activity was assayed every six days.

Tissue Collection and Enzyme Assay: The blood samples were collected by both the cardiac puncture method and the severance of the caudal peduncle using the disposable hypodermic syringe (Oluah, 1999). The liver was excised and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized as described by Devi *et al.* (1993). The liver was homogenized in ice-cold 0.25 M sucrose. The liver homogenate was centrifuged at 5000 rpm for 15 minutes at 4^{o}C.The blood was similarly centrifuged for 15 minutes at 1000 rpm to obtain the serum. The liver supernatant and serum were used for the lactate dehydrogenase (LDH) assay. The lactate dehydrogenase activity was calorimetrically determined at 445 nm using Sigma protocol number 500 (Sigma Chemical Company, St Louis, MO). The data from the replicate experiments were averaged and the mean (± SD) presented. The analysis of variance (ANOVA) was used to analyze the data for statistical significance ($P < 0.05$).

RESULTS AND DISCUSSION

The changes in the liver lactate dehydrogenase (LDH) activity in *Clarias albopunctatus* exposed to sublethal concentrations of Gammalin 20 and Actellic 25 EC are shown in Table 1.The results showed that exposing *C. albopunctatus* to 0.3 and 1.0 μg/l of both pesticides caused significant ($P < 0.05$) increases in the liver LDH activity when compared with the control. The liver LDH activity in the fish exposed to 0.3 μg/l Actellic 25 EC increased from 98.42 ± 1.06 μ/g on the 6th day to 319.8 ± 1.12 μ/g liver on the 18th day. In the fish exposed to 1.0 μg/l Actellic 25 EC, the enzyme activity increased from 246.25 ± 1.08U/g liver on the 6th day to 442.89 ± 1.22 μ/g liver at the end of the study.

The liver LDH activity increased from 147.63 ± 1.15 and 246.05 ± 1.1 μ/g on the 6th day to 393.68 ± 2.04 μ/g and 738.15 ± 1.14 U/g on the 18th day in the fish exposed to 0.3 and 1.0 μg/l Gammalin 20, respectively. In the group exposed to the mixture of 0.3 μg/l Actellic 25 EC and Gammalin 20, the enzyme activity increased from 98.42 ± 1.6 μ/g on day 6 to 442.89 ± 1.06 μ/g on the 18th day. The mean liver LDH activity in the Gammalin 20- exposed fish was significantly ($P < 0.05$) higher than in the fish exposed to Actellic 25 EC. When compared with the control, the liver LDH activity differed significantly ($P < 0.05$) in the treatment groups.

The serum LDH activity increased from 49.21 ± 1.36 U/l on day 6 to 124.61 ± 2.08 U/l on day 18 in the fish exposed to 0.3 μg/l Actellic 25 EC (Table 2). When the fish was exposed to 1.0 μg/l Actellic 25, the LDH activity increased from 123.03 ± 2.12 U/l on the 6th day. When the fish was treated with Gammalin 20, the serum LDH activity increased from 113.18 ± 1.76 U/l and 147.63 ± 1.88 U/l on day 6 to 196.84 ± 2.8 U/l and 270.66 ± 1.06 U/l on the 18th day in the fish exposed to 0.3 and 12.0 μg/l Gammalin 20, respectively. Similarly, the mean serum LDH activity increased from 260.81 ± 2.03 U/l on day 6 to 590 ± 1.2 U/l on the 18th day in the fish treated with a mixture of equal concentrations (0.3μg/l) of Actellic25 and Gammalin20. In this group, there was a 3-fold increase in the LDH activity when compared with the activity in the groups exposed only to 0.3 μg/l of either Gammalin 20 or Actellic 25 EC. The serum LDH was significantly higher ($P < 0.05$) in the in the fish treated with Gammalin 20.When compared with the control, the serum LDH activity was significantly higher ($P< 0.05$) in the fish exposed to the pesticides.

The result showed that the increase in the tissue LDH activity was concentration-dependent and also increased with duration of exposure. The result of the study is consistent with the reports of earlier studies. Simon *et al.* (1983) reported that paraquat caused increased phosphorylase and glucose-6-phosphatase activities in *Cyprino carpio.* Similarly, Reddy *et al. (*1983) reported increased malate dehydrogenase and lactate dehydrogenase activities in the crab *Oziotelphusa senex senex* exposed to sumithion, an organophosphate insecticide. Similar increase in LDH and alkaline phosphatase activities were observed in the English sole *Parophrys vetulus* treated with carbon tetrachloride (Casillas and Ames, 1986). Also, cadmium was reported to have elicited increased muscular LDH activity in Fiddler crab, *Uca pugilator* (Devi *et al.,* 1993) and in the brook trout, *Salvelinus fontinalis* (Christensen *et al.,* 1977). On the other hand, some agrochemicals and heavy metals inhibit tissue enzymes. Thebault and De Caris (1983) reported that trichlorophenoxyacetic acid (2, 4, 5-T), an auxin herbicide, inhibited gill Ca^{2+} ATPase activity in the trout.

Similarly, cholinesterase activity was inhibited in the fish *Callchthys cacllichtys* treated with methyl parathion (Da Silva *et al.,* 1993) and in *Funulus heteroclitus* due to chorphyrifos intoxication (Thirugnanam and Forgash, 1977). Inhibition of Acetylcholinesterase activity was also reported in the Walleye following exposure to chlorphyrifos (Philips *et al.,* 2002). Hilmy *et al.*

Table 1: Changes in the concentrations of liver lactate dehydrogenase of *C. albopunctatus* exposed to varying concentrations of Actellic 25 EC and Gammalin 20

Pesticide	Concentration (μg/l)	Duration of Exposure (days)		
		6	12	18
		Enzyme concentration (U/mg)		
Control	0	49.21 ± 2.0	98.42 ± 1.6	98.42 ± 1.6
Actellic 25 EC	0.3	98.42 ± 1.06	196.84 ± 2.0	319.87 ± 1.12
Actellic 25 EC	1.0	246.05 ± 1.08	275.58 ± 1.1	442.89 ± 1.22
Gammalin 20	0.3	147.63 ± 1.15	275.58 ± 1.3	393.68 ± 2.04
Gammalin 20	1.0	246.06 ± 1.10	344.47 ± 1.7	738.15 ± 1.44
Actellic 25 EC and Gammalin 20	0.3/0.3	98.42 ± 1.6	113.18 ± 1.1	442.89 ± 1.06

Values= (mean ± sd) of LDH activity in each group for 3 determinations

Table 2: Changes in the concentrations of the serum lactate dehydrogenase of *C. albopunctatus* exposed to varying concentrations of Actellic 25 EC and Gammalin 20

Pesticide	Concentration (μg/l)	Duration of Exposure (days)		
		6	12	18
		Enzyme Concentration (U/l)		
Control	0.0	26.61 ± 2.4	24.6 ± 1.8	24.61 ± 2.8
Actellic 25 EC	0.3	49.21 ± 1.36	96.42 ± 2.6	124.61 ± 1.8
Actellic 25 EC	1.0	123.03 ± 2.12	196.84 ± 1.5	246.05 ± 3.04
Gammalin 20	0.3	113.18 ± 1.76	147.63 ± 1.2	196.84 ± 2.8
Gammalin 20	1.0	147.63 ± 1.8	240.05 ± 2.0	270.66 ± 1.06
Actellic 25 EC and Gammalin 20	0.3/0.3	260.81 ± 2.03	344.47 ± 1.8	590.52 ± 1.2

Values= (mean ± sd) LDH activity in each group for 3 determinations

(1985) reported that cadmium inhibited LDH activity in the heart, liver and gills but not in the serum of the fish *Mugil cephalus*. Reduced LDH activity was also observed in the hepatopancreas of the crab *Uca pugilator* (Devi *et al.,* 1993). The result of this study is consistent with the reports of the effect of agrochemicals on LDH activity in mammals.

Parathion was found to elicit increased LDH activity in rats (GalloandLawryk, 1991). Similarly, Junge *et al. (*2001) reported a 2-fold increase in liver myelopreoxidase activity in the rat exposed to lindane.

Lactate dehydrogenase is known to catalyse the biochemical process of converting pyruvate to lactate with the attendant oxidation of NADPH. Thus, the increased LDH activity in both the liver and serum are indications of a shift in the carbohydrate metabolism from the glucose and glycogen catabolism to lactate synthesis. This shift in carbohydrate metabolism reflects the possible dependence of *C. albopunctatus* on anaerobic pathway during exposure to sublethal Actellic 25 EC and Gammalin 20. This goes to confirm the report of Omoregie *et al.* (1990) that these insecticides induce increased plasma lactate concentration in *Oreochromis niloticus.* This situation would predispose the fish to lactic acidosis, which may impact adversely on the health of the fish.

In conclusion, Actellic25 EC and Gammalin 20 were found to elicit increased lactate dehydrogenase activity in the fish *C. albopunctatus* with its attendant physiological stress.

REFERENCES

CASILLAS, E. and AMES, W. (1986). Hepatoxic effects of carbon tetrachloride on the English sole (*Parophrys vitulus):* possible indicator of liver dysfunction. *Comparative Biochemistry Physiology, 84 c:* 397 – 400.

CHRISTENSEN, G., MUNT, E. and FIANDT, J. (1977). The effect of methyl mercuric chloride, cadmium chloride and lead nitrate on six biochemical factors of brook trout (*Salvelinus fontinalis*). *Toxicology and Pharmacology, 42:* 523 – 530.

DA SILVA, H. C., MEDINA, H. S. G., FAQNTA, E. and BACILA, M. C. (1993). Sublethal effects of the organophosphate Folidol 600 (Methyl Parathion) on *Callichthys callichthys* (Pisces: Teleostei). *Comparative Biochemistry Physiol*ogy, *105 c:* 197 – 201.

DEVI, M., REDDY, P. S. and FINGERMAN, M. (1993). Effect of exposure on lactate dehydrogenase activity in the hepatopancreas and abdominal muscles of the Fiddler crab *Uca pugilator*.

*Comparative Biochemistry Physiol*ogy, *106c:* 739 – 742.

GALLO, M. A. and LAWRYK, V. J. (1991). *Organic phosphorous pesticides studies.* Williams and Wilkins, Baltimore. 1123 pp.

HILMY, A. M., SHABAMA, M. B. and DAABEES, A. Y. (1985). Effect of cadmium toxicity upon the *in vivo* and *in vitro* activity of proteins and five enzymes in the blood serum and tissue homogenates of *Mugil cephalus. Comparative Biochem*istry *Physiol*ogy, *81 c:* 145 – 153.

JUNGE, B., CARRION, Y., BASIO, C., GALLANO, M., PRINTARNIO, S., TAPIA, G. and VIDELA, L. A. (2001). Effect of iron over load and lindane intoxication in relation to oxidation stress, kuffer cell function and liver injury in the rat. *Toxicology and Applied Pharmacology, 170:* 23 – 28.

OLUAH, N. S. and AMALU, C. C. (1998). The effect of heavy metals on the aminotranserase activity in the freshwater catfish *Clarias albopunctatus*. *Journal Aquatic Sciences 13:* 5 – 8.

OLUAH, N. S. (1999). Plasma aspartate aminotrasferase activity in the catfish *Clarias albopunctatus* exposed to sublethal zinc and mercury. *Bulletin Environmental Contamination Toxicology, 63:* 343 – 349.

OMOREGIE, E., UFODIKE, E. B. C. and KEKE, I. R. (1990). Tissue Chemistry of *Oreochromis niloticus* exposed to sublethal concentrations of Gammalin 20 and Actellic 25 EC *Journal Aquatic Sciences,* 5: 33 – 36.

PHILIPS, J. A. WU, J. S., SUMMERFLET, H. and ATCHISON, G. (2002). Acute toxicity and cholinesterase inhibition in larval and early juvenile walleye exposed to chlopyrifos. *Environmental Toxicology and Chemistry, 21:* 1469 – 1474.

RASHATWAR, S. and ILYAS, R. (1983). The effect of chronic herbicide intoxication on in vivo activities of certain enzymes of freshwater fish *Nemachelinus. Toxicological Letters, 16:* 249 – 252.

REDDY, P. S., BHAGYALAKSHMI, A. and RAMASMENITHI, R. (1983). Chronic sumithion toxicity 1: Effect on carbohydrate metabolism in the crab muscle. *Toxicological Letters, 15:* 113 – 117.

SIMON, L. M., NEMOSOK, J. and BOROSS, L. (1985). Studies on the effect of paraquat on glycogen mobilization in the liver of common carp (*Cyprino carpio*) *Comparative Biochemistry Physiology, 75 c:* 167 – 169.

THEBAULT, M. T. and DE CARIS, F. X. (1983). Calcium ion ATPase inhibition in the trout gill microsome following in vitro and in vivo treatment with 2, 4, 5-T. *Comparative Biochemistry Physiology, 75c:* 369 – 372.

THIRUGNANAM, M. and FORGASH, A .T. (1977). Environmental impact of mosquito pesticide toxicity and anticholinesterase activity of choryopyrifos to fish in a salt marsh habitat. *Archives Environmental Contamination and Toxicol*ogy, 5: 415 – 425.

VERMA, S. R., RANI, S. and DALELA, R. C. (1981). Pesticide induced physiological alteration in certain tissues of a fish *Mytulus vitatus. Toxicological Letters, 9:* 327 – 332.

WORTHING, C. R. and HANCE, R. J. (1991). *Pesticide manual.* 8th edition. British Crop Protection Council, Thomton Heath, London.

EFFECT OF EFFLUENT FROM A VEGETABLE OIL FACTORY IN SOUTHEASTERN NIGERIA ON THE *MMIRIELE* STREAM

ATAMA, Chinedu and MGBENKA, Bernard Obialo
Fish Nutrition, Aquaculture and Hydrobiology Unit, Department of Zoology, University of Nigeria Nsukka.

Corresponding Author: Dr. MGBENKA, B. O. Fish Nutrition, Aquaculture and Hydrobiology Unit, Department of Zoology, University of Nigeria Nsukka. Email: bo_mgbenka@yahoo.co.uk.

ABSTRACT

Environmental monitoring of effluent discharged from a vegetable oil factory and route to receiving Mmiriele stream, Nnewi Anambra State, Nigeria was conducted bi-weekly for 12 months. The physicochemical parameters examined in the effluent assessment were dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total hardness, hydrogen ion concentration (pH), and ammonia-nitrogen. Others were copper (Cu), zinc (Zn), lead (Pb) and arsenic (As). Concentration of each of the parameters at the various sampled points indicated significant variation among the points (P < 0.05). Comparing the results to international effluent quality standards for municipal and industrial effluents discharged into surface inland waters and the Federal Ministry of Environment (FMENV) standards for such effluents showed that the mean values of each of the parameters was within acceptable limits except for very high distribution of lead recorded in all samples. Arsenic was notably not detected. The significance of the results is discussed.

Keywords: Vegetable oil factory effluent, Physicochemical parameters, Dissolved metal pollutants

INTRODUCTION

Industrial waste contains toxic substances that damage biological activity and kill desirable forms of life (Suter and Loar, 1992). One of such was as reported by Sandra (2000) and Jøorgensen and Johnsen (1989) for industrial waste waters. Very many physicochemical parameters are associated with effluent assessment most of which were considered.

From human health perspective, high levels of elements such as zinc, lead, arsenic and nitrogen are of great concern. For instance, nitrate may cause infant hemoglobinemia or blue-baby syndrome in which the oxygen-carrying capacity is blocked, causing suffocation. Lead is particularly toxic to young children and its hazards include kidney damage, metabolic interference and depressed biosynthesis of protein (Craun *et al*., 1981; Meybeck, 1982; Meybeck *et al*., 1989). Brown blood disease due to excessive nitrite nitrogen has also been reported in fish (Lovell, 1987).

However, industrial pollutants are difficult to characterize and detailed inventories of industrial wastes are rare. In addition to organic decomposable matter of complex composition with high biological oxygen demand, the waste from industries usually contain traces or larger quantities of raw materials, intermediate products, final products, by-products and processing chemicals. From the above problems associated with industrial pollutants, there is need to monitor waters in which industrial effluents are discharged.

In Nigeria, the Federal Environment Protection Agency (FEPA), now known as the Federal Ministry of Environment (FMENV) was created in 1988. It is charged with the statutory responsibility for overall protection of the environment. Among the guidelines of FMENV is mandatory provision of on-site or contractual industrial pollution effluent monitoring facilities within the set up of any industry (Ugochukwu and Leton, 2004). This law is often breeched. FMENV and State governments' environmental protection agencies (EPAs) are also charged with crosschecking effluent characteristics from factories and companies to ascertain the degree of compliance with the law. Too often, this is not done or is poorly done. The result is that the environment suffers from likely hazardous pollution with effluent discharged from factories.

Against this background, the need for the assessment of a vegetable oil factory effluent which enters the *Mmiriele* stream, Nnewi, used domestically by the people living around it cannot be over-emphasized. There is little or no recorded information on the physicochemical parameters of this body of water. The present study was therefore an independent study conducted to determine the physicochemical parameters of the section of the *Mmiriele* stream into which a

vegetable oil factory discharges its effluent. This is to establish some baseline information for this stream.

MATERIALS AND METHODS

Effluents from a vegetable oil factory were properly channeled into the receiving *Mmiriele* steam. Thus, the physicochemical parameters of the effluent that gets into the stream and the stream were monitored. Representative samples were collected from the effluent discharge route of the vegetable oil factory, Nnewi, Anambra States, Nigeria into the *Mmiriele* stream (Figure 1).

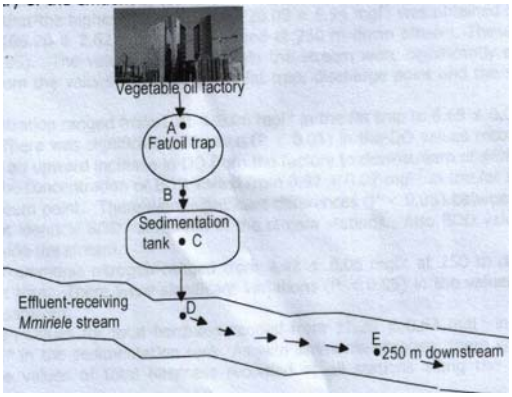

Figure 1: Effluent route from the RIMCO vegetable oil factory into the receiving *mmiriele* stream showing sampled points, namely; A station for collection of effluent immediately in the oil/fat trap, B oil discharge point station, C station for collection of effluent in the sedimentation tank, D station for collection of effluent in *mmiriele*, E 250 meters downstream of *mmiriele*, ● represents effluent collection stations indicated by letters, Arrows represent direction of effluent flow.

Additionally, representative samples were collected from the point of entry of the effluent in the stream and from 250 m downstream. Clean dry one litre wide mouthed transparent glass bottles with Teflon covers were used to collect the samples. The glass bottles were appropriately labelled with sample location, date and time of collection. For dissolved oxygen determination, water from the bottle was siphoned through the Winkler dissolved oxygen determination bottle and the water fixed in the field for the azide modification of the Winkler's method using the Hach test kit (Model FF3, Hach Company, Loveland Co., USA). Triplicate sampling unit were used. All samples were preserved at low temperature in ice chest, and analysed within 24 hours of collection. Sampling was done bi-weekly for a period of 12 calendar months. The following physicochemical parameters were determined: dissolved oxygen (DO), biochemical oxygen demand BOD), chemical oxygen demand (COD), ammonia–nitrogen (NH_4-N), hydrogen ion concentration (pH), and total hardness. Other parameters studied include the following heavy metals: copper (Cu), zinc (Zn), lead (Pb) and arsenic. All analyses for biochemical oxygen demand, chemical oxygen demand, total hardness, and heavy metals were done by using the standard methods described in APHA (1976, 1980) and Owen (1974). Most of the characteristics were determined with unfiltered samples except dissolved heavy metals in which filtered samples were used. Values obtained from the stream were compared with Federal Ministry of Environment Standards (FEPA, 1988).

Statistical Analysis: Means and standard error of means of the physicochemical parameters of the different sampled sites were calculated using descriptive statistics. The one way analysis of variance (ANOVA) was employed to test for any significance differences ($P < 0.05$) and the Fisher's least significant differences (F-LSD) and the Duncan's Multiple Range Test were employed to partition the differences of sampled means (Steel and Torrie, 1980).

RESULTS

The results of the mean physicochemical parameter are shown in Table 1. Comparison of physicochemical parameters in the *Mmiriele* stream, Nnewi with the Federal Ministry of Environment Standards is presented in Figure 2. Table 1 showed that the highest mean COD of 720.00 ± 5.94 mgl^{-1} was obtained in the fat trap and the least mean COD of 106.20 ± 2.62 mgl^{-1} was obtained at 250 m down stream. These values were significantly different ($P < 0.05$). The values obtained within the stream were significantly different ($P < 0.05$) from each other and from the values recorded in the fat trap, discharge point and the sedimentation tank.

The mean DO concentration ranged from 1.81 ± 0.06 mgl^{-1} in the fat trap to 6.69 ± 0.07 mgl^{-1} at 250 m downstream. There was significant difference ($P < 0.05$) in the DO values recorded between the all the sampled sites with an upward increase in DO from the factory to downstream of *Mmiriele*.

On the other hand, the concentration of BOD varied from 0.97 ± 0.07 mgl^{-1} in the fat trap to 4.41 ± 0.08 mgl^{-1} in the receiving steam point. There were significant differences ($P < 0.05$) between all sampling points outside the stream but identical BOD values within the stream stations. Also, BOD values within the stream were higher than outside the stream.

The concentration of ammonia-nitrogen ranged from 2.42 ± 0.06 mgl^{-1} at 250 m downstream to 15.30 ± 0.09 mgl^{-1} in the fat trap.

Table 1: Mean distribution of physicochemical parameters along a vegetable oil factory effluent discharge route in Nnewi, Nigeria

Sites	Water quality parameters								
	Chemical oxygen demand (mgl^{-1})	Dissolved oxygen (mgl^{-1})	Biochemical oxygen demand (mgl^{-1})	Ammonia nitrogen (mgl^{-1})	Total hardness (mgl^{-1})	pH	Copper (mgl^{-1})	Zinc (mgl^{-1})	Lead (mgl^{-1})
Fat trap	720.00 ±5.94[a]	1.81 ±0.06[a]	0.97 ±0.07[a]	15.30 ±0.09[a]	18.30 ±0.63[a]	7.53 ±0.02[a]	0.41 ±0.04[a]	0.54 ±0.02[a]	14.38 ±0.17[a]
Oil discharge point	196.00 ±3.57[b]	2.00 ±0.08[a]	1.82 ±0.08[b]	4.68 ±0.06[b]	20.00 ±0.82[b]	6.86 ±0.09[b]	0.46 ±0.05[a]	0.56 ±0.02[a]	15.43 ±0.46[b]
Sedimentation tank	148.70 ±2.27[c]	3.43 ±0.07[b]	2.94 ±0.04[c]	2.62 ±0.04[c]	24.00 ±0.68[c]	6.69 ±0.02[b]	0.47 ±0.04[a]	0.64 ±0.03[b]	16.73 ±0.31[c]
Effluent-receiving stream (*Mmiriele*)	117.30 ±2.36[d]	5.00 ±0.08[c]	4.41 ±0.08[d]	3.67 ±0.06[d]	11.20 ±0.04[d]	6.94 ±0.04[c]	0.31 ±0.06[b]	0.56 ±0.02[a]	14.61 ±0.21[a]
250 m downstream	106.20 ±2.62[e]	6.69 ±0.07[d]	4.28 ±0.09[d]	2.42 ±0.06[e]	16.40 ±0.62[e]	7.29 ±0.06[d]	0.18 ±0.03[a]	0.57 ±0.02[a]	14.68 ±0.29[c]

[1]*Mean values in a column followed by the same superscripts are not significantly different (P > 0.05).*

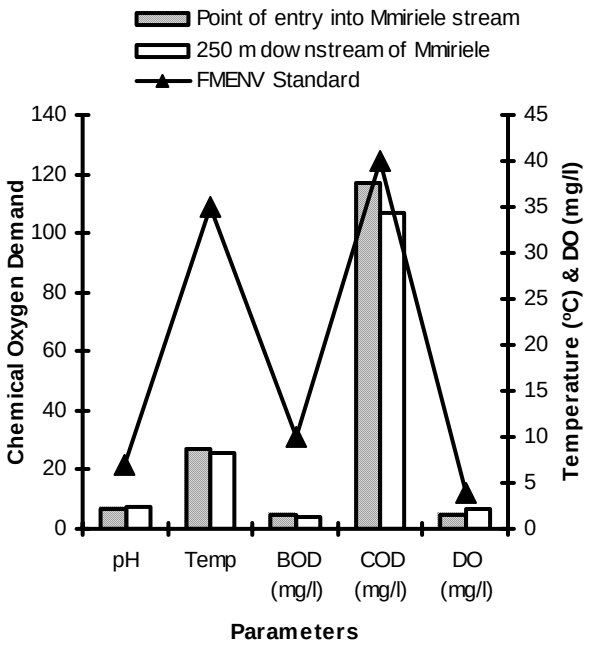

Figure 2: Water quality parameters of Rimco vegetable oil factory effleunts receiving mmiriele stream compared with Federal Minbistry of Environment Standards.

There were significant variations (P < 0.05) in the values recorded for the parameter at all stations sampled.

In addition, the mean values for total hardness ranged from 11.20 ± 0.53 mgl^{-1} in the receiving stream to 24.00 ± 0.53 mgl^{-1} in the sedimentation tank. As with ammonia-nitrogen, there were significant variations (P < 0.05) in the values of total hardness recorded at all stations along the effluent route sampled.

The pH values along the effluent route ranged from 6.69 ± 0.02 pH to 7.53 ± 0.02 pH in the sedimentation tank. For the pH, all points outside the stream were not significantly different but were different form the values of sampling points within the stream. The 250 m downstream station had a significantly different pH value from the pH at the effluent receiving point in *Mmiriele* stream (P < 0.05).

The highest mean value of dissolved copper recorded was 0.47 ± 0.04 ppm in the sedimentation tank and the least value was 0.18 ± 0.03 ppm at 250 m down stream. All points outside the stream were not significantly different (P > 0.05) in values but were significantly different from the values of the stations within the stream. The 250 m downstream station of the *Mmiriele* stream and the effluent receiving station recorded significantly different (P < 0.05) value of copper.

Dissolved zinc concentration ranged between 0.64 ± 0.03 ppm in the sedimentation tank to 0.54 ± 0.02 ppm in the fat trap (Figure 3). The F-LSD separation of means showed that there were no significant difference (P > 0.05) between values recorded in the discharge point of the receiving stream and 250 m downstream but these were significantly different (P < 05) from the values got in the fat trap and sedimentation tank.

The values for dissolved lead differed between 14.33 ± 0.17 ppm in the fat trap and 16.73 ± 0.31 ppm in the sedimentation tank. There were significant variations (P < 0.05) in the values of lead recorded in the effluent route stations (Figure 3). The value in the fat trap station was however not significantly different (P > 0.05) from the values of lead from the *Mmiriele* stream stations. No trace of arsenic was recorded in any of the sampled points.

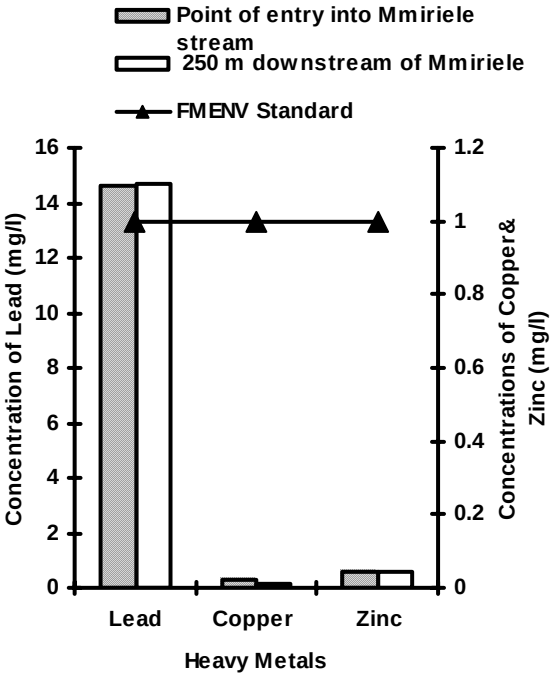

Figure 3: Concentration of heavy metals in the Rimco vegetable oil factory effluent receiving mmiriele stream compared with Federal Ministry of Environment Standards

DISCUSSION

The COD, BOD and DO levels at the point of entrance of the effluent into *Mmiriele* stream and at 250 m downstream compared to International Standards for water (GESAMP, 1988) and the Federal Ministry of Environment standards for municipal and industrial effluents discharge into surface inland waters (Figure 2) showed that the values are within acceptable limits. Maitland (1990) reported that clean cold water holds only about 12 mgl^{-1} of oxygen. Thus the results of DO in *Mmiriele* represent usable oxygen in the effluent. However, high temperature around 30 ºC in Nigeria, not only reduced the amount of oxygen which can dissolve in water, thereby minimizing the oxygen supply available to micro-organisms. Also, increase in the rate at which oxygen is utilized by micro-organisms is exacerbated by the high temperature. According to the traditional dissolved oxygen sag curve, a high rate of oxygen use might result in low or zero dissolved oxygen concentration in a particular stream reach. This would impair the stream's subsequent capacity to received further polluting discharges. Kolo and Yiza (2000) observed that increased organic matter decomposition in water can reduce the BOD to less than 4 mgl^{-1}. As can be seen from the values of BOD in the effluent entry point (4.41 ± 0.08 ppm) and the value at the 250 m downstream (4.28 ± 0.09) the stream was tending to the marginal 4 mgl^{-1} BOD.

In addition, the pH, ammonia-nitrogen and total hardness concentration were within Nigeria Federal Ministry of Environment set guidelines for waste disposal (FEPA, 1988). The source of hardness in the effluents could be through wash-offs from the machines with soap. Whatever source, hardness is an important factor for fish production. The recommended level for total hardness in fish pond is 20 mgl^{-1} (Boyd and Lichtkopler, 1979). The hardness level in this effluent may be adequate for fish production.

On the other hand, the levels of zinc and copper were considerably low while arsenic was notably absent. The level of lead was very high and remained stable throughout the study period. The lead in the stream may be contributed by effluent from either a battery factory located upstream or mobilization from the soil. This high level of lead may have resulted mainly from a lead-acid accumulator (battery) manufacturing activity in the vicinity, and from washings of leaded containers and high traffic density in the stream in this heavily industrialized city. The battery factory is located in the upstream section of the town. The GESAMP (1988) observed that low lead concentration of less than 0.02 mgl^{-1} may affect photosynthesis, delay embryonic development, and reduce growth in adult fish, mollusks and crustaceans. Mombeshora *et al*. (1983), however, reported a lower level of lead in their studies of stream and lakes and Ibadan, Nigeria indicating that the level of lead in the *Mmiriele* stream was high indeed.

From the above result it becomes necessary that factory wastes into the aquatic environment be monitored, to put a check on pollution. Furthermore, the quality requirements of water use could be the only constraints governing the choice of stream standards for particular surface water. Although, there was no direct link of lead to the Rimco Vegetable Oil factory, we strongly recommend that the high lead levels in the stream be reduced forthwith through reduced loading of the stream with lead.

ACKNOWLEDGEMENTS

We are very grateful to some members of the Department of Zoology, University of Nigeria, Nsukka, namely Prof. F. C. Okafor for very useful advice and facilitation of effluent collection from the factory, Mr. N. S. Oluah for assistance with the water analyses and Dr. J. E. Eyo for assisting in sampling during one of the several trips to Nnewi. We are indebted to the management of the factory especially Engr. L. O. Ilozue for allowing us access to the factory for collection of samples. The management of the factory by their action and

utterances showed some willingness to protect the environment.

REFERENCES

APHA (1976). *Standard methods for examination of water and waste water.* 16th edition. America Public Health Association and Water Pollution Control Federation, Washington DC, 1086 pp.

APHA (1980). *Standard methods of the examination of water and waste water*. 17th Edition. America Public Health Association, Washington D. C. 1134 pp.

BOYD, E. C. and LICHTKOPLER, F. (1979). *Water quality management in pond fish culture*. International Centre for Aquaculture, Agricultural Experiment Station, Research and Development Series 22. Auburn University, Alabama, USA. 30 pp.

CRAUN, G. F., GREATHOUSE, D. G. and ZUNDERSON, D. H, (1981). Haemoglobin levels in young children consuming high nitrate well water in the United States. *International Journal of Epidemiology, 10*: 309 – 317.

FEPA (1988). *National interim guidelines and standards for industrial effluents, gaseous emission and hazardous waste management in Nigeria.* Federal Environmental Protection Agency, Lagos Nigeria.

GESAMP (1988). *Review of potentially harmful substances.* Joint Group of Experts on the Scientific Aspect of Marine Pollution (GESAMP). *Reports of Ships. GESAMP*, 28: 27.

JØRGENSEN, S. E. and JOHNSEN, I. (1989). *Principles of environmental science and technology: Studies in environmental science 33,* Elsevier, Oxford, 628 pp.

KOLO, R.J and YISA, J. (2000). Preliminary base line assessment of water quality of River Suka, Niger State. *Journal of Fishery Technology. 91*: 105.

LOVELL, R. T. (1987). *Fish nutrition and feeding of fish.* Van Nostrand, New York. 260 pp.

MAITLAND, P. S. (1990). *Biology of freshwater*. Chapman and Hall. New York. 76 pp.

MEYBECK, M. (1982). Carbon, nitrogen and phosphorus transport by world rivers. *American Journal of Science, 28 (2)*: 401 - 450.

MEYBECK, M., FRIEDRIC, G., THOMAS, R. and CHAPMAN, D. (1989). Rivers. Pages 65 - 160. In: CHAPMAN, D.V. (Editor). *Water quality assessment: A guide to the use of biota sedimenta and water in environment monitoring*. Basil Blackwell Limited, Oxford.

MOMBESHORA, C. O., OSIBANJO O. and AJAYI, S. O. (1983). Pollution studies on Nigeria rivers. The onset of lead pollution of surface water in Ibadan. *Environment International, 9*: 81 - 84.

OWEN, T. L. (1974). *A handbook of common methods in limnology.* C V Mosby Company, Waco, Texas. 153 pp.

SANDRA, C. (2000). *Managing phosphorus to protect water quality.* Alberta agriculture, food and rural development. 315 pp.

STEEL, R. G. D. and TORRIE, J. H. (1980). *Principles and procedures of statistics: A biometrical approach*. Second edition. McGraw-Hill, New York, NY. 633 pp.

SUTER, G. W. and LOAR, J. M. (1992). Weighing the ecological risk of hazardous waste sites: The Oak Ridge Case. *Environmental Science and Technology* 6: 432 - 438.

UGOCHUKWU, C. N. C. and LETON, T. G. (2004). Effluent monitoring of an oil servicing company and its impact on the environment. *Ajeam-Ragee* 8: 27-30.

BAOBAB (*Adansonia digitata* L.) SEED PROTEIN UTILIZATION IN YOUNG ALBINO RATS I: BIOCHEMICAL INGREDIENTS AND PERFORMANCE CHARACTERISTICS

EZEAGU, Ikechukwu Edwin
Nutrition Unit, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, P. O. Box 15676, Enugu, Nigeria. Email: ikezeagu@yahoo.co.uk

ABSTRACT

Raw, cooked and HCl-extracted baobab, Adansonia digitata seed meals were used for biological and nutritional evaluation studies. The seed is low in protein (16.60g/100g DM) but could be a good source of oil (17.50g/100g) and minerals, particularly sodium, potassium and phosphorus, which contained 228.0, 1429.0 and 924.5 mg/100gDM respectively. Low levels of antinutritional factors such as tannin, phytate, cyanide, oxalate, nitrate/nitrite and absence of trypsin inhibitors were observed. Seed protein is high in sulfur-amino acid, with a chemical score (CS) of 126.80, but marginally limiting in lysine and threonine, with CS of 64.31 and 85.59 respectively based on the preschool age (2-5yrs) reference protein requirement. The seed oil contain appreciable level of unsaturated fatty acids with oleic and linoleic acids making up 66.32% of total fatty acids. The raw diet was similar to the casein diet in weight gain, feed intake, net protein retention (NPR) and true digestibility (TD) but significantly inferior in protein efficiency ratio (PER). Cooking did not have any significant effect on feed intake but significantly lowered the weight gain relative to the raw and casein diets. HCl-extracted meal exerted significantly lower weight gain compared to the raw, cooked and casein diets. It is concluded that the raw seed showed promise as a source of food supplement and is likely to be satisfactory in supporting growth and maintenance in livestock feeding.

Keywords: Adansonia digitata, Baobab seed protein, Biochemical ingredients, Performance, Rats

INTRODUCTION

The majority of sub-Saharan African countries including Nigeria are faced with acute food shortages. The solution to the food problem must be sought through a combination of all available sources. Food and agricultural scientists are beginning to screen wild and under-exploited native plants for possible potential sources of food in an attempt to widen the narrow food base (Vietmeyer and Janick 1996; Oelke *et al.,* 1997). Several reports have also indicated that lots of lesser-known native crop species are high in nutrients and could possibly relieve critical food shortages if given adequate promotion and research attention (Madubuike *et al.,* 1994; Murray *et al.,* 2001). Working on the prospects of utilizing such lesser known and neglected plants, research reports have revealed that quite a large number have useful qualities – either for direct use as animal feed ingredient or as a raw material for seed protein extraction (Ezeagu *et al.,* 2000, 2003). However, prior to utilization of such unconventional resources data indicating the nutrient composition and toxic factors should be available. Toxicological evaluation of possible epidemiological response to the ingestion of novel food sources and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization (Longvah *et al.,* 2000).

Baobab is a well-adapted deciduous tree native to the arid parts of Central Africa and widely spread in the savannah regions in Nigeria (Wickens, 1980; FAO, 1988). Its leaves, bark and fruit are used as food and for medicinal purposes in many parts of Africa. In the Sahel, for example, baobab leaf is a staple the Hausas used to make "*miyan kuka",* a soup prepared by boiling the leaf in salt water and reported to be a rich source of Vitamin C. During acute seasonal food supply fluctuations or famine periods, the leaves and fruit of baobab are of particular importance as supplementary and emergency food (Humphrey et al. 1993). The seed has a relatively thick shell, which is not readily separated from the kernel. The kernel is edible but the difficulties of decorticating seem to have limited its use as food/feed and consequently large quantities go into waste. But the increasing pressure of population and predictable food shortages are creating a demand for new food sources of human nutrition. Few reports have indicated the composition of the baobab fruit pulp and leaves (Nour *et al.,* 1980;

Yazzie *et al.,* 1994; Obizoba and Anyika, 1994), but reports on the nutritional and/or biochemical evaluation of the whole seed are scarcely available. An earlier report had indicated the potential of its use as food component or feed supplement (Proll *et al.,* 1998). This study seeks to verify further the nutritional qualities of baobab seed as a protein source and the effect of processing on the nutritional quality in albino rats

MATERIALS AND METHODS

Treatment of Sample: About 2 kg of the matured fruits were harvested from different locations around the city of Ibadan, Nigeria and the seeds were manually separated from the pods. Cooking was done by immersing in boiling water and allowing boiling for 30 min. For acid-extraction, 500g of seeds were immersed in 0.25 M HCl at 60 ^{o}C for 4 h according to the method of Tasneem *et al.* (1982). The acid extract was decanted and the residue washed free of acid using tap water and then dried. Raw and treated samples (350 g each) were ground to flour using a Wiley Mill with the 1 mm mesh sieve and stored in plastic bags at -4^{o} C until analysis.

Proximate Analysis: Nitrogen, fat, ash, micro- and macro-minerals were determined by standard methods (AOAC, 1990). Crude proteins (CP) and total carbohydrates were calculated by N x 6.25 and difference respectively. Total soluble sugars and starch were determined by the combined methods of Duboise *et al.* (1956) and Kalenga *et al.* (1981). Soluble sugars were extracted with ethanol (95 %) and residual starch was then hydrolysed with perchloric acid into monosaccharides. The sugars were then colourimetrically determined with phenol-sulphuric acid. Gross energy was calculated from the Atwater conversion system (FAO, 1982).

Analysis of Antinutritional Factors: Tannin was determined by the Folin-Denis method (AOAC, 1990); Phytic acid by the method of Wheeler and Ferrel (1971); trypsin inhibitor activity by the method of Kakade *et al.* (1974) using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. Phytohaemagglutinating activity was determined by the serial dilution method of Liener and Hill (1953) using trypsinized rabbit erythrocytes and expressed as haemagglutinating unit (HU)/mg sample. Cyanide was extracted with 0.1 M ortho-H_3PO_4 acid and estimated using an auto analyzer according to the method of Rao and Hahn (1984). Nitrate and nitrite were determined as previously described (Ezeagu and Fafunso, 1995) and oxalate was determined by the method of Baker (1952).

Amino Acid Analysis: Amino acids were determined according to the recommendations of FAO/WHO (1991) by triple hydrolysis (Pellet and Young, 1980) as previously described (Petzke *et al.,* 1997).

Fatty Acid Analysis: For fatty acid analysis the lipid extract was transmethylated with trimethylsulfonium-hydroxide (TMSH) as described by Schulte and Weber (1989). Aliquots (10 mg) of fat were dissolved in 250 µl trichloromethane, followed by addition of 250 µl of the internal standard and 250 µl of TMSH solutions. The fatty acid methyl esters were analyzed using a GLC (model 5890 series II, Hewlett Packard Co., Palo Alto, CA.) equipped with a flame-ionization detector and a 30 m capillary column (DB-Wax, id 0.32mm). The initial oven temperature was 140 ^{o}C followed by temperature programming in three steps: a first rate of 4^{o}C/min until 170^{o}C, followed by a second rate of 1.5^{o}C/min until 185^{o}C and a third rate of 4^{o}C/min until 220 ^{o}C. The final temperature was maintained for 33 min. The injection temperature was 225 ^{o}C and the detector temperature was 250 ^{o}C. Helium was used as the carrier gas. Peak areas were integrated using Hewlett-Packard 3365 Series II ChemStation software, and the fatty acids were expressed as percentage of total fatty acid pool. Fatty acids were identified by comparison of their retention time with those of known standards. Quantitative data were obtained using tricosanoate ($C_{23:0}$) as an internal standard.

Animals and Diets: Experimental diets were prepared according to the method of Chapman et al. (1959) with adequate provision of vitamins and minerals (Miller, 1963). Twenty weanling male albino rats, about 24 days old with mean weights of 26-28 g were obtained from the Preclinical Laboratories of the University of Ibadan, Ibadan. The animals were housed individually in all-aluminum screen metabolic cages with provision for urine, faecal collection and unrestricted access to water and food. The rats were assigned four per group, equalized for body weight in a randomized block design. One group was fed the basal protein-free diet, another group was given a 10% protein diet based on casein, the other three groups were assigned to diets with 10% protein supplied by the raw, cooked or acid-extracted meals, respectively. Weighed amounts of diets were daily offered to the animals for 21 days. The food residues were collected, dried and weighed. The faeces were oven-dried at 60 ^{o}C and stored in plastic containers until analyzed. A drop of dilute H_2SO_4 was added to urine samples to prevent any loss of nitrogen. The rats were weighed weekly and protein efficiency ratio (PER), net protein retention (NPR) and true digestibility (TD) were computed from total feed intake, total faeces voided, as well as the nitrogen determination.

Table 1: Proximate composition and mineral components of baobab seed meal compared to soybean, cowpea and maize

	Baobab	Soybean*	Cowpea*	Maize*
Proximate Composition				
Crude protein	16.60	36.70	23.1	8.9
Crude fat	17.50	20.10	15.0	3.9
Ash	5.50	4.60	3.4	1.2
Carbohydrates	60.40	33.95	67.8	74.2
Total sugars	2.52	-	-	-
Starch	22.60	-	-	-
Crude fibre	14.94	-	-	-
Energy, kJ (kcal)/100g	1883 (450)	1816 (434)	2016 (482)	1490 (356)
Minerals (mg/100g)				
Sodium	228.0	10.0	20.0	
Potassium	1429.0	192.0	96.0	
Calcium	212.0	260.0	130.0	
Magnesium	353.0	320.0		
Phosphorus	924.5	750.0	430.0	
Iron	11.13	-	-	
Copper	2.55	-	-	
Zinc	8.41	-	-	
Manganese	2.10	-	-	

** FAO 1982.[1] Mean of two independent analyses, - Not available*

All analysis was done in duplicate. Data were analyzed by one-way analysis of variance. Treatment means were compared by the Duncan's (1955) multiple range tests.

RESULTS AND DISCUSSION

Chemical analysis of the whole baobab seed is presented and compared to some common staples in Table 1. The results seem to be on the same level with the previous report (Proll et al., 1998). Comparing protein contents, baobab seed is lower in protein (16.60g/100g) than soybean (36.70 g/100g) and cowpea (23.10g/100g) but higher than maize (8.90g/100g). Total sugar is low (2.52g/100g) but starch content of (22.60 g/100g) is higher than the 18.44 g/100g reported for soybean (Ezeagu et al., 2000). With a total fat and carbohydrate contents of 17.50 and 60.40 g/100g respectively, baobab seeds could be a good source of energy and edible oil, and thus a useful supplement in animal feed formulation. There are appreciable levels of minerals, potassium (1429.0) and phosphorus (924.5mg/100g) being the most abundant. The seed meal seems to be higher in iron (11.13), copper (2.55) and zinc (8.41mg/100g) than conventional staples and will easily satisfy animal needs, assuming that they occur in readily available forms. About 34% of total P occurred as phytate-P, which is lower than 80% value reported for most legumes (Rackis and Anderson, 1977). Gross energy (1883.28) was higher than that of maize (1490.22) but comparable to those of soybean (1815.89) and common beans (2016.40 kJ/100g).

The results on antinutritional components (Table 2) showed absence of trypsin inhibitor, which could be considered as a nutritional advantage. Tannin (0.29 mg/g), phytate (1.20 g/100 g), total oxalate (42.0 mg/100 g) and cyanide (0.25mg/100g) appeared low and in reasonable agreement with values reported for commonly consumed food articles.

Table 2: Antinutritional components of baobab seed

Parameters	Baobab* seed
Tannin, mg/g	0.29
Phytate, g/100g	1.20
Phytate-phosphorus	0.34
Phytate-P as % total P	1.0
Trypsin inhibitor, TIU/mg	ND
Haemagglutinins, HU/mg	0.250
Cyanide, mg/100g	0.25
Total oxalate, mg/100g	42.0
Water soluble Oxalate	26.0
Soluble oxalate as % of total oxalate	61.9
Nitrate, mg/g	19.45
Nitrite, mg/g	0.104

*ND: Not Detected, *Means of two independent analyses*

Amino acid profile as shown in Table 3 indicates a fair complement of essential amino acids. Using the FAO/WHO/UNU (1985) preschool age (2-5yrs) reference amino acid requirement as a guide in calculating the chemical score (CS) of amino acids, the seed seems marginally limiting in lysine and threonine (CS 64.31 and 85.59% respectively) but

Table 3: Essential amino acid profile of baobab seed (g/100g Protein)

	Baobab	Chemical score	FAO/WHO/UNU 1985 (reference pattern)	
			Child* 2-5yr	Adult
Lysine	3.73	64.31	5.80	1.60
Methionine	1.25			
Cystein	1.92			
Total S-Amino Acids	**3.17**	**126.80**	**2.50**	**1.70**
Isoleucine	3.54	126.43	2.80	1.30
Leucine	6.54	99.09	6.60	1.60
Phenylanine	4.54			
Tyrosine	2.72			
Total Aromatic Amino Acids	**7.26**	**115.24**	**6.30**	**1.90**
Threonine	2.91	85.59	3.40	0.90
Tryptophan	1.38	125.45	1.10	0.5
Valine	4.99	142.57	3.50	1.30
Histidine	1.98	104.21	1.90	1.60
[1]**Total EAAs**	**33.52**	**43.30**	**33.90**	**12.70**

**For calculating the chemical score of amino acids, the FAO/WHO/UNU (1985) reference pattern for children 2-5 years old was used, Total EAAs: Total essential amino acids*

high in sulphur-amino acids (CS 126.8%). Acceptable CS is considered to be in the order of 60 and above (Nordeide *et al.,* 1994). However, the seed protein was quite adequate in total essential amino acids (EAAs) and compared favorably to the reference protein in total EAAs and in meeting the recommended adult requirements.

Oleic and linoleic acids are the most abundant unsaturated fatty acids (Table 4). With low polyunsaturated/saturated ratio (P/S) (1.1) compared to soybean (3.6) and other high linoleic sources (Sinclair, 1964), the baobab seed oil may not be considered a good source of essential fatty acids.

Table 4: Fatty acid composition of oil (Area %)*

Fatty acids	Baobab
Lauric C_{12}	-
Myristic $C_{14:0}$	0.25
Palmitic $C_{16:0}$	22.06
Palmitoleic $C_{16:1n-7}$	0.27
Hexadecadienic $C_{16:2n-4}$	0.95
Stearic $C_{18:0}$	4.02
Oleic $C_{18:1n-9}$	34.97
Oleic (isomer) $C_{18:1n-7}$	1.00
Linoleic $C_{18:2n-6}$	26.14
Linolenic $C_{18:3n-6\ \gamma}$	0.49
Linolenic $C18:3n-3\ \alpha$	2.00
Arachidic $C_{20:0}$	0.86
Gadoleic $C_{20:1n-9}$	0.22
Benhenic $C_{22:0}$	0.42
Lignoceric $C_{24:0}$	-
Sum	93.65
Sat[a]	27.61
P/S ratio[b]	1.07

*[a]Sum of $C_{14:0}$ +$C_{16:0}$+ $C_{18:0}$ +$C_{20:0}$+$C_{22:0}$, [b]Polyunsaturated ($C_{16:2}$+$C_{18:2}$+$C_{18:3}$ / Saturated ($C_{16:0}$+$C_{18:0}$+$C_{20:0}$+$C_{22:0}$+$C_{24:0}$), *Mean of two independent analyses*

Results of the feeding experiment (Table 5) showed total weight gain, feed and protein intakes (13.45, 70.40 and 8.42 g respectively) of rats maintained on the raw meal were statistically similar ($P < 0.01$) to those on the casein control diet (17.70, 71.53 and 7.15 g respectively). Rats on the raw meal recorded a significantly ($P < 0.01$) superior weight gain (13.45) compared to those on the cooked (9.80 g) and HCl-extracted (3.53 g) meals. Cooking did not have significant effect on feed and protein intakes but significantly ($P < 0.01$) lowered the weight gain compared to the raw and casein diets. It is possible heat damage may have occurred, even though, prolonged cooking was avoided in this experiment (Amadi and Hewilt, 1975). Heat is reported to enhance the nutritive value of proteins by making the sulfur-containing amino acids more available to the animal (Hayward et al., 1936). It was also observed that animals on HCl-extracted meal have significantly ($P < 0.01$) lower feed intake relative to those on the raw, cooked and control diets. PER values of 1.63, 1.57 and 0.49 obtained for raw, cooked and HCl-extracted meals respectively differed significantly ($p<0.01$) compared to the casein diet (2.47). PER for the raw and cooked meals seems to be on the same level with 1.96 previously reported for autoclaved baobab meal (Proll *et al.,* 1998).

While cooking did not improve the PER over the raw meal, acid-extraction significantly lowered the PER relative to the raw and cooked meals. Kawatara *et al.* (1969) has reported low growth of rats fed HCl-extracted meals. Acid–

Table 5: Protein quality indices of raw and processed baobab seed meal

Diets	Weight gain (g)	Feed intake (g)	Protein intake (g)	PER	NPR	TD %
Casein	17.70 ±1.71[a]	71.53±4.71[a]	7.15±0.47[ab]	2.47±0.08[a]	3.07±2.34[a]	93.17±5.37[a]
Raw	13.45 ±2.64[a]	70.40±4.14[a]	8.42±1.26[a]	1.63±0.29[b]	2.18±2.96[a]	80.64±15.23[a]
Cooked	9.80 ± 1.27[b]	62.90±4.98[a]	6.29±0.47[b]	1.57±0.21[b]	2.28±2.48[a]	85.53±1.49[a]
HCl-Extracted	3.53 ± 3.07[c]	59.88±10.09[b]	6.35±0.99[b]	0.49±0.62[c]	0.69±7.90[b]	79.91±1.69[b]

abc (Means not followed by the same subscript on the same column are significantly different (P<0.05) Mean ±SD (Standard Deviation)

extraction may have affected palatability negatively and/or caused loss of nutritional components resulting to poor quality meal. But this observation however, contradicts the report of Tasneem and Subramanian (1986) that acid extraction leached out antinutritional substances from guar seed and significantly improved growth parameters of experimental animals. Effect of acid extraction may therefore depend on the nature of the food substrate. NPR and TD values were lower, but only significantly ($P < 0.05$) for the acid-extracted meal, compared to casein. The fiber content of baobab seed and residual antinutritional factors may have hindered attack of proteins by digestive enzymes, thus reducing digestibility.

It may be inferred from this study that uncooked baobab seed is well tolerated by experimental rats and thus could be recommended as a potential protein source. However, factors of amino acid digestibility and/or availability may need further investigation.

REFERENCES

AMADI, S. C. and HEWITT, D. (1975). The digestibility and availability of lysine and methionine in isolated soybean protein after severe heat damage. *Proceedings of Nutrition Society, 34(1):* 26A - 35A.

AOAC (1990). Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

BAKER, C. J. C. (1952). The determination of oxalates in fresh plant material. *Analyst, 77:* 340 - 344.

CHAPMAN, D. G., CASTILLO, R. and CAMPBELL, J. A. (1959). Evaluation of protein foods. I. A method for the determination of protein efficiency ratio. *Canadian Journal of Biochemistry and Physiology, 37:* 679 - 686.

DUBOISE, M. C., GRUILLIES, K. A., HAMILTON, J. K., ROGERS, P. A. and SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28:* 350 - 356.

DUNCAN, D. B. (1955). Multiple range and multiple F-tests. *Biometrics, 11:* 1 - 42

EZEAGU, I. E. and FAFUNSO, M. A. (1995). Effect of wilting and processing on the nitrate and nitrite contents of some Nigerian leaf vegetables. *Nutrition and Health, 10:* 269 - 275.

EZEAGU, I. E., PETZKE, K. J., OLOGHOBO, A. D., METGES, C. C. and AKINSOYINU, A. O. (2000). Compositional evaluation and feed potential of the African kudzu (Pueraria phaseoloides, Roxb. Benth) seed meal. *Journal of Food Science and Technology, 37:* 191 - 195.

EZEAGU, I. E., MAZIYA-DIXON, B. and TARAWALI, G. (2003). Seed characteristics and nutrient and anti-nutrient composition of 12 *Mucuna* accessions from Nigeria. *Tropical and Subtropical Agroecosystems, 1:* 129 - 139.

FAO (1982) Food Composition tables for the Near East. Food and Agricultural Organization (FAO/UN), Rome. *Food and Nutrition Paper, 26:*

FAO/WHO (1991) Protein Quality Evaluation, Report of a joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Consultation, Rome. Food and Agriculture Organisation, *FAO Food and Nutrition Paper, No.51:*

FAO/WHO/UNU (1985). Energy and Protein Requirements. Report of Joint Food and Agricultural Organization/World Health Organization/United Nations University (FAO/WHO/UNU) Expert Consultation, Geneva. *WHO Technical Report, Series No. 724:*

FAO (1988). Traditional Food Plants. Food and Agricultural Organization (FAO/UN), Rome*. Food and Nutrition Paper, 42:*

HAYWARD, J., STEANBLACK, H. and BOHSTEDT, O. (1936). Effect of heat on protease inhibitor and nutritive value of heat-treated legume proteins. *Poultry Science, 15:* 180 – 182.

HUMPHREY, C. M., CLEGG, M. S., KEEN. C. L. and GRIVETTI, L. E. (1993). Food diversity and drought survival; The Hausa example. *International Journal of Food Science and Nutrition, 44:* 1 – 16.

KAKADE, M. L., RACKIS, J. J., MCGHEE, J. E. and PUSKI, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chemistry, 51:* 376 - 382.

KALENGA, K., HOOD, L. F. and VANSOEST, P. J. (1981) Characterisation of starch and fibre of banana fruit. *Journal of Food Science, 46:* 1885 - 1890.

KAWATRA, B. L., GARCHA, J. S. and WAGLE, B. S. (1969). Studies on Guar (*Cyamopsis tetragoloba*). *Journal of Nutrition and Dietetics, 6:* 91 – 97.

LIENER, I. E. and HILL, E. G. (1953). The effect of heat treatment on the nutritive value and haemagglutinating activity of soybean oil meal. *Journal of Nutrition, 49:* 609 – 619.

LONGVAH, T., DEOSTHALE, Y. G. and KUMAR, P. U. (2000). Nutritional and short-term toxicological evaluation of perilla seed oil. *Food Chemistry, 70:* 13 - 16.

MADUBUIKE, F. N., OJIMELUKWE, P. C. and AJAH, P. O. (1994). Proximate composition, energy content and physicochemical properties of *Afzelia africana* and *Brachystegia eurycoma* seeds. *Plants Foods for Human Nutrition, 46:* 339 - 334.

MILLER, D. S. (1963). A procedure for determination of NPU using rat body technique. Pages 34 – 36. *In: Evaluation of Protein Quality.* National Academy Science (NRC), Washington DC, USA.

MURRAY, S. S., SCHOENINGER, M. J., BUNN, H. T., PICKERING, T. R. and MARLETT, J. A. (2001). Nutritional composition of some wild plant foods and honey used by Hadza foragers of Tanzania. *Journal of Food Composition and Analysis, 14:* 3 - 13.

NORDEIDE, M. B., HATLOG, A., FOLLING, M., LIED, E. and OSHANG, A. (1994). Nutrient composition and nutritional importance of green leaves and wild food resources in an agricultural district, Koutiala, in Southern Mali. *International Journal of Food Science and Nutrition, 47(6):* 455 - 468.

NOUR, A. A., MAGBOUL, B. I. and KHEIRI, N. H. (1980). Chemical composition of baobab fruit (*Adansonia digitata* L.). *Tropical Science, 22:* 383 – 388.

OBIZOBA, I. C. and ANYIKA, J. U. (1994). Nutritive value of baobab milk (gubdi) and mixtures of baobab (*Adansonia digitata* L.) and hungry rice acha (*Digerati exilis*) flours. *Plant Foods for Human Nutrition, 46:* 157 – 165.

OELKE, E. A., PORTER, R. A., GROMBACHE,R A. W. and ADDIS, P. B. (1997). Wild rice – New interest in an old crop. *Cereal Foods World, 42(4):* 234 - 247.

PELLET, P. L. and YOUNG, V. R. (1980). Nutritional evaluation of protein foods. *UN University Food Nutrition Bulletin, (Supplementary 4):*

PETZKE, K. J., EZEAGU, I. E., PROLL, J., AKINSOYINU, A. O. and METGES, C. C. (1997). Amino acid composition, available lysine content and in vitro protein digestibility of selected tropical crop seeds. *Plant Foods for Human Nutrition, 50:* 151 – 162.

PROLL, J., PETZKE, K. J., EZEAGU, I. E. and METGES, C. C. (1998). Low nutritional quality of unconventional tropical crop seeds in rats. *Journal of Nutrition, 128:* 2014 – 2022.

RACKIS, J. J. and ANDERSON, R. L. (1977). Mineral availability in soy protein products. Food *Products Development, 11:* 44 – 47.

RAO, P. V. and HAHN, S. K. (1984). An automated enzymatic assay for determining the cyanide content of cassava products. *Journal of Science of Food and Agriculture, 35:* 426 – 436.

SCHULTE, E. and WEBER, K. (1989). Schnelle Herstellung der Fettsäuremethyester aus Fetten mit Trimethysulfoniumhydroxid oder Natriummethylat. *Fat Science and Technology, 91:* 181 – 183.

SINCLAIR, H. M. (1964). Carbohydrate and fats. Pages 41 - 56. *In:* BEATON, G. H. and MCHENRY, E. W. (Eds.). *Nutrition,* Volume 1, Academic Press, New York.

TASNEEM, R. and SUBRAMANIAN, N. (1986). Nutritional quality of detoxified Guar (*Cyamopsis tetragonoloba*) meal. *Journal of Agriculture, Food and Chemistry, 34:* 853 – 857.

TASNEEM, R., RAMAMANI, S. and SUBRAMANIAN, N. (1982). Functional properties of Guar seed (*Cyamopsis tetragonoloba*) meal detoxified by different methods. *Journal of Food Science, 47:* 1323 – 1328.

VIETMEYER, N. and JANICK, J. (1996). New crops. Pages 2 – 8. *In:* Proceedings of the Third National Symposium of American Society of Horticultural Scientist, Alexandria, USA, 22 – 25 October 1996.

WHEELER, E. L. and FERREL, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry. 48:* 312 – 320.

WICKENS, G. E. (1980). The use of the baobab (*A. digitata* L) in Africa. Page xx – zz. *In:* LE HOUEROU, H. N. (Ed.). *Browse in Africa: The current state of knowledge*. ILCA, Addis Ababa, Ethiopia.

YAZZIE, D., VANDERJAGT, D., PASTUSZYN, A., OKOLO, A. and GLEW R. H. (1994). The amino acid and mineral content of baobab (*Adansonia digitata* L) leaves. *Journal of Food Composition and Analysis, 7:* 189 - 193.

EFFECT of *Dermestes maculatus* INFESTATION ON SOME NUTRITIONAL COMPOSITION OF SMOKED AFRICAN CATFISH, *Clarias gariepinus* BURCHELL, 1822

[1]UGWU, Lawrence Linus Chukwuma, [2]NWAMBA, Helen Ogochukwu and [2]KANNO, Linda Uchenna

[1]Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria

Corresponding author: Dr. UGWU, L. L. C., Department of Animal Production and Fisheries Management, Ebonyi State University, P.M.B.053, Abakaliki, Ebonyi State. Nigeria

ABSTRACT

Studies on pest infestation of some nutritional composition of smoked-dried Clarias gariepinus were carried out, to assess the effect of exposing preserved fish products to different levels of infestation by Dermestes maculatus and the resultant effect on pH, crude protein (CP), free fatty acid (FFA) and tissue contents. Graded levels (5, 10 and 15) of larval and adult D. maculatus were used to infest pieces of C. gariepinus placed in 7 groups of 3 bottles per group. The experiment lasted 8 weeks (56days). There were consistent decreases in the pH and CP as well as the tissue contents of fish with storage time, although the CP content of the samples with the larval pests did not differ significantly from those without larval pests (P > 0.05). Fish samples exposed to adult pests showed significant variation (P < 0.05) in their pH and CP contents while the FFA content increased with storage time but was not significant (P > 0.05). The longer the storage periods of the infested smoked fish the more the tissue was degraded.

Keywords: *Dermestes maculatus, Clarias gariepinus* infestation

INTRODUCTION

The African catfish, genus *Clarias* has very high commercial value in Nigeria owing to its flavour and taste. The good quality coupled with its ability to feed on virtually anything makes the fish a highly recommended species for aquaculture development in Nigeria (Reed *et al*., 1967; Bard *et al*., 1976; Olatunde, 1983).

The phenomenon of fish preservation is enunciated by the simple principle of making fish unfavourable for the growth of spoilage organisms. In Nigeria, fish drying is the most adopted technique since it is within the socio-economic levels of artisanal fishermen contributing over 90% of domestic fish supply (Mabawonku and Ajayi, 1982). Many materials subjecting fish to different preservation techniques notably: traditional smoking, traditional solar drying, oven and Ife solar drying have been carried out (Afolabi *et al*., 1984). Analyses of the preserved fish showed the occurrence of a high proportion of predominantly unsaturated fatty acids in oven and Ife solar-dried fish compared to the traditionally smoke-dried fish. In spite of the shortcoming, there is an insatiable market for dried fish products in the country.

An estimated 95% of the total artisanal fish landings are smoked or sun-dried; the problem of large scale infestation often results in more than 50% losses due to inadequate packaging and storage (Moses, 1983). The need to further investigate the extent and rate of damage of the nutritional quality of dried fish products becomes imminent. Studies on the action of *D. maculatus* as a pest of dried fish and meat in Nigeria and Zambia have been carried out (Osuji, 1975; Proctor, 1977). Although much work has been done on the prevalence and rate of degradation of this pest to host species, little has been done on the effect of pest infestation on nutritional quality of *Clarias gariepinus*. The objective of this study therefore was to determine the effect of varying levels of pest infestation on the crude protein, free fatty acid, pH and tissue degradation of smoked *C. gariepinus*.

MATERIALS AND METHODS

Fish Collection and Preservation: Twenty-one (21) live specimens (450 ± 2.30g) of African catfish, *C. gariepinus* (Burchell, 1822) were bought from Artisan market, Enugu, Nigeria. *The Practical Manual for the Culture of the African Catfish,*

Clarias_gariepinus. (Viveen *et al*., 1986) was used in the identification of the fish specimens. The specimens were later killed, gutted and decapitated. The carcasses were cut into small pieces, washed thoroughly with water and immersed in 20% saline solution for 10 minutes in accordance with the methods specified by Horn (1974). The immersion in salt solution was to reduce the water activity (A_w) in the specimens and retard microbial development. They were subsequently smoked over a smoking kiln for 48 hours at 87^0C, packed into small polythene bags and kept in the Research Laboratory of Enugu State University of Science and Technology, Enugu.

Collection of D. maculatus: *D. maculatus* were obtained from 10kg of heavily infested dried C. gariepinus purchased from fish sellers at Enugu main market, Enugu, Nigeria. The larval and adult stages of this pest were disengaged from the tissues and placed in 21 clean reagent bottles (500ml) with perforated covers. In the laboratory, the pests were placed in another set of 21 clean reagent bottles (50ml) whose open ends were covered with mosquito-mesh nets.

Infestation of *C. gariepinus* with *D. maculatus*: The study was designed to have smoked pieces of *C. gariepinus* (0.50 kg) subjected to four treatment groups (A, B, C and D) of pest infestation. Group A represented the control with no pest infestation, while groups B, C and D represented low (LI), medium (MI) and high (HI) infestations of both the adult and larval stages of *D. maculatus*. Both the control (A) and the pest infested treatments (B, C and D) were replicated thrice to give a total of 21 experimental replicates.

Smoked pieces of *C. gariepinus* (10.50 kg) were measured out with a chemical balance and randomly placed in 21 clean reagent bottles (500 ml) at 0.50 kg of fish per bottle. Fish samples contained in 3 bottles under group B were infested with 5 adult *D. maculatus* while the remaining 3 bottles (under the same group) were infested with 5 larval pests. This level of infestation was regarded as low infestation (LI). Fish samples under group C were infested with 10 adult pests (3 bottles) and 10 larval pests (3 bottles) and this was also regarded as medium infestation (MI). Fish samples in group D were infested with 15 adult pests (3 bottles) and 15 larval pests (3 bottles) regarded as high infestation (HI). Group A fish samples (3 bottles) were uninfested and served as the control. Each group of bottles with infested fish samples was appropriately labelled according to treatment and left for investigation for 56 days.

Fish samples from each bottle were disengaged from larval and adult pests every 2 weeks (14 days), weighed and analysed chemically for crude protein (AOAC, 1995), free fatty acid (Marinetti,1967) and pH using a pH meter.

Chemical Analysis: Fat contents of fish samples were extracted by Bligh and Dyer (1959) method. The fatty acids were determined by gas-lipid chromatography (GLC) through esterification by refluxing in 4% sulphuric acid (H_2SO_4) and methylation with methyl-hydroxide (MeOH) for 16h at 79^0C (Marinetti, 1967). Complete esterification was confirmed by thin layer chromatography (TLC) on silica gel G plates using a solution of petroleum ether, diethyl ether, and acetic acid (90:10:1) as solvent system.

Total nitrogen was measured by micro-kjeldahl method and the crude protein content determined by multiplying by 6.25 (AOAC, 1995). The pH was determined with a pH meter immersed in a suspension of finely ground fish samples. All the data obtained were subjected to analysis of variance to determine the levels of significance (Steel and Torrie, 1990).

RESULTS

The crude protein (CP) content of *C. gariepinus* exposed to larvae of *D. maculatus* decreased consistently from a pre-infestation protein value of 78.52% to a least value of 45.23 % (Table 1). This result varied with smoked fish sample exposed to adult pest and whose crude protein content decreased from 78.52 % to 38.10 % within 8 weeks (56 days) experimental period. The least CP content was found in the control experiment after 8 weeks (56 days). This was compared to the least CP value (45.23 %) for high larval infestation (HI) and 53.61 % for medium larval infestation (MI) (Table 1). There was no significant difference $F(4,8) = 1.36$ ($P > 0.5$) in the CP content between various levels of larval infestation. However, there was a significant difference $F(4, 8) = 9.84$ ($P < 0.05$) in CP content of smoked fish exposed to adult *D. maculatus*.

The greatest loss (21.99 %) in tissue weight was recorded in *C. gariepinus* infested with adult *D. maculatus* (Table 2). This value indicated that the adult pests were more destructive than the larval pests. In addition, the fast rate of loss in tissue weight could be due to increase in fat content of the fish sample. Two-way analysis of variance indicated a significant difference between periods of exposure of infested fish samples and levels of treatments ($P < 0.05$). The control experiment showed uninfested fish samples with a declining trend of protein values and there was a

Table 1: Percentage (%) mean crude protein of smoked *C. gariepinus* infested with *D. maculatus*

Treatments	Weeks of Study				
	0	2	4	6	8
Larvae					
L1	78.52 ± 0.14	72.62 ± 0.25	68.40 ± 0.24	66.40 ± 0.09	61.20 ± 0.39
M1	78.52 ± 0.13	70.32 ± 0.19	67.01 ± 0.32	55.78 ± 0.49	53.61 ± 0.59
H1	78.52 ± 0.35	71.20 ± 0.65	69.14 ± 0.47	48.10 ± 0.18	45.23 ± 0.16
			F (4,8) = 1.36 (P > 0.05)		
Adults					
LI	78.52 ± 0.18	61.22 ± 0.23	61.25 ± 0.18	58.46 ± 0.28	55.03 ± 0.05
MI	78.52 ± 0.45	67.50 ± 0.37	60.02 ± 0.48	56.74 ± 0.66	43.72 ± 0.48
HI	78.52 ± 0.36	60.04 ± 0.36	46.02 ± 0.34	43.72 ± 0.44	38.10 ± 0.26
			F (4,8) = 9.84 (P < 0.05)		
CTRL	78.52 ± 0.12	66.04 ± 0.66	55.87 ± 0.45	52.81 ± 0.56	45.81± 0.27

LI= low infestation, MI =medium infestation, HI = high infestation, CTRL = control

Table 2: Mean weight losses (g) of smoked *C. gariepinus* infested with *D. maculatus*

Treatments	Weeks of Study				
	0	2	4	6	8
Larvae					
LI	15.46 ± 0.34	14.26 ± 0.41	13.06 ± 0.14	10.66 ± 0.31	9.47 ± 0.22
MI	15.46 ± 0.26	13.06 ± 0.47	10.86 ± 0.47	9.30 ± 0.27	6.80 ± 0.45
HI	15.46 ± 0.55	11.66 ± 0.24	9.86 ± 0.24	7.86 ± 0.47	4.68 ± 0.10
	F - value (treatment) = 5.91 (P > 0.05); F - value (period) = 82.92 (P > 0.01)				
Adults					
LI	15.46 ± 0.08	13.66 ± 0.27	11.94 ± 0.34	10.59 ± 0.31	7.38 ± 0.40
MI	15.46 ± 0.45	12.48 ± 0.36	10.81 ± 0.48	7.80 ± 0.41	5.39 ± 0.31
HI	15.46 ± 0.35	12.96 ± 0.23	10.10 ± 0.22	7.34 ± 0.51	3.40 ± 0.41
	F - value (treatment) = 132.97 (P < 0.01); F-Value (period) = 3.63 (P < 0.05)				
CTRL	15.45 ± 0.35	15.43 ± 0.54	15.37±0.41	15.32±0.35	15.32 ± 0.35
	F - value (treatment) = 4.63 (P < 0.05); F-value (period) = 135.97 (P < 0.01)				

LI = Low infestation, MI = Medium infestation, HI= High infestation, CTRL = control

Table 3: Free fatty acids (as % total fatty acid weight) of total lipids of smoked *C. gariepinus* subjected to various levels of *D. maculates* infestation.

Treatments	Weeks of Study				
	0	2	4	6	8
Larvae					
LI	8.42 ± 0.13	9.15 ± 0.16	12.17 ± 0.32	16.96 ± 0.07	18.36 ± 0.14
MI	8.42 ± 0.28	10.85 ± 0.18	12.43 ± 0.21	15.97 ± 0.17	18.01 ± 0.58
HI	8.42 ± 0.15	9.36 ± 0.18	14.60 ± 0.20	18.71 ± 0.35	20.87 ± 0.43
	F - value (treatment) = 1.23 (P > 0.05); F-Value (period) = 108.38 (P < 0.01)				
Adults					
LI	8.42 ± 0.32	9.14 ± 0.66	11.25 ± 0.40	15.64 ± 0.32	19.50 ± 0.19
MI	8.42 ± 0.11	10.20 ± 0.33	13.03 ± 0.58	16.58 ± 0.26	20.00 ± 0.59
HI	8.42 ± 0.21	10.35 ± 0.43	13.48 ± 0.57	16.74 ± 0.35	21.78 ± 0.38
	F - Value (treatment) = 3.89 (P < 0.05); F - Value (period) = 398.25 (P < 0.01)				
CRTL	8.42 ± 0.05	9.00 ± 0.12	12.70 ± 0.10	16.30 ± 0.40	20.10 ± 0.30
	F - Value (treatment) =3.82 (P < 0.05); F- Value (period) = 39.4 (P < 0.01)				

LI = Low infestation, MI = medium infestation, HI = High infestation, CTRL = Control

significant difference between these values and storage period ($P < 0.05$). However, no significant difference was obtained for fish infestation levels ($P > 0.05$)

The quantity of free fatty acids in *C. gariepinus* in response to the period of exposure to infestation of larval and adult *D. maculatus* is presented in Table 3. The result of the control experiment is also presented. The highest mean value of 21.78 ± 0.43 % is obtained from larval infestation. Two-way analysis of variance revealed a significant effect ($P > 0.05$) of both larval and adult infestation levels over storage time. However, the different levels of larval treatment

Table 4: Percentage (%) total lipids of smoked *C. gariepinus* infested with *D. maculatus*

Treatments	weeks of study				
	0	2	4	6	8
Larvae					
LI	8.60 ± 0.12	8.69 ± 0.14	8.05 ± 0.29	8.40 ± 0.08	8.70 ± 0.16
MI	9.31 ± 0.16	8.71 ± 0.26	8.71 ± 0.22	8.61 ± 0.18	8.71 ± 0.56
HI	8.60 ± 0.15	8.60 ± 0.18	8.73 ± 0.21	8.70 ± 0.36	8.70 ± 0.45
F - value (treatment) = 1.60 (P > 0.05); F-value (period) = 38.77 (P < 0.01)					
Adults					
LI	8.39 ± 0.32	8.64 ± 0.64	8.72 ± 0.38	8.59 ± 0.33	8.60 ± 0.18
MI	6.73 ± 0.43	6.88 ± 0.32	6.94 ± 0.56	7.02 ± 0.24	7.58 ± 0.57
HI	6.24 ± 0.54	6.64 ± 0.41	6.98 ± 0.58	7.07 ± 0.35	7.46 ± 0.30
F-value (treatment) = 2.63 (P < 0.05); F-Value (period) = 49.79 (P < 0.01)					
CTRL	6.68 ± 0.44	6.96 ± 0.42	7.24 ± 0.36	7.13 ± 0.32	7.49 ± 0.31
F-value (treatment) = 3.38 (P < 0.05); F- Value (period) = 37.45 (P < 0.01)					

LI = Low infestation, MI = medium infestation, HI= High infestation, CTRL= control

Table 5: Mean pH values of smoked *C. gariepinus* infested with *D. maculatus*

Treatments	Weeks of Study				
	0	2	4	6	8
Larvae					
LI	6.70 ± 0.01	6.55 ± 0.04	6.51 ± 0.04	6.47 ± 0.01	6.02 ± 0.04
MI	6.70 ± 0.03	6.66 ± 0.01	6.40 ± 0.05	6.14 ± 0.02	6.93 ± 0.02
HI	6.70 ± 0.10	6.54 ± 0.02	6.31 ± 0.04	6.17 ± 0.03	6.01 ± 0.08
F-Value (treatment) = 1.25 (P > 0.05); F-Value (period) = 61.25 (P < 0.01)					
Adults					
LI	6.70 ± 0.50	6.42 ± 0.01	6.33 ± 0.15	6.04± 0.20	5.87 ± 0.07
MI	6.70 ± 0.60	6.54 ± 0.17	6.28 ± 0.12	6.17 ± 0.38	5.91 ± 0.28
HI	6.70 ± 0.37	6.41 ± 0.18	6.29 ± 0.32	6.00 ± 0.54	5.86 ± 0.45
F-Value (treatment) = 1.00 (P < 0.01); F-Value (period) = 62.00 (P < 0.01)					
CTRL	6.59 ± 0.06	6.43 ± 0.23	6.27 ± 0.10	6.02 ± 0.12	6.02 ± 0.12
F-value (treatment) = 3.00 (P < 0.05); F- value (Period) = 44 (P < 0.01)					

LI = Low infestation, MI = medium infestation, HI= High infestation, CTRL= control

did not effect any significant difference in the quantity of free fatty acid in the smoked fish (P > 0.05) (Table 3). The smoked *C. gariepinus* responded similarly to larval and adult infestations with regard to its percent total lipids content (Table 4). The values of the lipids were significantly different with storage period both for the larval and adult infestation (P < 0.01) (Table 4). The values of the total lipids were significantly difference with storage period both for the larval and adult infestations (P < 0.01) (Table 4). The values of the total lipids were not significantly affected by levels of larval treatments (P > 0.05) but by adult treatments (P < 0.05). Contrary to what obtained for the free fatty acids, the highest mean value of total lipids (9.31± 0.16%) was recorded with larval infestation (Table 4).

Table 5 which shows the mean variations in pH values of fish infested with larval and adult *D. maculatus* varied from a pre-infested value of 7.00 to a reduced value of 6.01± 0.08 (for larval infestation) and 5.86 ± 0.04 (for adult infestation). A least pH value of 6.02 ± 0.12 was recorded for fish under the control experiment. Both larval and adult infestations of the fish affected significantly the pH value of the fish with storage period (P < 0.01). The various levels of larval treatments did not significantly affect the pH values of the smoked fish (P > 0.05) whereas adult infestations significantly affected the pH values of the fish (P < 0.01).

DISCUSSION

The decreased crude protein (CP) content of fish samples within 8 weeks (56days) of this study (Table 1) contradicted an earlier report that pest infestations did not affect the nutritional quality (proteins, fatty acids) of smoked fish tissues (Nduh,1984). The decrease in the CP content of fish from 78.52 % to 45.81 % of the control experiment was a clear manifestation that the nutritional qualities of fish depleted where pest infestation was absent.

This result was probably due to fish spoilage and deterioration resulting from the combined activities of micro-organisms and tissue enzyme (Shewan, 1961; Frazier, 1976). Hydrolytic activities of fish tissue enzymes (cattepsins and glutamic dehydrogenases) have been known to

breakdown proteins into peptones, polypeptides and amino acids resulting in fish deterioration (FAO, 1968).

The result of fish tissue degradation led to loss in weight of smoked *C. gariepinus* (Table 2). *C. gariepinus* infested with adult *D. maculatus* suffered the greatest loss in tissue weight contrary to Osuji (1975) and Nduh (1984) views that the larvae of *D. maculatus* are the most destructive of dried stored fish products. The well-developed biting mouth-parts of adult *D. maculatus* in comparism with those of the larvae must have contributed to the rapid loss in tissue weight of fish samples infested with the adults in this study. The reason was that the well-developed mouth-parts of the adults conferred on them a more destructive tendency than the larvae.

The treatment of smoked fish samples to varying levels of larval infestation did not affect the free fatty acids (FFA) (Table 3) and percent total lipids (% TL) (Table 4) of the tissue. FFA and % TL of fish infested by both larval and adult pests increased with prolonged period of storage. These results compared favourably with the observations made by Olley and Watson (1962) and Nduh (1984) that attributed these increases to the hydrolysis of Phospholipids by lipases and also agreed with Chen *et al.* (1974) report that during storage, fats become rancid owing to peroxide formations at the double bond by atmospheric oxygen. The authors further stated that rancidity may also be as a result of hydrolytic breakdown by micro-organisms leading to the liberation of free fatty acids.

The pH of the infested fish samples and the control decreased with periods of storage (Table 5). This was probably due to variations in the storage medium, which enhanced fast activities and the release of metabolic by-products (CO_2, urea and uric acid) from the pests. This decrease in pH could have prevented the proliferation of pathogenic micro-organisms such as *Clostridium botulinum* and *Bacillius stearothermophilus*. The result was the abundance of pest food for healthy growth of the pests, as well as the gycolytic breakdown of fish tissue to give lactic acid. This assertion is in accordance with the views of Frazier (1976), who reported a decrease in pH post-mortem fish tissues owing to glycolysis. This study, however, varied with that of Emokpae (1978) who reported an increase in pH of smoked *C. gariepinus*: something he attributed to the breakdown of protein to amino acids and consequently to ammonia.

Conclusion: The results of this study showed that the crude protein contents of infested fish decreased with increase in storage time. This may be attributed to the activities of certain micro-organisms which facilitated enzymatic breakdown of proteins to amino acids. Storage time may be due to the oxidation of fats to fatty acids resulting in rancidity. In addition, the decrease in pH values of infested fish was attributed to the metabolic by-products (CO_2, urea and uric acid) by micro-organisms in the fish tissue. Tissue degradation of smoked fish samples was related to the infestation levels and exposure time. Thus, the longer the periods of storage of infested smoked fish the more the tissues are degraded.

ACKNOWLEDGEMENT

The authors wish to acknowledge the contributions of the following persons towards the successful execution of the research: Dr. C. E. Eze, Head of Department, Applied Biology, Enugu State University of Science and Technology, Enugu; who provided necessary laboratory facilities for the study; Professor Okonkwo, Dean of the Faculty of Natural Sciences of the same institution, who offered useful advice. We are extremely grateful to them.

REFERENCES

AFOLABI, A. O., OMOSOLA, A. A. and OKE, O. L. (1984). Quality changes of Nigerian traditionally processed freshwater fish species. 1. Nutritive and organoleptic changes. *Journal of Food Technology, 19:* 333 – 340.

AOAC (1995). *Official Methods of Analysis of the AOAC*, 16th Edition, Association of Official Analytical Chemists, Washington DC, USA. 928 p

BARD, J., DE KIMPE, P., LAZARD, J., LEMASSON, J. and LESSENT, P. (1976). *Handbook of tropical fish culture*. Centre Technique Frostier Tropical, 45 bis, avenue, de ca Belle Gabrielle 94130, Nogent–Sur marine, France. 90 pp.

BLIGH, E. G. and DYER, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37:* 911 – 917.

CHEN, F. C., WAREN, W. and LEVIN, R. E. (1974). Identification of major high boiling volatile compounds produced during refrigerated storage of gaddock fillets. *Applied microbiology, 28:* 680 – 769.

EMOKPAE, A. O. (1978). Organoleptic assessment of the quality of fresh fish. Pages 12 – 15. *In:* 1978 Annual Report of Nigeria Institute of Oceanography and Marine Research (NIOMR), Lagos, Nigeria.

FAO (1968). Ice in fisheries. Food and Agricultural Organization. *FAO Fisheries Report Series, 59:* 1 – 68.

FRAZIER, W. C. (1976). *Food microbiology*. Mc-Graw Hill Book Co. New York. 285 pp.

HORN, H. J. (1974). Processing of lake Tanganyika sardines in Burundi. Pages 218 – 221. *In:* KRENZER, R. (ed). *Fishery Products.* Fishing News Books, Survey, England.

MABAWONKU, A. F. and AJAYI, T. O. (1982). Economic survey of artisanal fisheries in Bendel, Cross River, Lagos, Ogun, Ondo and Rivers State. Pages 64 – 76. *In: Proceeding of the National Seminar on Integrated Development of Artisanal and Inshore Fisheries in Nigeria*, Federal Development of Fisheries, Lagos, Nigeria.

MARINETTI, G. C. (1967). *Lipid chromatographic analysis.* Volumes 1 and 2, Marcel Decker, Incorporated, New York.

MOSES, B. S. (1982). Problems and prospects of artisanal fisheries in Nigeria. Pages 76 – 90. *In: Proceedings of Second Annual Conference of the Fisheries Society of Nigeria (FISON).* Calabar, January, 1982.

NDUH, T. A. (1984). *Insect pests of dried fish sold in Jos Metropolitan market*. MSc. Thesis, University of Jos, Nigeria, 126 pp.

OLATUNDE, A. A. (1983). Strategies for increasing freshwater fish production in the Green Revolution Programme. Pages 70 – 75. *In: Proceedings of Second Annual Conference of the Fisheries Society of Nigeria (FISON).* Calabar, January, 1982.

OLLEY, J. and WATSON, H. (1962). Phospholipids activity in herring muscle. Pages 56 – 66. In: LEITH, J. M.(ed). *Proceeding of the First International Conference of Food Science and Technology.* Gordon and Breach Publication Limited, London.

OSUJI, F. U. C. (1975). Recent studies on beetle infestation of dried fish in Nigeria with reference to Lake Chad district. *Nigerian Journal of Entomology, 1:* 63 - 68.

PROCTOR, D. L. (1977). The protection of smoked dried freshwater fish from insect damage during storage in Zambia. *Journal of Stored Products Research, 8:* 139 -149.

REED, W., BURCHAR, J., HOPSON, A. J., JONATHAN, J. and IBRAHIM, Y. (1967). *Fish and Fisheries of Northern Nigeria.* Government Press, London. 226 pp.

SHEWAN, J. M. (1961). The microbiology of water fish. Pages 487 – 560. *In:* G. BERGSTORM (ed). *Fish as Food.* Academic Press Incorporated, New York.

STEEL, R. G. D. and TORRIE, J. H. (1990). *Principles and Procedures of Statistics, A Biometrical Approach..* 2nd edition, Mc Graw-Hill Books, Tokyo, Japan. 633 pp.

VIVEEN, W. J. A. R., RICHTER, C .J. J., VAN OORDT, P. G. W. J., JANSEEN, J. A. L. and HUISMAN, E. A. (1986). *Practical Manual for the Culture of the African catfish, Clarias gariepinus*

THE EFFECT OF SALINITY STRESS ON BUCCAL VENTILATORY RATE IN THE AFRICAN LUNGFISH, *Protopterus annectens* OWEN

[1]OKAFOR, Anthony Ikechukwu and [2]CHUKWU, Lucian Obinnaya
[1]Department of Zoology, University of Lagos, Lagos, Nigeria
[2]Department of Marine Sciences, University of Lagos, Lagos, Nigeria

Corresponding Author: Dr. OKAFOR, Anthony Ikechukwu, Department of Zoology, University of Lagos, Lagos, Nigeria

ABSTRACT

The buccal ventilatory rate of the African lungfish, Protopterus annectens (Owen) following acclimation to diluted seawater was investigated under laboratory conditions for six days. Healthy adult specimens of African lungfish, Protopterus annectens (Owen) (mean weight 299.4g and mean length 38.9 cm) procured from Anambra river at Otuocha were subjected to the following concentrations of dilute seawater: 0%, 5% (s = 1.8 ‰), 10% (s = 3.5 ‰), 15% (s = 5.3 ‰), 20% (s = 7.0 ‰), 30% (s = 10.5 ‰) and 40% (s = 14.0 ‰) respectively. The results revealed that increase in salinity had a significant positive correlation (r = 0.92, p < 0.05) with increase in buccal ventilatory rate. The mean least and highest buccal ventilatory rates were 5.32 and 12.26 times per hour at 1.8‰ and 14.0‰ salinities respectively. The implications of the findings for the culture of this fish species in estuarine ecosystems are discussed.

Keywords: Salinity Stress, Buccal Ventilation, *Protopterus annectens*

INTRODUCTION

The African lungfish, *P. annectens* lives in shallow parts of West African rivers and lakes Their range of distribution spreads from Nigeria to Senegal and beyond on the West African coast line. (Dupe and Godet, 1969; Dupe, 1973; Daffala *et al.,* 1985; Otuogbai and Ikhenoba, 2001; Okafor and Odiete, 2002 a, b). Recent studies show that it can tolerate seawater up to a maximum of 30% (Okafor, 2004). Thus, the fish has got the potential of being cultured in brackish water. When some fresh water fish species are cultivated in brackish water, they exhibit enhanced hatching, growth and survival rates (Canagaratnam, 1959; Otto, 1971; Nwigwe, 1985; Sugiyama, 2002). Consequently, there is the need to evaluate the physiological adjustments which *P. annectens* can make whilst subjected to brackish water regimes. The paper therefore determines changes in buccal ventilatory rate of *P. annectens* subjected to different concentrations of diluted seawater. The information obtained may serve as guidelines for studies on the osmoregulatory abilities of the species in estuarine ecosystems.

MATERIALS AND METHODS

Live fish specimens of the African lungfish *P. annectens* procured from Anambra river at Otuocha in Anambra State of Nigeria were transported to the Zoology laboratory of the University of Lagos, Lagos, Nigeria and acclimated at room temperature for 28 days inside eight glass tanks that measured 0.54 x 0.30 x 0.30 m which were neither covered nor aerated. Each tank contained 3 litres of dechlorinated water. The fish were fed daily on fish feed obtained from the Nigerian Institute of Oceanography and Marine Research (NIOMR), Victoria Island, Lagos, *ad libitum* until used for the experiment.

The water in all tanks was changed twice weekly to prevent the accumulation of waste materials, uneaten food and the fish's mucous secretions. Seawater was collected at high and low tides from the Bar Beach (Atlantic Ocean) at Victoria Island, Lagos and filtered through a fine 0.5 mm sieve.

Three litres of each of the percentages of the seawater were prepared thus: 0%, 5% (s = 1.8 ‰), 10% (s = 3.5 ‰), 15 % (s = 5.3 ‰), 20% (s = 7.0 ‰), 30% (s = 10.5 ‰) and 40 % (s = 14.0 ‰) by diluting seawater of 100% salinity (s = 35 ‰) with an appropriate volume of dechlorinated water. The salinity of each prepared diluted seawater was determined using a salinometer (Table 1).

Seawater above 40 % in concentration was not prepared since *P. annectens* tolerates 30% seawater indefinitely and 40% seawater for only about 4 to 5 days (Okafor, 2004).

Fourteen specimens of *P. annectens* chosen from amongst those that survived acclimation were now introduced into seven glass tanks containing the above concentrations of

Table 1: The salinities (‰) of the various concentrations of dilute seawater that were prepared

Prepared seawater Concentration (%)	Corresponding salinity (‰)	Volume of dechlorinated Water (litres)	Volume of100 % seawater (35 ‰)
40	14.00	1.80	1.20
30	10.50	2.10	0.90
20	7.00	2.40	0.60
15	5.30	2.55	0.45
10	3.50	2.70	0.30
5	1.80	2.85	0.15
0	0.00	3.00	0.00

diluted seawater at a stocking rate of two specimens per tank.

Buccal Ventilatory Rate: Only healthy and active fishes with no external signs of disease or physical injuries were selected for the experiment. The buccal ventilatory rate was estimated as the number of times each fish would remove its head out of the water, opened its mouth in order to breathe atmospheric oxygen, closed it and dipped its head back into the water, within an hour. This was repeated five times and the mean values noted.

RESULTS

Increase in salinity was directly correlated with increase in buccal ventilatory rate Coefficient of linear correlation r = 0.9162767 at 0.05 probability (Figure 1). At 0 %, 5%, 10 %, 15 %, 20 %, 30 % and 40 % salinities, the mean buccal ventilatory rates were 4.04, 5.32, 6.39, 7.50, 9.86, 10.16 and 12.26 respectively.

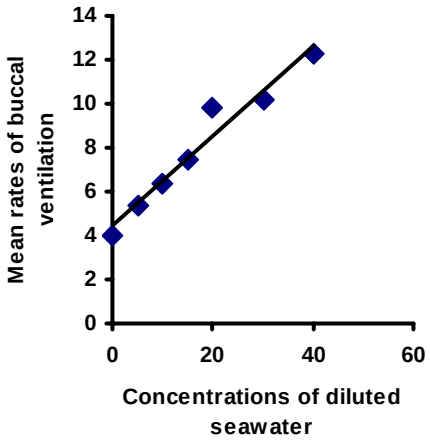

Figure 1: The mean rates of buccal ventilation at various concentrations of diluted seawater

DISCUSSION

The result clearly indicates a positive linear correlation between salinity and buccal ventilatory rate. Holliday (1969) reported reduction in oxygen content of saline waters. The present study opined that the increased buccal ventilatory rate when immersed in saline water could be a means of increasing the total amount of oxygen that would reach the tissues via the lungs since the amount received from the saline water via the gills was highly reduced.

Similar findings have been documented by Enajekpo (1989), Ebele *et al.* (1990), Onusiriuka and Ufodioke (1994), Chukwu (2001) and Chukwu and Ugbeva (2003). For example, Enajekpo (1989) reported an increase in respiration rate (opercular ventilation) in *Tilapia zilli* and *Oreochromis niloticus* exposed to sublethal concentrations of water soluble fractions of Bonny light crude.

Thus in the attempt to culture *P. annectens* in brackish or estuarine waters, farmers should put into consideration the increased demand for atmospheric oxygen in the new habitats. Since this process requires the expenditure of energy, this may be compensated by increasing the rate of feeding.

There is no lungfish that naturally inhabits brackish or estuarine waters within this Cenozoic era. However, fossil records have established that lungfishes that lived during the Permian, Carboniferous and Devonian periods such as *Gnathorhiza, Megapleuron* and *Soederberghia* were estuarine and shallow marine dwellers (Carroll, 1988; Ahlberg, *et al.,* 2003). *Soederberghia* gulped air and probably inhabited a shallow near-shore marine environment (Ahlberg, et al., 2003). The occurrence of *Soederberghia groenlandica* in the Famennian old red sandstone of North America, Greenland and Australia respectively thus furnishes evidence of contact or close proximity between North and South Pangaea during the Palaezoic era (Ahlberg *et al.,* 2003). In fact the evolution of air breathing by lungfishes has traditionally been associated with their entry into freshwaters (Carroll, 1988).

REFERENCES

AHLBERG, P. E., JOHANSEN, Z., ZERINA, J., and DAESCHLER, E. B. (2003). The late Devonian lungfish, *Soederberghia* (Sarcopterygii, Dipnoi) from Australia and

North America and its biogeographical implications. *Journal of Vertebrate Palaeontology*, *21(1):* 1 – 12.

CANAGARATNAM, P. (1959). Growth of fishes in different salinities. *Journal of Fish Research Board of Canada, 16:* 121 – 130.

CARROLL, R. L. (1988). *Vertebrate Palaeontology and Evolution*. W. H. Freeman and Company, New York. 1996 pp.

CHUKWU, L. O. (2001). Acute toxicity of treated industrial effluent to decapod crustacean, *Macrobrachium vollenhoevenii*. *Journal of Science, Technology and Environment*, *1(2):* 21 – 29.

CHUKWU, L. O. and UGBEVA, B. O. (2003). Acute toxicity of textile mill effluents to estuarine macro-invertebrate, *Clibinarius africanus* (Aurivillus) and *Tilapia zilli* (Gerr) fingerlings. *Journal of Nigerian Environmental Society, 1(2):* 223 – 228.

DAFFALA, A. A., ELIAS, E. E. and AMIN, M. A. (1985). The lungfish, *Protopterus annectens* (Owen) a bio-control agent against *Schistosome* vector snail. *Journal of Tropical Medicine and Hygiene, 88:* 131 – 134.

DUPE, M. and GODET, R. (1969). Conditioning of the responsiveness of the central nervous system in the life cycle of the lungfish, *Protopterus annectens*. *General and Comparative Endocrinology, 2:* 275 – 283.

DUPE, M. (1973). Factors conditioning the awakening reaction of olfactory origin in the lungfish, *Protopterus annectens*. *Comparative Biochemistry and Physiology*, *44A:* 647 – 653.

EBELE, S., OLADIMEJI, A. A. and DARAMOLA, J. A. (1990). Molluscicidal and piscicidal properties of copper (II) tetraoxosulfate (VI) on *Bulinus globosus* (Morelet) and *Clarias anguillaris* (L). *Aquatic Toxicology, 17:* 231 – 238.

ENAJEKPO, H. O. T. (1989). *Effects of water soluble fractions (WSFs) of Bonny Light crude oil on some physiological parameters in Oreochromis niloticus.* M.Sc. Thesis, University of Jos, Nigeria.

HOLLIDAY, F. G. T. (1969). The effects of salinity on the eggs and larvae of teleosts. Pages 293 – 312. *In:* W. S. Hoar and D. J. Randall (eds), *Fish Physiology*, Vol. 1, Academic Press, New York.

NWIGWE, C. A. (1985). *Effects of salinity on survival and growth of the catfish, Clarias lazera.* M. Tech. Thesis. Rivers State University of Science and Technology, Nigeria. 56 pp.

OKAFOR, A. I. and ODIETE, W. O. (2002 a). The process of aestivation in the laboratory by the African lungfish, *Protopterus annectens* (Owen). *African Journal of Science, 3(2):* 764 – 767.

OKAFOR, A. I. and ODIETE, W. O. (2002 b). Seasonal changes in Gonadosomatic and Hepatosomatic indices of the African lungfish, *Protopterus annectens* (Owen) of Anambra river, Otuocha, Nigeria. *African Journal of Science, 3(2):* 668 – 672.

OKAFOR, A. I. (2004). Salinity tolerance of the African lungfish, *Protopterus annectens* (Owen). *African Journal of Science, 5(1):* 1073 – 1083.

ONUSIRIUKA, B. C. and UFODIKE, E. B. C. (1994). Acute toxicity of water extracts of sausage plant, *Kigelia africana* and akee apple, *Blighia sapida* on African catfish, *Clarias gariepinus* (Teugels). *Journal of Aquatic Sciences, 9:* 35 – 41.

OTTO, R. G. (1971). Effects of salinity on the survival and growth of pre-smolt coho salmon, *Oncorhynchus kisutch*. *Journal of Fish Research Board of Canada, 28:* 343 – 349.

OTUOGBAI, T. O. S. and IKHENOBA, A. (2001). Food and feeding habits of the lungfish, *Protopterus annectens* (Owen) and the possible role of the paired fins in feeding. *World Journal of Biotechechnology, 2(2):* 233 – 238.

SUGIYAMA, C. R. (2002). The effects of salinity on spermatozoa, eggs and larvae of *Paracottus kessleri* (Dybowski). *Japanese Journal of Fish Physiology, 3:* 342 – 345.

PARASITIC DISEASES AND SEXUAL DISABILITY: A CRITICAL REVIEW OF SOME PARASITIC DISEASES WITH SERIOUS SEXUAL REPERCUSSIONS

[1]OKAFOR, Fabian Chukwuemenam and [2]OMUDU, Edward Agbo
[1]Department of Zoology, University of Nigeria, Nsukka
[2]Department of Biological Sciences, Benue State University, Makurdi

Corresponding Author: OMUDU, E. A. Department of Biological Sciences, Benue State University, Makurdi

ABSTRACT

A wide range of parasitic diseases even though not sexually transmitted, invade male and female reproductive organs causing direct pathological damages leading to impaired fertility and sexual dysfunction. This paper provides a framework for thinking about the psychological impact and burden of these parasitic infections. It begins by providing the etiology of these diseases and a brief overview of the socio-cultural and psychological implications of infected and affected individuals. The article concludes with reflections as to how interactions of parasitological and anthropological factors produce multi-dimensional reproductive health problems requiring urgent multi-disciplinary investigation and intervention.

Keywords: Parasitic infection, Sexual repercussion

INTRODUCTION

A range of parasitic diseases has different reproductive health consequences for men and women. Parasites may affect sex and/or sexual organs in two ways: the infecting organism may produce sufficient debilitation or anatomic deformities to make sex impossible as in onchocercrasis and lymphatic filariasis or may cause direct damage to male and female reproductive organs impairing fertility as a result of inhibition of gamete production as in trichomoniasis, schistosomiasis and toxoplasmosis. Of the vast array of parasitic diseases endemic in Nigeria, only trichomoniasis caused by the protozoan parasite *Trichomonas vaginalis* is known to be sexually transmitted. A wide range of other parasitic diseases, even though not sexually transmitted, may however invade male and female reproductive organs causing direct pathological damages leading to impaired fertility and sexual disability (Burrow and Ferris, 1975; Hartigan, 1999; Omudu and Amali, 2003).

Other parasitic diseases may not directly affect reproductive organs but their various pathological manifestations may resemble diseases generally believed to be sexually transmitted with its attendant stigma. It is often the stigma that exacerbates the medical and psychological burdens of infected and/or affected individuals. Genital symptoms and manifestations of a variety of protozoan and helminthic infections which are not usually sexually transmitted may mimic classic sexually transmitted infections by producing ulceration (for example, amoebiasis, leishmaniasis), wart-like lesions (schistosomiasis), or lesions of the upper genital tract as a result of amoebiasis and schistosomiasis.

Richens (2004) reported a variety of other genital symptoms less suggestive of Sexually Transmitted Diseases (STDs), these include hydrocele (seen with filariasis) and haemospermia (seen with schistosomiasis). Considering the pathological damage to sex organs and the social and psychological burden borne by affected and infected individuals, there is urgent need to focus specific attention on their clinical presentation with the aim of providing holistic management and intervention.

Schistosomiasis for instance, though not sexually transmitted, has been reported to have serious sexual implications when the eggs invade tissues lining the reproductive organ causing anatomic deformities enough to reduce sexual pleasure for both partners (Hartigan,1999; Hanson, 1999; TDR News 1996). Female Genital Schistosomiasis (FGS), as it is commonly referred to, has also been associated with a range of pathologies including infertility, abortion and ectopic pregnancy (WHO, 1998). Mondaini *et al* (2004) reported cases of testicular pains resulting from parasitic infection, especially among non-immuned individuals who have visited endemic countries. Though they maintained that scrotal swelling may not be a specific diagnosis for filariasis, it may however be a pointer to genital filariasis.

The main focus of this paper is to discuss the sexual implications of some parasitic diseases and how socio- cultural beliefs combine with parasitological manifestations to make life a wretched existence for infected and/or affected individuals. It is hoped that medical experts will

begin to address some of these socio-cultural beliefs when developing intervention strategies rather than focusing only on the biological mechanism through which these diseases operate.

SOME PARASITIC DISEASES WITH SEXUAL REPERCUSSIONS

Onchocerciasis: Onchocerciasis, or river blindness is caused by a filarial parasite O*nchocerca volvulus* and transmitted by the bites of blackfly S*imulium* species. This disease is rated as a leading cause of blindness and about 20 million people are infected worldwide while one million of those infected are totally or partially blind (Edungbola and Parakoyi, 1991). Onchocerciasis can manifest in several dramatic and bizarre forms with an incredible adverse impact on agriculture, demography and socio-economic conditions. It causes extensive skin disfiguration with unbearable discomfort. However, its most dreadful and terminal complication is blindness.

Besides blindness, hernias, leopard skin, hanging groins and elephantiasis have been identified as important complications of onchocerciasis in areas where the disease is endemic (Edungbola *et* a*l.*, 1991). While the association between hanging groins and hernias has been reported (Williams and Williams, 1966) it has been established that hernia is a definite and major complication of onchocerciais (Nelson 1958, Edungbola *et al.,* 1991).

The medical and socio-economic seriousness of these complications of onchocerciasis such as hernias, elephantiasis, hanging groins are often associated with sexual disability because of the severe enlargement of organs and disfiguration. Scrotal elephantiasis and hernias constitute formidable psychological burden for infected individuals as a result of obvious sexual repercussions. Partner desertion and abandonment often characterize such situations. In addition to the sexual incapacitation, scrotal elephantiasis and hernias also lower the productivity and wage earning capacity of those afflicted.

Gender roles also influence the consequences of these manifestations of onchocerciasis (Hartigan,1999, Harnson, 1999). For example masculinity is often demonstrated through multiple sexual conquests and control of resources. Men who develop scrotal elephantiasis, hernia or hanging groins find it very difficult, if not impossible, to engage in sexual intercourse in addition to lower productivity. Women, on the other hand depend more on their physical appearance to enhance their prospects for marriage and sustaining a relationship with male partners, developing any of these onchocercal manifestations jeopardise these aspirations.

The skin manifestations in onchocerciasis are characterised by severe dermatitis and itching, the thickening and atrophy of skin and pigmentary aberrations. Obikeze (1992) reported that these pigmentary aberration (onchodermatitis) has very grave social, economic and psychological implications for victims of onchocerciasis. Young women and men with the disease are discriminated against, humiliated by peers, avoided by friends and stigmatized by society. Married women with onchodermatitis lose the affection of their husbands while marriage chances of infected persons are adversely affected, if not totally ruined. Psychologically, onchodermatitis engenders withdrawal behaviour, isolationism and societal maladjustment on the part of the affected individual.

Ovuga *et al.* (1995) investigated some aspects of social anthropological implications of onchocercal skin disease in Nebbi district in Uganda. Results indicated that onchocerciasis was considered to be mysterious disease, especially the dermal manifestations of lizard and leopard skin. The disease was often mistaken for measles and leprosy and as such affected individuals suffered from discriminatory practices applied on sufferers of measles and leprosy. The belief systems of the community were said to be responsible for the discriminatory practices of the people against those affected by onchocercal skin diseases. Persons who had these skin conditions ranked separation from spouses as the most painful aspect of the stigma.

Lymphatic Filariasis: Lymphatic filariasis is caused by the filarial nematode *Wuchereria bancrofti* and transmitted by the bites of C*ulex* mosquito species. Although the disease caused by this parasite is rarely fatal, the morbidity due to the disease is high due to lymphoedema and hydrocoele which are results of impairment in lymphatic drainage. The socio-economic implications of this disease include social isolation or stigma in reaction to the enlarged limbs or hydrocoel (WHO 1998).

It is estimated that 40 million people suffer from the chronic, disfiguring manifestations of this disease, including 27 million men with testicular hydrocoele, lymph scrotum or elephantiasis of the scrotum (Dreyer *et al.* 1997). An estimated 13 million people, the majority of which are women have filarial-associated lymphoedema or elephantiasis of the leg, arm or breast (WHO 1998). Although lymphatic filariasis is ranked as the second leading known cause of disability worldwide (WHO 1998) little attention has been paid to the important but hidden

disability associated with the genital manifestations of this disease leading to sexual disability.

Immobility, clumsiness, embarrassment and depression have been observed in many sufferers (Mbah and Njoku, 2000). Generally these problems have led to severe functional impairment of occupational and sexual activities. Hydrocoele, the genital manifestations of lymphatic filariasis in men, present as a chronic swelling of the scrotum and victims find it very difficult to engage in sexual intercourse. About 27 million men are infected worldwide with 75 % in subsaharan Africa (Hartigan, 1999). The experience of the disease is significantly influenced by socio-cultural beliefs in endemic communities. Hydrocoele is associated with sexual disability and infertility; women often suffer greater social and psychological consequence of limb and genital enlargement. The fear of stigmatization drives many victims underground; as a result this disease condition is rarely reported at health centres (Hanson, 1999; Hartigan, 1999; Vlassoff and Bonilla 1994).

Ahorlu *et al.* (2001) assessed the consequences of hydrocoele and benefits of hydroceolectomy on the physical activity and social life in three lymphatic filariasis endemic villages in Ghana and they reported that hydrocele, especially large ones, severely reduced the patients' work capacity and impaired sexual function, and that overall, it had a considerable negative effect on the quality of life for the patients, their family and the community. Reasons why patients refused hydrocoeletomy in the past were the high cost of surgery, fear of death and impotence and /or sterility that might result from the operation. Patients that underwent hydrocoelectomy reported remarkable improvement in quality of life, work capacity and sexual function. Other benefits of hydrocoelectomy included the restoration of self-esteem, thus enabling affected individuals to participate more in community activities.

Other social anthropological studies on lymphatic filariasis in Northern Ghana by Gyapong *et al.* (2000) revealed that complications of lymph scrotum and ridicule from community members were ranked highest among problems of patients. Unmarried men in particular found it difficult to find a spouse with their condition, and various degrees of sexual dysfunction were reported amongst married men.

Schistosomiasis: Schistosomiasis is an excreta/urine-water borne parasitic disease transmitted through fresh water snail intermediate host. It is caused by the trematode *Schistosoma haematobium* or *S. mansoni*. Of all the parasitic diseases with sexual repercussion, schistosomiasis enjoys the greatest attention. Eggs of both *S. mansoni* and *S. haematobium* are often found in reproductive organs of the infected female. This disease has been associated with infertility, extra-uterine pregnancy (Hartigan, 1999; Burrow and Ferris, 1975). Acute infection of the reproductive organ may result to Vesico Vaginal Fistula and chronic inflammation of the vaginal epithelium leading to painful discomfort during sexual intercourse (Burrow and Ferries, 1975).

Female Genital Schistosomiasis (FGS) has been associated with increased vulnerability to HIV infection. This association is because the symptoms of urinary and genital schistosomiasis are sometimes confused with other sexually transmitted diseases. A study conducted in Malawi in 1994 by some researchers from the World Health Organization on FGS assessed the extent of pathological damage in the genital area of women infected with urinary schistosomiasis. They investigated the relationship between urinary schistosomiasis and infertility and the impact of the disease on women's marital and sexual life. Fifty-one women with urinary schistosomiasis underwent thorough gynecological examinations including colposcopy and photographic documentation of lesions. Microscopy of the genital biopsies revealed that 33 had *S. haematobium* eggs in their cervix, vagina and /or vulva. There was a significant correlation between size of genital lesions and the number of ova counted. Tumors in the vulva were seen with naked eyes. The report published in TDR news (1996) observed that though the sample was very small, significant cases were found in women who had fewer children than desired and whose husbands had children with other women, suggesting some sort of sexual dissatisfaction with partners. It was reasoned that their husbands were pushed into extra marital affairs because of loss of sexual pleasure with partners who had FGS.

Schistosomiasis is a disease with serious gender bias, women are differentially exposed to the disease as a result of their water carrying responsibilities. Men are not left out of the sexual repercussions of this disease. The commonest diagnostic feature in male urinary schistosomiasis is the passage of bloody urine. This is sometimes confused with some symptoms of STDs and as a result infected individuals are stigmatized by peers and avoided by the opposite sex.

Trichomoniasis: This is the most prevalent sexually transmitted parasitic infection, and the most prevalent non-viral and bacterial sexually transmitted disease in the world (Obiajuru *et al.,* 2002, Njoku *et al.,* 2000). The parasite *Trichomonas vaginalis* is basically a flagellate that exists commonly in the vegetative form. The prevalence of trichomoniasis has continued to rise in Nigeria and other sub-saharan countries especially as the greater percentage of the

population become sexually active (Hanson, 1999). The parasite inhabits the vagina and cervix of females and urethra of males. Although sexually active male and female are at risk, it is more frequently encountered in females than males because it is generally assymptomatic in men.

The symptoms could be severe such as intense inflammation of the vagina, with itching and copious discharge from the vagina or urethra (Acholonu, 1998). *T. vaginlis* may impair fertility in women by causing direct damage to the fallopian tube and may induce watery sperm, premature ejaculation and prostates in men (Ukoli, 1990). Lesions of cervix, vagina and vulva resulting from infection with *T. vaginalis* are painful during intercourse and cause chronic inflammation of the fallopian tubes which may result in infertility, tubal pregnancy and abortion (Burrows and Ferries, 1975; Ukoli 1990). These more serious pathological effects result from long-lasting infection, though they may be reversed after successful treatment. The psycho-social implications of this disease is very serious with vaginitis and urethritis causing severe discomfort and purulent discharge which mess-up inner wears of infected persons. Some scholars are of the opinion that this parasite can also be transmitted through contaminated inner wears, towels, toilet seat (Obiajuru *et al.* 2002).

The offensive odour of the discharge discourages initiation and sustenance of sex. Spouses of infected partners are likely to resort to alternative sex partners as the case with other known sexually transmitted diseases. Individuals with trichomoniasis are blamed for being promiscuous, and since it is often reported among women, there is a gender bias in the stigma. The inflammation of the vaginal wall results in severe pains during sexual intercourse thus exacerbating the biological and psychological problems faced by infected individuals.

The manifestations of other parasitically induced diseases may also have serious repercussions for sexual harmony. Cutaneous leishmaniasis may disfigure/destroy body parts resulting to lose of sensation. Because of the similarity of this disease to leprosy, affected individuals are ostracised and denied sexual privileges. Studies in a number of different regions of the world indicate that both cutaneous and visceral leishmaniasis are more likely to be detected in men than women. It is however, important to note that in the case of cutaneous leishmaniasis, the disease does not result in permanent incapacitation (Hartigan, 1999).

THE IMPORTANCE OF ADDRESSING PSYCHOLOGICAL ISSUES WHEN ASSESSING IMPACT AND BURDEN OF PARASITIC DISEASES

Communicable disease experts tend to focus exclusively on the biological mechanisms through which disease operates when they develop their management and intervention strategies; rarely do they broaden their vision to include an examination and investigation of how these diseases impact on the holistic reproductive health needs. To accurately assess the magnitude, depth and profound implications of sexual disability for both men and women suffering from parasitic diseases with sexual manifestations, social anthropological investigation on the impact of the disease on damaged male and female identity need to be undertaken. Gyapong *et al.,* (2000) suggested the inclusion of psychological issues in the calculation of Disability Adjusted Life Years (DALYs). The social and psychological consequences of parasitic diseases are often excluded from the burden of disease calculation. In order to address this biomedical bias, the WHO recently listed a range of conditions that should be considered for global burden of disease (GBD) revisions. These include indirect obstetric complication, reproductive tract infection, psychological morbidity and other reproductive health concerns (WHO, 1998).

Anthropological input in terms of community perceptions of these diseases including local taxonomies and etiology is very valuable in developing health education materials to support interventions. While it is important to develop and administer chemotherapeutic remedies, a more sophisticated understanding of cultural, psychological and social dimensions of endemic parasitic diseases with genital manifestations is also crucial in introducing sustainable community intervention. Anthropological involvement in disease management ensures that some account is taken of knowledge and cultural influence on the patterns of disease and coping mechanism employed by sufferers. Gubler (1997) therefore noted that in order to achieve substantive successes in disease prevention and control, there is the need to include social scientists in the control process primarily due to socio-cultural and psychological factors that contribute in the spread and experience of these infections.

It is therefore medically, epidemiologically and psychologically sensible to address the sexual and reproductive health implications of parasitic disease in endemic communities. Participatory research methodologies are appropriate for better understanding of psychological burden associated with sexual disability and incapacitation induced by these parasitic infections. This is crucial for the

development of multi-disciplinary strategy to tackle parasitic diseases and their impact on agriculture, socio-economic well-being and reproductive health. Intensifying public education can help reshape traditional beliefs that often determine community behaviour as a result of fear, ignorance and socio-cultural practices.

CONCLUSION

The interaction of parasitological and socio-cultural factors produces multi-dimensional health problems that require multi-sectoral intervention. The prevalence, manifestation, natural history and severity of consequences of these parasitic diseases vary from place to place, this is because responses, attitudes and beliefs (which influence overall disease consequence and burden) differ. Though the medical and socio-economic impact of parasitic diseases has enjoyed attention from bio-social scientists, the linkage of these diseases to sexual disability with its attendant deprivation and stigma has not enjoy similar patronage. The 'compartmentalization of knowledge' has made it difficult for medical and social experts to communicate across disciplines. The urgent need therefore is for integrated disease control approach that address the whole 'web of causation' and overall repercussion of diseases on individuals and endemic communities.

REFERENCES

ACHOLONU, A. D. W. (1998). *Trichomoniasis*: *A little recognized sexually transmitted disease but with grave consequences*. Public lecture, Nigeria Academy of Science, Lagos.

AHORLU, C. K., DUNYO, S. K., ASAMOUH, G. and SIMONSEN, P. E. (2001). Consequences of hydrocele and the benefits of hydrocoelectomy. *Acta Tropica, 80(3):* 215 – 221.

BURROW, G. N. and FERRIS, M. D. (1975). *Medical Complications during pregnancy*. W. B. Saunders Company. London. 962 pp.

DREYER, G., NOROES, J. and ADDIS, D. (1997). The silent burden of sexual disability associated with lymphatic filariasis. *Acta Tropica, 63:* 57 – 60.

EDUNGBOLA, L. D. and PARAKOYI, D. B. (1991). Onchocerciasis and dracunculiasis: A review of the global status. *Nigerian Journal of Parasitology, 12:* 1 – 27.

EDUNGBOLA, L. D., BABATA, A. L., PARAKOYI, D. B., OLADIRAN, A. O., JANYO, M. S. and ASAOLU, S. O. (1991). Hernias and onchocerciasis. *Nigerian journal of Parasitology, 12:* 45 – 50.

GUBLER, D. T. (1997). Human behaviour and cultural context in disease control. *Tropical Medicine and International Health, 24(A):* I – 2.

GYAPONG, M., GYAPONG, J., NEISS, M. and TANNER, M. (2000). The burden of hydrocele on men in Northern Ghana. *Acta Tropica,* 77*(3):* 287 – 294.

HANSON, K. (1999). *Measuring up: Gender, burden of disease, and priority setting techniques in the health sector*. Working Paper Series No. 99(12). Population and Development Studies. Harvard School of Public Health, USA.

HARTIGAN, P. (1999). *Communicable disease, gender and equity in health*. Working Paper Series No. 99(08). Population and Development Studies. Harvard School, of Public Health, USA.

MBAH, D. C. and NJOKU, O. O. (2000). Prevalence of lymphatic filariasis in Oraeri, Anambra State, Nigeria. *Nigerian Journal of Parasitology, 21:* 95 – 102.

MONDAINI, N., HOPSTER, D., CHOI, W. H., BORLEY, N. C. and THOMPSON, P. M. (2004). Parasitic manifestations of testicular pain. *Archieves Ital Urol Androl, 76(1):* 121 – 126.

NELSON, G. S. (1958). "Hanging groin" and hernia, complications of onchocerciasis. *Transactions of the Royal Society of Tropical medicine and Hygiene, 52:* 272 – 275.

NJOKU, A. J., OBIAJURU, I. O. C., NJOKU, J. C., NWOKOLO, E. A., UWAEZUOKE, J. C. and ANOSIKE, J. C. (2000). Prevalence of *Trichomonas vaginalis* among students of tertiary institutions in Imo State, Nigeria. *Nigerian Journal of Parasitology, 21*: 83 – 94.

OBIAJURU, I. O. C., NJOKU, A J. and OGBULIE, J. N. (2002). Prevalence of Trichomoniasis and the impact of sex education, knowledge and attitudes in relation to trichomoniasis and other sexually transmitted diseases in Imo States Nigeria. *Nigerian Journal of Parasitology,* 23: 81 – 90.

OBIKEZE, D. S. (1992). Social impact of onchocercal skin disease in rural Nigeria: Pages 23 – 45. *In: Methods for community diagnosis of onchocerciasis to guide Ivermectin based control in Africa*. UNDP/WORLD BANK/WHO/ TDR. Geneva.

OMUDU, E. A. and AMALI, O. (2003). Poor, pregnant and parasitized: A pathological and socioeconomic appraisal of the impact of parasitic diseases on pregnant women in Nigeria. *International Journal of Gender and Health, 1(1):* 21 – 31.

OVUGA, E. B. L., OGWAL-OKENY, J. W. and OKELLO, D. O. (1995). Social anthropological aspect of onchocercal skin disease in Nebbi District, Uganda. *East African Medical Journal, 72(10):* 649 – 653.

RICHENS, J. (2004). Genital manifestations of tropical diseases. *Sexually Transmitted Infections, 80:* 12 –18.

TDR (1996). Malawi study signals, need for more research on female genital schistosomiasis. Pages 3 – 4. *In:* Bruyere, J. (ed.). *TDR news No. 50. June, 1996*. UNDP/WORLD BANK/WHO/TDR. Geneva.

UKOLI, F. M. A. (1990). *Introduction to Parasitology in Tropical Africa*. Textfolw Limited, Ibadan, Nigeria. 463 pp.

VLASSOFF, C. and BONILLA, E. (1994). Gender related differences in the impact of tropical diseases on women: what do we know? *Journal of Biosocial Sciences, 26:* 37 – 53.

WHO (1998). *Gender and health.* Technical Paper, WHO/FRH, WHD/98:16, Geneva.

WILLIAMS, E. H. and WILLIAM, P. H. (1996). A note on apparent similarity in distribution of onchocerciasis, femoral hernia and Kaposi's Sarcoma in the West Nile district of Uganda. *East African Medical Journal, 43:* 208 – 209.

SUPEROXIDE DISMUTASE (SOD) ACTIVITY AND SERUM CALCIUM LEVEL IN RATS EXPOSED TO A LOCALLY PRODUCED INSECTICIDE *"RAMBO INSECT POWDER"*

OTITOJU Olawale and ONWURAH Ikechukwu Noel Emmanuel
Pollution Control and Biotechnology Unit, Department of Biochemistry University of Nigeria, Nsukka, Enugu State, Nigeria

Corresponding Author: Dr. ONWURAH I. N. E. Pollution Control and Biotechnology Unit, Department of Biochemistry University of Nigeria, Nsukka, Enugu State, Nigeria. Email: iyknuelo@yahoo.com

ABSTRACT

Studies of superoxide dismutase (SOD) induction in rats exposed to locally produced insecticide; "Rambo" of which the active chemical compound is permethrin (0.6% w/w) was performed. The calcium levels in the blood plasma of the exposed rats were also evaluated. The rats were divided into three groups of five rats per cage. Each group of rats was fed with 1 %, 5 % or 10 % of the insecticide in their diets. The control group was fed normal diet. The effect of insecticide at various concentrations on superoxide dismutase (SOD) activity in the blood plasma was not significantly different (P > 0.05) in the newly weaned rats (NWR). However, in the middle-aged rats (MAR) and aged rats (AR) groups, the results were significantly different (P < 0.05) against the parallel controls. Comparison of the effect of the insecticide on SOD induction at various concentrations among the groups based on age difference showed significantly different result (P < 0.05), especially among the groups fed with 10 % (w/w) of the insecticide in the diet. Serum Ca^{2+} level (0.51 ± 0.22mg/ml) increased from newly weaned rats groups to (0.66 ± 0.24mg/ml) in the middle-aged rats and (0.63 ± 0.04mg/ml) for the aged rats. The observed Ca^{2+} increase was significantly high for rats fed 10 % (w/w) concentration of the insecticide in the diet (P < 0.05). This increase tends to suggest a concentration- dependent effect. The no-observed-effect-concentration (NOEC) was found to be 0.006 g permethrin per 100 g of the diet, which is equivalent to 1% of the "Rambo" insecticide per 100g of the feed. Results of this study show that in non-target organisms "Rambo" insect powder may induce superoxide dismutase activity, thus, suggesting, oxidative-stress related toxicity. The observed increase in calcium ion especially at 10 % (w/w) of the insecticide showed that permethrin may induce toxic effects associated with cell death via mitochondria uncoupling and loss in ATP metabolism.

Keywords: Superoxide dismutase, Calcium ion, Permethrin insecticide, Rambo insect powder

INTRODUCTION

In our enthusiasm for new successes, we have often overlooked the fact that we have polluted our environment and purposely or inadvertently have exposed ourselves and other life forms to hazardous chemicals (e.g. pesticides). Most of these chemicals have been implicated as causing cell injury and death especially in non-target organisms. (Sotherton, 1991 and Moreby *et al*., 2001). Toxicity may occur directly as a result of a chemical compound being converted to free–radicals or via superoxide anion formation (Bridges *et al*., 1983). Free radicals have been implicated in several human diseases such as cancer and heart diseases (Gutheridge, 1994). More so, they cause membrane damage (Onwurah and Eze, 2000), due to generation of lipid peroxidation product. Detoxification of reactive oxygen species is one of the prerequisites of aerobic life, and the multiple line of defence system. The repertoire to counteract the potentially hazardous reactions initiated by oxygen metabolites includes all levels of protection, prevention, interception and repairs. It comprises non-enzymatic and enzymatic systems. The enzymes involved in antioxidation are the superoxide dismutase, glutathione peroxides and catalases (Ledig and Doffoel, 1988). Superoxide dismutase (SOD) catalyses the destruction (dismutation) of superoxide free – radical ions. These ions are believed to be responsible for lipid peroxidation and peroxidative haemolysis of erythrocytes. The action of SOD therefore results in the protection of the biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Olusi, 2000). To ameliorate the damage caused by the hydroxyl radical formed from superoxide radicals and

hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. SOD is an important isoenzyme functioning as superoxide radicals' scavengers in the living organisms. Its activity is also induced by diverse stresses (Bowler *et al*., 1992), presumably, because of the increase in the concentration of superoxide radicals in cells. SOD is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress.

Calcium ions are important and are required for many physiological functions. Although, the role of Ca^{2+} as a mediator of toxicant induced cell death has been a subject of interest, intracellular Ca^{2+} homeostasis is of importance to cell viability (Palmeira, 1999). The critically important cellular calcium pool for regulation of intracellular events is the cytosolic Ca^{2+}, and this is control by hormones and growth factors (Thomas *et al.,* 1984). Loss of the ability to respond to such hormones and growth factors may result in cell death (Orrenius *et al*., 1989). The mechanisms by which Ca^{2+}-mobilising hormones, like vasopressin, induce intracellular Ca^{2+} transients have been extensively studied (Kawanishi *et al*., 1989 and Glennon *et al*., 1992). Normally, intracellular Ca^{2+} homeostasis is maintained by the concerted operation of cellular transport and compartmentation systems (Carafoli, 1987). Damages caused by free radicals or superoxide radical on the plasma membrane will lead to a rise in cytosolic Ca^{2+} concentration, which may cause cell injury and finally cell death (Comporti, 1993).

Most cells incorporate a variety of very active defense and repair systems when exposed to environmental toxicants, and these defense systems may be overwhelmed during prolonged exposure. The imposed damage may be qualitative or quantitative in nature. In this study, the effects of a locally produced insecticide *Rambo insect powder* on serum Ca^{2+} level and induction of SOD were investigated.

MATERIALS AND METHODS

Test Sample: The test sample for the experiment was a locally produced insecticide, Rambo insect powder that contains 0.60 % (w/w) Permethrin as the active ingredient. *Rambo insect powder* is produced by Gongoni Co. Limited, 89A Sharada Industrial Estate, Phase 111, Kano, Nigeria.

Formulation of Treatment: Different concentrations of the insecticide powder in the diet were prepared by weighing-out a definite amount of growers' mash (feed) and then mixed with the "Rambo" insect powder. The concentrations of the active ingredient of the "Rambo" insecticide (permethrin) in the feed were 0.006 g, 0.03 g and 0.06 g. This produced either 1 %, 5 % or 10 % (w/w) of the "Rambo" insect powder in the feed. The feed for control contains no "Rambo" powder. All the animals were given sufficient quantity of water daily.

Procurement and Management of Experimental Animal: Wilster albino rats weighing between 120 – 720 g were obtained from the Faculty of Veterinary Medicine, University of Nigeria Nsukka (UNN) and maintained on a commercial feed (growers' mash) for five days in the animal house of the Department of Biochemistry, UNN, before the commencement of the experiment. The animals were grouped into three; newly weaned rats (NWR; 2 – 4 weeks, weighing 150 – 185 g), middle aged rats (MAR; 7 – 12 weeks, weighing 290 – 335 g), and aged rats (AR; 13 – 16 weeks, weighing 570 – 642 g). Each group was fed with different concentrations of the Rambo contaminated diets (1 %, 5 % and 10 % w/w), the control groups were fed with the normal diet.

Protein Determination in the Plasma: Total protein concentrations (mg/ml) in the plasma were analysed with Follin-Ciocalteau reagent as described by Cunha-Bastos *et al*. (1999). Bovine Serum Albumin (BSA) was used as standard protein.

Superoxide Dismutase Assay: An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activities in blood plasma (Misra and Fridovich, 1971). Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent prepared as described by Onwurah, (1999) to a mixture of epinephrine (3×10^{-4} M), Na_2CO_3 (10^{-3}M), EDTA (10^{-4}M), and 1.0ml of deionized water at a final volume of 6 ml. The auto-oxidation was read in a spectrophotometer at 480 nm every 30 sec for 5 min. The experiment was repeated with 1.0 ml of the blood plasma from different blood samples collected from different groups of animals. A graph of absorbance against time was plotted for each, and the initial rate of auto-oxidation calculated. One unit of SOD activity was defined as the concentration of the enzyme (mg protein/ml) in the plasma that caused 50 % reduction in the auto-oxidation of epinephrine (Jewett and Rockling, 1993). Superoxide dismutase activity was subsequently calculated for each sample.

Serum Calcium Assay: This was based on the method of precipitation by chloranilic acid (Cerioti, 1974). To 0.5 ml of the serum in a centrifuge tube was added 0.5 ml of chloranilic acid. This was mixed thoroughly and centrifuged. The precipitate was washed in 3 ml of 50 % prapanol–water mixture, and later dissolved in 3.5 ml of citrate

buffer (0.2 mol/l). The mixture was shaken on a "cyclomixer", and the absorbance read at 530 nm against water as blank.

Statistical Analysis: Mean values (± SD) of duplicate experiment with duplicate sampling (N = 4) were taken for each analysis. Significantly different results were established by one – way ANOVA and differences between groups, age, and concentrations were determined by DUNCAN multiple range test. The accepted value of significance was $p<0.05$ (Duncan, 1955).

RESULTS

The inhibition of the initial rate of auto-oxidation of epinephrine brought about by SOD has been used in a rapid, sensitive, and convenient method of assessing the presence of this enzyme in protein extracts of cell homogenates (Misra and Fridovich, 1971). The corollary also holds: The activity of SOD was measured due to the presence of superoxide anion (McCord and Fridovich, 1970). Table 1 showed the results of inhibition studies on the auto-oxidation of epinephrine (pH 10.2) by blood plasma protein of rats exposed to "Rambo" insecticide contaminated diet at various concentrations of 1 %, 5 % or 10 % (w/w). The result showed that a plasma protein level was not significantly different ($P > 0.05$) within the groups (NWR, MAR and AR) of experimental rats and the controls.

The specific activity of SOD did not significantly increased in the NWR groups fed with 1 %, 5 % or 10 % (w/w) – contaminated diet relative to control ($P > 0.05$). In the contrary, the MAR groups and AR groups fed with 1 %, 5 % or 10 % - contaminated diet showed significant increase in the specific activity of SOD ($P < 0.05$) relative to their controls. Pair wise comparison between NWR/MAR, NWR/AR and MAR/AR groups, fed with 1 %, 5 % or 10 % (w/w) insecticide – contaminated diet between 7 – 21 days of exposure showed significantly different results ($P < 0.05$) on SOD only at 10 % (w/w) insecticide – contaminated diet; but the 1 % and 5 % (w/w) insecticide – contaminated showed non significant difference ($P > 0.05$) (Table 3).

Serum Ca^{2+} levels is shown in Figure 1. The results were not significantly different within the groups fed with 1 % and 5 % of the insecticide – contaminated diet ($P > 0.05$). The results were however significantly different within the groups NWR, MAR and AR fed with 10 % of the insecticide – contaminated diet ($P < 0.05$). Comparison of the effect of the insecticide on Ca^{2+} levels between the pairs of NWR/MAR, NWR/AR and MAR/AR groups showed significantly different results ($P < 0.05$) at 10 % concentration of insecticide-contaminated diet (Table 3).

DISCUSSION

The effect of pesticides on non-target organisms is well documented (Moreby and Southway, 1999). The present study reports the effect of permethrin (formulated as "Rambo" insect powder) on non-target organisms. Our results demonstrated that SOD activity decreased in the middle-aged rats (MAR) and aged–rats (AR) groups. The newly – weaned rats (NWR) groups showed a marked increase in the SOD activity when compared with the control. These differences in plasma SOD levels may be due to several factors, such as age, concentration of toxicants, sex, diet etc. The low levels of SOD in the plasma of MAR and AR rats fed with insecticide-contaminated diet may be due to the overwhelming influence of superoxide radicals or activated metabolites generated by the insecticide exposure on the cell membrane of the exposed rats. Determination of SOD in plasma protein samples is based on the ability of the enzyme to inhibit superoxide anion- dependent reactions (Marklund and Marklund, 1974).

The increase in SOD activity in the NWR groups may be due to an induction of the enzyme protein in the presence of reactive metabolites of permethrin (Ledig and Doffoel, 1988). This is obvious from the results in Table 2 where the plasma protein level for NWR fed with varying concentration of the insecticide in the diet were significantly high than that of the control. Similarly, Deuterman (1980) showed that at birth and at the earlier stage of life, there was a marked increase in the activity of many enzymes in the body system of rats. These enzymes are involved in many reactions relating to xenobiotic metabolism and more so, a number of them are age-dependent. The increase in enzyme activity at an earlier stage in life may suggest that NWR groups with increase level of SOD activity could metabolize the permethrin such that its putative toxic effect was not overwhelming to subjugate the mechanism of action of SOD. SOD is an extremely potent antioxidative enzyme that fights cellular damage arising from free radical induction of reactive metabolites from oxidation of hydrocarbon compounds (Onwurah and Eze, 2000). Hence, induction of SOD activity in rats' blood plasma when exposed to environmental toxicants such as "Rambo" insecticide may be an adaptive mechanism for its survival. The rate at which individual and/or groups of rats metabolized the toxicant is age-dependent. This is justified by the mortality ratio (1:4) of rats in favour of newly weaned rats when compared with aged rats. SOD activity is also induced by diverse stresses (Bowler *et al.*, 1992) which may include exposure to hydrocarbon compound, copper, ultra-violet radiation, thermal pollution, disease etc.

TABLE 1: Rate of auto-oxidation of epinephrine in rats exposed to insecticide-contaminated diet

Auto-oxidation mixtures (Am)	Auto-oxidation rate (Units/min)	Percent inhibition (%)
Am + 1.0 ml Distilled H_2O	0.078 ± 0.003	--
Am + 1.0 ml plasma NWR 1 %*	0.026 ± 0.014	66.67 ± 0.047
Am + 1.0 ml plasma NWR 5 %	0.073 ± 0.047	6.41 ± 0.047
Am + 1.0 ml plasma NWR 10 %	0.037 ± 0.013	52.56 ± 0.013
Am + 1.0 ml plasma NWR control	0.070 ± 0.030	10.26 ± 0.044
Am + 1.0 ml plasma MAR 1 %	0.066 ± 0.044	15.38 ± 0.044
Am + 1.0 ml plasma MAR 5 %	0.047 ± 0.003	57.69 ± 0.003
Am + 1.0 ml plasma MAR 10 %	0.066 ± 0.044	15.38 ± 0.044
Am + 1.0 ml plasma MAR control	0.033 ± 0.003	57.38 ± 0.003
Am + 1.0 ml plasma AR 1 %	0..021± 0.010	26.92 ± 0.032
Am + 1.0 ml plasma AR 5 %	0.042 ± 0.019	46.15 ± 0.019
Am + 1.0 ml plasma AR 10 %	0.048 ± 0.023	38.46 ± 0.023
Am + 1.0 ml plasma AR control	0.030 ± 0.010	61.54 ± 0.010

** Plasma taken from different groups of rats eg. Newly weaned rats (NWR) fed with 1% (w/w) contaminated diet. For details see materials and method.*

TABLE 2: SOD activity and total plasma protein levels in rats exposed to insecticide-contaminated diets

Group	Plasma Total Protein (mg/ml)	Superoxide Dismutse (SOD)	
		Activity (units[a] / ml)	*Specific activity Unit/mg protein
NWR 1 %	0.66 ± 0.14	1.33 ± 0.0003	2.02 ± 0.34
	0.64 ± 0.17	0.13 ± 0.0009	0.20 ± 0.26
NWR 10%	0.48 ± 0.04	1.05 ± 0.0003	2.19 ± 0.29
NWR control	0.43 ± 0.02	0.21 ± 0.0006	0.49 ± 0.11
	0.56 ± 0.11	0.31 ± 0.0009	0.55 ± 0.13
	0.68 ± 0.22	0.80 ± 0.0006	0.18 ± 0.10
MAR 10%	0.65 ± 0.18	0.31 ± 0.0009	0.48 ± 0.17
MAR control	0.67 ± 0.19	1.15 ± 0.0006	1.72 ± 0.24
AR 1 %	0.52 ± 0.08	0.23 ± 0.0008	0.44 ± 0.15
AR 5%	0.47 ± 0.05	0.92 ± 0.0004	1.96 ± 0.23
AR 10%	0.41 ± 0.01	0.77 ± 0.0005	1.88 ± 0.18
AR control	0.56 ± 0.07	1.23 ± 0.0002	2.20 ± 0.45

**Specific activity for the SOD in all the groups is not significantly different (P < 0.05) [a]One unit (of activity) of Sod is generally define as the amount of the enzyme that inhibits the autoxidation of epinephrine by 50 %.*

Table 3: Duncan multiple range test of one-way ANOVA for comparing the ages of rats exposed to varying concentrations of insecticide-contaminated diet on SOD activity and serum calcium levels

Combinations	Sod in Plasma		Serum Ca^{2+} Level	
	Differences	LSR	Differences	LSR
NWR 1% / MAR 1%	0.043	0.162	0.12	0.32
NWR 1% / AR 1%	0.003	0.162	0.23	0.32
AR1% / MAR 1%	0.043	0.162	0.11	0.32
NWR 5% / MAR 5%	0.026	0.130	0.23	0.36
NWR 5% / AR 5%	0.005	0.130	0.23	0.36
AR 5% / MAR 5%	0.031	0.130	0.00	0.35
NWR 10% / MAR 10%	0.037	- 0.049*	0.17	0.09*
NWR 10% / AR 10%	0.000	- 0.049*	0.16	0.09*
AR 10% / MAR 10%	0.037	- 0.049*	0.23	0.09*

**Significantly different results (P < 0.05)*

SOD is an important enzyme in living cells for maintaining normal physiological conditions and coping with oxidative stress.

The role of calcium ion (Ca^{2+}) as a mediator of toxicant-induced cell death is very important to this study because intracellular Ca^{2+} homeostasis is very important to cell viability. Our results demonstrated that there is an increase in the serum Ca^{2+} level from NWR to MAR and ARs. This agrees with the work of Thomas *et al.*, (1984). Similarly, there seems to be an inverse correlation between the SOD and serum Ca^{2+} level.

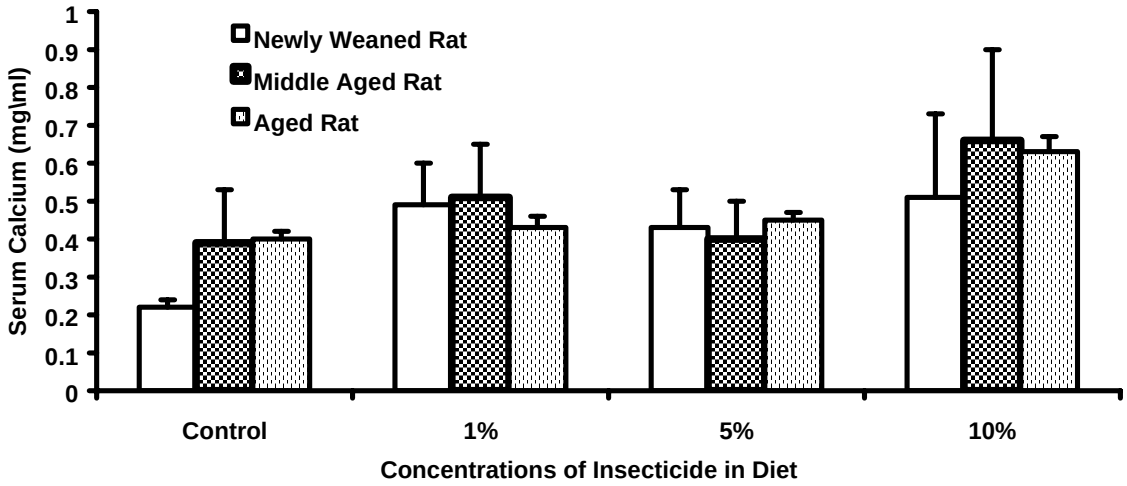

Figure 1: Serum calcium levels (mg\ml) of rats exposed to insecticide contaminated diets

The group with high SOD activity possessed very low level of serum Ca^{2+}. This may be as a result of the scavenging ability of SOD on the superoxide radicals generated by the increase in cytosolic Ca^{2+} level. The elevated Ca^{2+} level can cause several tissue injuries and subsequently affect membrane potential and mitochondrial uncoupling (Marklund and Marklund, 1974). Several studies with xenobiotics demonstrated mitochondrial energy uncouplers (Deuterman, 1980), which suggest disruption of energy supply as a common principal cause of cellular cytotoxicity.

Acute pesticide poisoning, particularly in developing countries, is frequent and thus of great importance in public health. The magnitude of the problems depends on a number of contributing factors, such as types of pesticide regulations, awareness of the degree of danger, training to minimized exposure and availability of medical treatment facilities. The use of pesticide in developing countries is often characterized by lack of vital knowledge of its toxicity and procedures for safe use. This plays a critical role in obtaining direct exposure by both target and non-target organisms.

The action of "Rambo" insecticide on non-target groups may vary widely in comparison to the other insecticides like paraquat (Palmeira, 1999); deltamethrin, zeta–cypermethrin and dimethoate (Moreby *et al.*, 2001). It has become apparent that both SOD and Ca^{2+} are important to both toxicological and physiological processes. The relative importance of the various Ca^{2+} dependent processes in cells needs to be further clarified and the toxicity of "Rambo" insecticide with other pyrethroid could be further compared using biochemical markers.

REFERENCES

BOWLER, C., MONTAGU, M. V. and IRIZE, D. (1992). Superoxide dismutase and stress tolerance. *Analytical Review Plant Physiology and Molecular Biology, 43:* 83 – 116.

BRIDGES, J. W., BENFORD D. J. and HUBBARD S. A. (1983). Mechanisms of toxic Injury. *Annals of New York Academy of Sciences, 83:* 42 – 63.

CARAFOLI, E. (1987). Intracellular calcium homeostasis. *Analytical Review in Biochemistry, 56:* 395 – 433.

CERIOTI, G. (1974). Inorganic substances. Pages 1566 – 1572. In: H. C. CUTINS (ed). *Clinical Biochemistry*. Principles and Methods, Volume 2, Blackwell Scientific Publications, Oxford.

COMPORTI, M. (1993). Lipid peroxidation, an overview. Pages 65 – 87. In: POLI, G., ALBANO, E. and DIANZANI, M. U. (Eds.). *Free radicals: from basic science to medicine*. Birkhauser Verlag Basell, Switzerland.

CUNHA-BASTOS, V. L. T., CUNHA-BASTOS, J., LIMA J. S. and CASTRO-FAIRA, M. V. (1991). Brain acetylcholinesterase as an "in-vitro" detector of organophosphorus and carbamate insecticide in water. *Water Resources, 25:* 835 – 840.

DEUTERMAN, W. C. (1980). Metabolism of toxicants: phase II reactions. Pages 92 – 104. In: Hodgson E. and Guthrie, F. E. (Eds.). *Introduction to Biochemical Toxicology*. Blackwell Scientific Publications, Oxford.

DUNCAN, D. (1955). Multiple range test and multiple F-tests. *Biometrics*, *11:* 1 – 42.

GLENNON, M. C., BIRD, G. J., KWAN, C. Y. and PUTNEY, J. W. (1992). Actions of vasopressin and the Ca^{2+} - ATPase Inhibitor, *thapsigargin*, on Ca^{2+} singling in hepatocytes. *Journal of Biological Chemistry, 267:* 8230 – 8233.

GUTTERIDGE J. M. C. (1994). Antioxidants, nutritional supplements and life-threatening diseases. *British Journal of Biomedical Science, 51:* 288 – 293.

JEWETT, S. L. and ROCKLIN, A. M. (1993). Variation in one unit of activity with oxidation rate of organic substrate in indirect superoxide dismutase assays. *Analytical Review of Biochemistry*, *212:* 55 – 59.

KAWANISHI, T., BLANK, L. M., HAROOTUNIAN, A. T., SMITH, M. T., and TSIEN, R. Y. (1989). Ca^{2+} oscillations induced by hormonal stimulation of individual fura – 2 – loaded hepatocytes. *Journal of Biological Chemistry, 264:* 12859 – 12866.

LEDIG M. and DOFFOEL M. (1988). Superoxide dismutase activity in erythrocytes of alcoholic patients. *Advances in the Biosciences, 71:* 125 – 129.

MARKLUND, S. and MARKLUND, G. (1974). Involvement of superoxide anion radical in the auto-oxidation of pyragallol, and a convenient assay for superoxide dismutase. *European Journal of Biochemistry, 47:* 469 – 474.

MCCORD, J. M. and FRIDOVICH, I. (1970). The utility of SOD in studying free radical reaction. (ii) The mechanism of the mediation of cytochrome C reduction by a variety of electron carriers. *Journal of Biological Chemistry, 245:* 1374 – 1377.

MISRA, H. P. and FRIDOVICH, I. (1971). The generation of superoxide radical during the autoxidation of ferredoxin. *Journal of Biological Chemistry*, *246:* 6886 – 6890.

MOREBY S. J., SOUTHWAY S., BARKER, A. and HOLLAND J. M. (2001). A comparison of the effect of new and established insecticides on non-target invertebrates of winter wheat fields. *Environmental Toxicology and Chemistry*, *20:* 2243 – 2254.

MOREBY, S. J. and SOUTHWAY, S. E. (1999). Influence of autumn applied herbicides on summer and autumn food available to birds in winter wheat fields in Southern England. *Agricultural Ecosystem and Environment, 72:* 285 – 297.

OLUSI, S. O., (2002). Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. *International Journal of Obesity*, *26:* 1159 – 1164.

ONWURAH I. N. E. and EZE M. O. (2000). Superoxide dismutase activity in *Azotobacter vinelandii* in the disposition of environmental toxicants exemplified by Fenton reagent and crude oil. *Journal of Toxic Substance Mechanisms, 19:* 111 – 123.

ONWURAH, I. N. E. (1999). Lipid peroxidation and protein oxidation in *Azotobacter vinelandii* exposed to mercury, silver, crude oil and Fenton reagent. *Journal of Toxic Substance Mechanisms, 18(4):* 167 – 176.

ORRENIUS, S., MCCONKEY, D. J., BELLOMO, G. and NICOTERA, P. (1989). Role of Ca^{2+} in toxic cell killing. *Trends in Pharmacological Sciences, 10:* 281 – 285.

PALMEIRA, C. M. (1999). Herbicide induced mitochondrial and cellular liver toxicity: A review of paraquat, dinoseb, and 2,4 – D effects. *Journal of Toxic Substance mechanisms,* 18: 187 – 204.

SOTHERTON, N. W. (1991). Conservation headlands and practical combination of intensive cereal farming and conservation. Pages 373 – 397. *In:* FIRBANK, L. G., CARTER, N., DERBY-SHIRE, J. .F. and POTTS G. R. (Eds.). *The ecology of temperate cereal fields*. Blackwell Scientific Publishers, Oxford.

THOMAS, A. P., ALEXANDER, J. and WILLIAMSON, J. R. (1984). Relationship between inositol polyphospate production and the increase of cytosolic free Ca^{2+} induced by vasopresin in isolated hepatocytes. *Journal of Biological Chemistry, 259:* 5574 – 5584.

ANIMAL WASTE MANAGEMENT STRATEGIES, A REVIEW

[1]UCHEWA, Emmanuel Nwafoagu, [1]OTUMA, Michael Oria and [2]BROOKS, Peter
[1]Department of Animal production and Fisheries Management, Ebonyi State University, PMB 053, Abakaliki, Ebonyi State, Nigeria
[2]Faculty of Food, Land and Leisure, University of Plymouth, Seale Hayne, Newton Abbot. TQ12 6NQ, United Kingdom

Corresponding Author: UCHEWA, E. N**.** Department of Animal production and Fisheries Management, Ebonyi State University, PMB 053, Abakaliki, Ebonyi State, Nigeria

ABSTRACT

The issue of pollution and environmental protection now command widespread interest and political attention. Increased concern over environmental destruction has led to the introduction of new anti pollution laws and regulations in many countries throughout the world. Some such regulations focus on curbing pollution caused by industrial and agricultural activities. Animals produce enormous quantities of waste per day. In areas supporting intensive livestock production, accumulation of such waste can pose a serious environmental hazard. A single animal pen of a moderate size will produce quantities of waste equal to that produced by a small town annually. Waste produced from these pens usually lead to soil, water and the atmosphere pollutions. Several nutritional advances have been reported which serve to reduce the excretion and pollutive effect of animal waste.

Keywords: Environmental pollution, Animal, Nutrition, Animal waste

INTRODUCTION

Intensive animal production systems are inefficient feed converters into. This is particularly true for nitrogen (N), phosphorus (P) and potassium (K) ratio in animal diet compare to the dietary intake. A large fraction of these elements in feed are not deposited in animal tissue, but wasted as a mixture of urine and faecal matter (Tamminga and Verstegen, 1992). Losses in animal excreta occur in form of solid, liquid and gases.

High animal stocking density often, results in high waste production per unit area, thus animal manure is becoming a burden on the environment (AFRC, 1991). This is particularly the case in areas where intensive systems are employed for animal husbandry such as in The Netherlands and Holland. Public pressure aimed at reducing environmental pollution, including that caused by the animal industry, is a growing concern. In order to avoid a forced, significant reduction in the size of the animal industry, measures will have to be taken to reduce its negative impact on the environment (Tamminga and Verstegen, 1992). A number of biological approaches may be pursued which can help reduce environmental pollution arising from animal waste.

Dietary manipulation designed to increase feed digestibility reduces the quantity of faecal matters produced by the animals. The incorporation of specific enzymes in diets has also solved some specific nutritional problems. Enzymes rich feed often reduces the level of pollutants excreted in the faecal matter (Bateman, 1998).

MAJOR POLLUTANTS

To sustain their growth, plants must assimilate a variety of nutrient, most notably nitrogen and phosphorus. These nutrients are invariably present in animal manure (Headon and Walsh, 1994). Manure thus serves as an effective fertiliser. However, if manure is applied to the soil at a rate, which exceeds plant assimilation, a build up of nutrients can occur (Tamminga *et al.,* 1992). Such nutrients, which include nitrogen, phosphorus and minerals, can cause serious pollution.

Phosphorus: Low efficiency in the utilization of dietary phosphorus is seen in pigs and other monogastric animals. This is reflected by the large quantity of phosphorus normally associated with animal waste (Cromwell, 1980). Dietary supplementation of phytase enzyme can effect the concentration of phosphorus in poultry and livestock wastes through its ability to liberate phytate phosphorus contained in the cell walls of feed grains (Edens *et al.,* 1999). However this liberation of phytate phosphorus can only be accomplished if a concomitant reduction is made in supplemented dietary inorganic phosphorus and calcium. Phytate forms acid salts with mineral cations such as calcium, magnesium, copper, Zinc,

iron and potassium thereby reducing mineral solubility and availability (Erdman, 1979). When acted upon by phytase enzyme, these cations are released much like phosphorus. Consequently, increased availability of these minerals will result in increased retention of phosphorus in chicken given phytase. In contrast with nitrogen, phosphorus generally remains in association with the surface layer of soil. This limits the extent to which phosphorus pollutes the ground water (MAFF, 1996). Soil erosion or manure run-off from the soil surface, however, can result in appreciable quantities of phosphorus entering the waterways. The presence of excess nutrients in such waterways invariably leads to pollution.

Nitrogen: Excess nitrogen present in manure is in inorganic form (often as ammonium ion NH_4^+). Some may be lost to the atmosphere as ammonia (NH_3) (MAFF, 1996). Because of its positive charge, NH_4^+ tends to associate electrostatically with the soil particles. This renders much of the applied nitrogen initially immobile in the soil. However, some of the NH_4^+ in the soil, which remains unassimilated by plants, is subsequently converted to nitrate in the soil. Although a proportion is converted to nitrogen gas (N_2) by the process of denitrification, much of the nitrate will find its way into ground water supplies (Headon and Walsh, 1994).

Although quantitatively, nitrogen and phosphorus represent the major pollutants present in animal wastes, several other waste constituents can have adverse environmental effects. Increasing concern has been voiced by many with regard to the quantities of minerals derived from animal faeces released in the environment (AFRC, 1991).

NUTRITIONAL APPROACHES TO REDUCE POLLUTION FROM ANIMAL WASTES

A number of nutritional approaches may be pursued which can help reduce the pollutive effect of animal waste (Vandergrift, 1992). In this regards, in piggery attention has been focused on reduction of nitrogen and phosphorus in the faecal matters, while maintaining health and high performance of the pigs. Nutritional management can substantially reduce the quantity of nitrogen and phosphorus excreted by pigs (VanKlooster *et al.,* 1998).

Dietary manipulation designed to increase feed digestibility reduces the qualities of manure produced by an animal (MAFF, 1991). Inclusion of probiotics in the diet may also assist the animal to utilise dietary nutrients more efficiently (Goransson, 1997). The presence of pathogens or potential pathogens (coliform) in the gut can render digestion and absorption of nutrients less effective. This in turn results in excessive excretion of such nutrients in the faeces (Van't Klooster *et al.,* 1998).

The addition of specific enzymes to diets may also solve specific nutritional problems. Feed enzymes can reduce the levels of nitrogen and phosphorus excreted in the faeces. Phytase renders phosphorus in the form of phytic acid, which is biologically available to the animal (Cromwell, 1980). Cellulases and protease may be used to enhance digestion of fibrous and proteinacious dietary components (Tamminga and Verstegen, 1992). Glucanases and pentosanases may be employed to destroy anti-nutritive molecules such asglucans and pentosans (Headon and Walsh, 1994). Anti-nutritional factors generally have an adverse effect on digestion and on assimilation. Their removal, therefore, exerts a positive effect on these physiological processes.

Ammonia is one of the most noxious pollutants associated with animal waste (Tamminga and Verstegen, 1992). Build up of ammonia concentrations in animal pen has a detrimental effect on both animals and animal keepers alike. Excess of ammonia into the atmosphere has an obvious pollutive effect (Horn and Squire, 1997). Beal *et al.* (2001) has shown that pre-treatment of pigs diet with protease increased the *in vitro* digestion of nitrogen in weaner pigs. There are four reasons why enzymes may be added in certain diets:

1. To remove or destroy anti-nutritional factors
2. To enhance overall feed digestibility
3. To render certain nutrients biologically available
4. To reduce the pollutive effect of animal excretes

Reducing Phosphorus Excretion through Nutrition: Inclusion of microbial phytase in pig diets is one of the initial successes in the utilization of enzyme to solve specific nutritional problem. Phytase currently represents the most exciting potential application of enzyme in the animal feed industry (Nasi, 1990). Two thirds of the phosphorus in cereal grain is in form of phytic acid, (phytate). This form of phosphorus is biologically unavailable to monogastric animals, as they do not produce digestive enzyme (phytase) capable of releasing the phosphate groups from phytate (Bateman, 1998).

Jongbloed *et al.* (2000) stated that since 1990 various experiments with exogenous microbial phytase have been reported to quantify their effect on the apparent digestibility/availability of phosphorus. One of the first and most interesting experiments was the dose-response effect of microbial phytase (Natusphos®) on the apparent digestibility of phosphorus in growing pigs from 20 to 55 Kg (Beers and Jongbloed, 1992). Six doses of phytase (from 0 to 1800 FTU/Kg) were used in two types of grower's diets

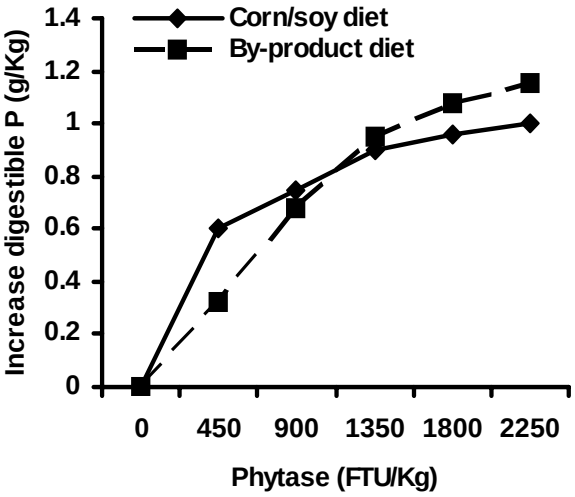

Figure 1: Improvement in digestible P by microbial phytase (Natuphos) in two diets for growing pigs (Beers and Jongbloed, 1992)

(based either on corn-soybean meal or phytate-rich by-products). The efficiency of microbial phytase appeared to be related to its dose and the type of diet (Figure 1).

From 0 – 400 FTU/Kg there was a rapid increase in microbial phytase efficacy, which flattened afterwards. In the experiment, it was shown that microbial phytase was considerably effective in enhancing phosphorus digestion and so increases the amount of digestible /available phosphorus in the feed for pigs.

Approximately 67 % of the phosphorus in plant tissue is in the form of phytate phosphorus (Myoinositol hexakisphosphate) (Cromwell *et al.,* 1993), which is only minimally available to monogastric animals since they lack the phytase enzyme that hydrolyses phytic acid to inositol and/or thophosphate (Peeler, 1972). Supplementation of phytase enzyme in corn-soybean meal diet of broiler chickens improves the availability of phytate bound phosphorus (Simons *et al*., 1992; Edens *et al.,* 1999). Phytate phosphorus content of corn is 68% of the total phosphorus, and in soybean meal phytate phosphorus represents 60 % of the total phosphorus (Edens *et al.* 1999). Simons et al (1990) demonstrated that in three-week-old broilers the availability of dietary phosphorus could be increased up to 65 % by means of supplemental dietary phytase while reducing fecal phosphorus by 50 %. Furthermore, inclusion of phytase activity in diets having wheat, triticale, rye, or their by-products resulted in better phosphorus utilization in poultry (Choct, 2001).

The efficacy of microbial phytase depends on animal related factor such as physiological status and housing condition (Kemme *et al.* 1997a). Kemme et al. (1997b) showed that the efficacy of phytase in generating digestible phosphorus decreased in the order of lactating sows, growing-finishing Pigs, sows at the end of pregnancy, piglets and sows at mid pregnancy.

A prerequisite for a good evaluation of microbial phytase efficacy is that the animal be fed below their phosphorus requirement. This is due to intestinal regulation of phosphorus absorption when animals are fed above their phosphorus requirement. It is commonly known that higher dietary calcium levels decrease apparent absorption of phosphorus. Thus a balance of calcium: phosphorus must be established for excellent performance (Jongbloed, 1993).

Reducing Nitrogen Excretion through Nutrition: To reduce the level of nitrogen in fecal matter through nutrition, two approaches are possible thus: (I) Enhancement of the deposition of nitrogen in animal products (meat, eggs, milk) and (ii) constant maintenance reduction of dietary nitrogen input while productivity is sustained (Tamminga and Verstegen, 1992). The first approach requires the intermediary metabolism to operate more efficiently, while the second approach largely depends on reducing nitrogen losses along the gastro-intestinal tract.

Both approaches will result in a reduction of Nitrogen in animal excreta by 20 %. A more efficient intermediary metabolism will reduce nitrogen excretion in urine by 10 %, whereas a reduction in losses from gastro-intestinal will reduce quantities present in both faeces and urine by 30 % (Lenis and Jongbloed, 1994). Feeding ration containing a poor balance of amino acids results in removal of excess nitrogen in the faeces. Taylor, *et al.* (1979) indicated that the dietary content of total crude protein could be reduced from 17.6 % to 14.5 % by the addition of crystalline lysine. This leads to improved balance of essential amino acids present in the diet and better protein utilisation.

Inclusion of protease in the diet may also promote more efficient utilization of dietary protein (Headon and Walsh, 1994). The endogenous proteolytic activities associated with the digestive tract are normally more than adequate to promote efficient degradation of dietary protein. For instance supplementation of feeds with exogenous microbial proteolytic activities can serve to improve protein utilization in animals subject to high protein intake.

Cellulase help in the breakdown of cell wall structure and make nutrients in vegetable materials much more available to the animal, they also break down xylan in cereal grains and reduce viscosity of the digester. The increase in the transit time in the gut leads to increase efficiency of utilization. The enzyme activation may also help

promote more efficient digestion of poorly digestible proteins, such as those found in intimate association with some other dietary factors (Bateman 1998).

In monogastric animals, protein digestibility is low for some legume seeds, due to the presence of anti-nutritional factors (ANF) like lectins and protease inhibitors (Tamminga and Verstegen 1994). Low protein digestion can be overcome by technological treatment of the diet in an optimal combination of temperature, moisture and time. Short treatment at high temperature is more effective in reducing the antinutritional factor content of the dietary ingredient. Reduction in the activity of proteinous ANF and further breakdown of non-starchy polysaccharides can be accomplished using enzymes, during germination and grinding to finer particle size.

Beal *et al*. (1998) in a factorial analysis demonstrated the difference in the *in vitro* nitrogen digestibility between raw soybean and different full fat soybean meals both with and without enzyme treatment at different p^H (Table 1). Surprisingly, they observed that raw soybean appeared to be more digestible in pigs than processed soybean meal. However, the pre-treatment of soybean with exogenous enzymes increases protein digestibility. This is because large molecular weight proteins are partially hydrolyzed before the commencement of digestion. The difference in nitrogen digestibility between the raw and processed soybean meal could be due to a number of factors. Heat denaturation preventing digestive enzymes to act on amino acid residues, differences in solubility due to the pH of the stomach and loss of available protein due to heat induced interactions with other substances.

Nitrogen excretion can also be reduced substantially by supplying dietary amino acids in accordance with the animal's requirement and by incorporating free amino acids in the feeds and lowering crude protein content.

Multi phase feeding, in which diets can be automatically adjusted by means of a computer controlled feeding system may reduce excretion of nitrogen and phosphorus by 10 and 15 to 22%, respectively. Bourdon and others achieved nitrogen and phosphorus reduction applying multi phase feeding to castrated male pigs weighing between 25 and 100kg with decreased dietary protein levels, and supplementary addition of limiting amino acids (VanKlooseter *et al.,* 1998). From the experiments they concluded that the amount of nitrogen excreted was reduced by 50 %, with multi phase feeding accounting for 0.10 %. Van-der Peet-schwering *et al.* (1996) using multiphase feeding for growing and castrated male pigs between 25 and 110Kg live weight. Reported that multi phase feeding reduced ammonia emission by 45%, compared to the single control diet. Further more, multi phase feeding lead to 22 % reduction in phosphorus excretion by growing pigs (Beers and Jongbloed 1992). Results of Kemme *et al.* (1997a, b) indicated that multi phase feeding does not always lead to optimum performance and slaughter quality of pigs.

Requirements of nitrogen and phosphorus for breeding sows are much lower during pregnancy than during lactation. The use of separate diets for pregnancy and lactation compared with one diet for both reduced the excretion of nitrogen and phosphorus by 20 % (VanKlooseter *et al.,* 1998).

Growth promoters, because of improved feed conversion ratios have an estimated 7 and 3% reduction on nitrogen and phosphorus excretion per weaned piglet and growing pig respectively, according to Jongbloed et al (1992). Both nitrogen and phosphorus excretion can be further reduced with recombinant porcine somatotropin (rPST) (Bateman 1998); unfortunately, the use of growth promoters in feed is banned.

The source and level of fermentable carbohydrates in the diet influence ammonia volatilisation of pig slurry (Coppoolse *et al.* 1990). In an experiment, using three different treatments, Aarnink and Lenis (1998) fed soluble maize starch in treatment 1 and replaced it with coconut expeller and soybean hulls in treatment II and III respectively, ammonia volatilisation was decreased under laboratory conditions by 0.35%, 0.51% and 0.36% respectively. In a second experiment, the same authors examined the effect of electrolyte balance (Na + K – Cl), Ca-level and Ca-salt on ammonia emission from slurry. When CaSO4, Ca – benzoate or CaCl2, replaced CaCO3 respectively, the ammonia emission of slurry under laboratory conditions was reduced by 30%, 54% and 33% respectively.

EFFECT ON THE PERFORMANCE OF THE ANIMAL

There have been numerous reports on the effects of microbial phytase and phosphorus utilization. In addition to the general established improvements in phosphorus digestibility, significantly higher live weight gain and better-feed conversion efficiency have often been reported (Beers and Jongbloed, 1992; Cromwell, 1980; Dungelhoef and Rodehutscord, 1995 and Kemme *et al.,* 1997a). This could be explained by interference of phytic acid with the digestion of other essential minerals and protein. Phytate complexes in acid and alkaline media have been described (Dierick and Decuypere (1994). *In vitro* studies have shown that phytate-protein complexes involving amino groups of lysine, histidine and arginine are formed, which are insoluble and biologically unavailable in

Table 1: In vitro N digestibility (%) in different full fat soybean meals pre-treated with proteases P2, P3, or P4 with pepsin digestion at p^H 2

Protease 20,000 units g/N	% N digestibility				
	RSB	SPC	MIC	TSD	AUT
Control	78.5	80.3	74.7	67.8[1]	70.1[1]
P2	85.8[a1]	84.0[a1]	79.1[a1]	73.5[a]	81.1[a2]
P3	88.9[a]	84.9[a1]	82.7[1]	74.4[a]	78.1
P4	85.8[a1]	87.8[a1]	77.8[a]	74.8[a]	82.3[a]

Values in the same column with the same letter are not significantly different P<0.05. Values in the same row with the same number are not significantly different P< 0.05 (Source: Beal et al., 1998)

Table 2: Relative performance of pigs using positive control diets as 100(n=11) and effect of phytase when added to the positive control diet (n=6)

	Negative control	Positive control	Phytase effect	Positive control	Phytase effect
Growth rate	100	115.0 ± 6.5	116.7 ±10.6	100	106.6 ± 5.5
Feed intake	100	105.4 ± 5.2	107.6 ± 7.8	100	103.0 ± 3.2
FCR	100	93.0 ± 4.9	93.2 ± 5.0	100	95.7 ± 4.9

Source: Jongbloed et al. (1999)

Table 3: Mean magnitude of effects of addition of enzymes to poultry and pig diets

Enzyme	Poultry		Pigs	
	LWG	FCR	LWG	FCR
Protease	1.05	0.97	Small	Small
Amylase	1.12	0.93	1.04	0.96
Pentosanase	1.17	0.93	1.05	0.95
β-glucanase	1.18	0.91	1.01	0.98
Cellulase/Hemicellulase	1.07	0.94	1.03	0.91
Blends	-	-	1.09	0.93

Results and treatment /control ratio (Source: Dierick and Decuypere, 1994)

normal physiological conditions (Nasi, 1990). In addition, these protein complexes are less liable to be attacked by proteolytic enzymes than the free proteins (Jongbloed and Mroz, 1999; Dierick and Decuypere, 1994). Increased protein deposition and amino acid digestion in the small and large intestine of pigs by phytase may help to explain increased performance in pigs (Edens *et al.* 2000, Schoner *et al.* 1993 and Jongbloed 1999). Jongbloed *et al.* (1999) presented results on the effect of microbial phytase on performance of pigs compared. Data in table 2 shows that the performance of both the positive control and the phytase groups were superior to the negative control group. The positive control group and the phytase-supplemented group were almost identical. Performance of the pigs receiving the positive control group with supplementary phytase was slightly better than those without phytase. The relative ratios compared with the positive control diet for growth rate, feed intake and feed conversion ratio were 106.0 ± 5.6, 103.0 ± 3.2 and 95.7 ± 4.9 respectively. This may imply that either the phosphorus requirement was not yet met, which is unlikely, or there is another positive effect of microbial phytase on performance.

Several researchers have demonstrated improved performance of broiler chickens given feeds supplemented with a phytase product. Improved growth performance, assessed by increased body weight, feed intake and better feed conversion efficiency, has been reported in chickens (Edens *et al.,* 2000; Schoner *et al.,* 1991; and Schoner *et al.,* 1993) and turkeys (Qian *et al*, 1996). They further demonstrated improved performance of broilers given supplemental dietary phytase, which was correlated with improved bone growth and mineralisation. This response was attributed to increase retention of certain minerals and nutrients in addition to phytase-liberated phytate phosphorus (Swick and Ivey, 1990). The responses of pigs to supplementation of diets with proteolytic enzymes have been studied (Partridge, 2001; Chot, 2001; Dierick and Decuypere, 1994). In piglets did proteolytic enzymes improve live weight gain (LWG) and feed conversion ratio (Chot, 2001).

The underlying rationale for enzyme supplementation is the fact that the proteolytic and amylolytic digestive system is not fully developed until 4 – 6 weeks of age (Dierick and Decuypere, 1994).

In addition, nutritional stress associated with abrupt changes in the diet of the piglet may justify the use of supplemental enzymes at such times. However, as recovery and adaptation of

endogenous enzyme production is very rapid, benefits of enzyme addition will be short-lived and of the order of two weeks (Headon and Walsh, 1994). α - Amylase addition to a barley diet for young pigs improved live weight gain and feed conversion ratio by about 4 %. In grower and finishing and growing pigs, amylase supplementation to cereals did not affect pig performance (Dierick and Decuypere, 1994). Choct (2001) reported significant increase in the live weight gain and feed conversion efficiency of chickens fed barley diets. Since it is established that the starch in barley is totally digestible by the amylase secreted by chickens, improvements with amylase supplementation were probably due to the added enzymes used; i.e. the crude enzyme used contained β - glucanase activity.

The effects of added enzymes are greater in poultry than in pigs and more apparently in young than in older animals (Dierick and Decuypere, 1994). However, it is difficult to draw definite conclusions. Table 3 shows the mean effects of simple addition of enzymes to poultry and pigs diets.

EFFECT ON THE ENVIRONMENT

In the absence of microbial phytase, only approximately 16 % of phosphorus in corn and approximately 36 % of phosphorus in soybean meal is digested by pigs (Jongbloed *et al.,* 2000). Because of the large amount of undigested dietary phosphorus, a substantial amount of phosphorus is removed via the faces. Based on the estimates of Cromwell *et al.* (1993), a dose of microbial phytase equal to 1000FTU/g converted approximately one third of the unavailable phosphorus to an available form. About 500FTU/Kg of diet generates approximately 0.8 g digestible Phosphorus per kilogram of diet, which is equivalent to 1.0 g phosphorus from monocalcium phosphate or 1.23 g phosphorus from dicalcium phosphate, which is often used in the United States.

A significant reduction in poultry manure phosphorus can be achieved via the use of microbial phytase in feed. This can reduce the nitrogen: phosphorus ratio in poultry wastes. Blander and Flegal (1997) studied the effect of feed supplemented with allzyme phytase in layer diets and reported a 16 % reduction in fecal phosphorus from laying hens fed inorganic phosphorus at 80 % of NRC requirements and a 25 % reduction in fecal phosphorus from laying hens fed 60 % of NRC requirements. A 35 % decrease in fecal phosphorus from laying hens given microbial phytase product at 250 FTU/Kg diet was reported by Coppoolse *et al.* (1990). Similarly, Blanda and Flegal (1996) reported decreased phosphorus excretion in turkeys given allzyme phytase. The fecal reductions from laying hens and market turkeys given allzyme phytase supplemented feeds were similar to the phosphorus reduction found in pigs given another phytase feed supplement, (Simons and Versteegh, 1992) and broilers (Yi *et al.,* 1996).

Conclusion: Many of the environmental impact of pig farming are known and ultimately controllable. The use of enzymes in pig production is an well-accepted practice today. Generally, most of the enzymes effectively depolymerise the soluble NSP into smaller polymers, though some products with affinity for both soluble and insoluble NSP are also used. It is wise to test economic policy and regulatory changes against the environmental consequences and ensure proper planning and implementation of control measures. That said, it is clear that whilst the potential for damage on the environment is great, nutritional manipulation to enhance efficient utilisation of Phosphorus and nitrogen by monogastric animals will reduce the damage done to the environment by these animals by at least 30 %.

REFERENCES

AFRC (1991). Live stock feeds and feeding. *Agricultural and Food Research Council. Nutrition Abstract and Review, Series B, 61:* 683 – 722.

BATEMAN, G. (1998). Environmental impact of pollutants from pig farming and current legislative developments. Pages 251 – 268. *In:* WISEMAN, J., VARLEY, M. A. and CHADRWICK, J. P. (Ed). *Progress in Pig Science*. Nottingham University Press. Nottingham.

BEERS, S. and JONGBLOED, A. W. (1992). Apparent overall (total) digestibility of phosphorus in relation to doses of *Aspergillus niger* phytase in diets for pigs. *Journal of Animal Science, 70:* 237 – 242.

BEAL, J. D., BROOKS, P. H. and SCHOLETZE, H. (1998). The effect of pre-treatment with different proteases on the *in-vitro* digestibility of nitrogen in raw soyabean and four different processed full fat soybean meals. Pages 112 -115. *In:* VAN ARENDONK, J. A. M. DUCROCQ, V., VAN DER HONING, Y., MADEC, F., VAN DER LENDE, T., PULLER, D., FOLCH, J., FERNANDEZ, E. W. and BRUNS, E.W. (ED). *Book of Abstract of the 49th meeting of the European Association of Animal Production.* Warsaw 24th – 29th August, 1998.

BLANDER, R, J. and FLEGAL, C. (1996). The effect of using phosphate enzyme on the performance of growing market Turkeys

and excreted phosphorus. *Poultry Science, 75:* 52 – 60.

BLANDER, R, J. and FLEGAL, C. (1997). The effect of Phytase on egg production and egg specific gravity in laying hens. *Poultry Science, 76(1)*: 3.

CHOCT, M. (2001). Enzyme supplementation of Poultry diets based on viscous cereals. Pages 145 - 160. *In:* BEDFORD M. R. and PARTRIDGE, G. C. (Ed). *Enzymes in farm animal nutrition.* CABI Publishing. Wallingford, Oxon.

COPPOOLSE, J., VAN VUREN, A. M., HUISMAN, J., JANSSEN, W. M. M., JONGBLOED, A. W., LENIS, N. P and SIMONS, P. C. M. (1990). The excretion of nitrogen, Phosphorus and potassium by farm animals, now and tomorrow. *Landbouwtijdchrift, 45:* 909 – 927.

CROMWELL, G. L. (1980). Biological availability of Phosphorus in foodstuffs for swine. *Foodstuffs, 52:* 38 – 42.

CROMWELL, G. L., COFFEY, R. D., MONEGNE, H. J. and RANDOLPH, J. H. (1993). Efficacy of low activity phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. *Journal of Animal Science, 73:* 449 – 456.

DUNGELHOEF, M and RODEHUTSCORD, M. (1995). Effect of phytase on the digestibility of phosphorus in pigs. *Ulbers Tierernhrg, 23:* 133 – 157.

DIERICK, N. A and DECUYPERE, J. A. (1994). Enzymes and growth in pigs. Pages 169 – 195. *In:* COLE, D. J. A., WISEMAN, J. and VARLEY, M. A. (Ed). *Principles of Pig Science.* Nottingham University Press, Leicestershire.

EDENS, F, W., PARKHURST, C. R. and HARVENSTEIN, G. B. (1999). Allzyme phytase reduces phosphorus and nitrogen excretion by caged broilers and by broilers in conventional housing. Pages 491 – 150. *In:* LYON, T. P. and JACQUES, K. A. (Ed). *Biotechnology in the feed industry.* Proceedings of Alltech's 15th Annual Symposium. Nottingham University Press. Nottingham.

ERDMAN, J. W. (1979). Oil seed phytases. Nutritional implications. *Journal of Animal Science, 56:* 736 – 741.

GORANSSON, L. (1997). Alternatives to antibiotics – The influence of new feeding strategies for pigs on biology and performance. Pages 451 - 462. *In:* WISEMAN, J. and GARNSWORTHY, P. C. (Ed). *Recent development in pig nutrition 3.* Nottingham University Press. Nottingham.

HEADON, D. R. and WALSH, G. (1994). Biological control of pollutants. Pages 196 – 213. *In:* COLE, D. J. A., WISEMAN, J. and VARLEY, M. A. (Ed). *Principles of Pig Science.* Nottingham University Press, Leicestershire.

HORN, D. B. and SQUIRE, C. R. (1997). An improved method for the estimation of ammonia in blood plasma. *Journal of Veterinary Science, 17:* 99 – 108.

JONGBLOED, A. W., KEMME, P. A. and MROZ, Z. (1993). The role of microbial phytase in pig production. Pages 173 – 180. *In:* WENK, C. and BOESSINGER, M. (Ed). *Enzyme in Animal production.* Proceedings of the 1st Symposium, Kartanse Ittingen, Switzerland.

JONGBLOED, A. W. and MROZ, Z. (1999). Influence of phytase on availability of phosphorus, amino acids and energy in swine. Pages 1 – 20. *In:* COLE, D. J. A., WISEMAN, J. and VARLEY, M. A. *(Ed). Principles of Pig Science.* Nottingham University Press, Nottingham.

JONGBLOED, A. W., KEMME, P. A., MROZ, Z. and DIEPEN, H. T. M. (2000). Efficacy, use and application of microbial phytase in pig production, a review. Pages 111 – 130. *In:* LYONS, T. P. AND JACQUES, K. A. (ED). *Biotechnology in the feed industry.* Proceedings of Alltech's 16th Annual Symposium. Nottingham University Press, Nottingham.

KEMME, P. A., RADCLIFFE, J. S., JONGBLOED, A. W. and MROZ, Z. (1997a). The effect of body weight, housing, and calculation method on mineral digestibility and the efficacy of microbial phytase in diets for growing finishing pigs. *Journal of Animal Science, 75:* 2139 – 2146.

KEMME, P. A., RADCLIFFE, J. S., JONGBLOED, A. W., MROZ, Z. and BREYNEY, A. C. (1997b). The efficacy of *Aspergillus niger phytase* in reducing phytate phosphorus available for absorption in pigs influenced by their physiological status. *Journal of Animal Science, 75:* 2129 – 2138.

LENIS, N. P. and JONGBLOED, A. W. (1994). Modelling animal feed and environment to estimate nitrogen and mineral excretion by pigs. Pages 342 – 351. *In:* COLE, D. J. A., WISEMAN, J. and VARLEY, M. A. (Eds). *Principles of Pig Science.* Nottingham University Press, Nottingham.

MAFF (1991). *Code of good agricultural practice for the protection of water*. Ministry of Agriculture, Fisheries and Food, United Kingdom. 120 pp.

MAFF (1996). *Agriculture in the United Kingdom.* Ministry of Agriculture, Fisheries and Food United Kingdom. 110 pp.

NASI, M. (1990). Microbial phytase supplementation for improving availability of plant phosphorus in the diet of the growing pigs. *Journal of Agricultural Science, 62:* 435 – 443.

PEELER, H. T. (1972). Biological availability of nutrients in feed. Availability of major mineral ions. *Journal of Animal Science, 35:* 695 – 699.

QIAN, H. E., KORNEGAY, T. and DENBOW, D. M. (1996). Phosphorus equivalence of microbial phytase in Turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. *Poultry Science, 75*: 69 – 81.

SCHONER, B. R. J., HOPPE, P. P., SCHWARZ, G. and WIEESCHE, H. (1991). Comparative effects of microbial phytase and inorganic phosphorus on performance and retention of phosphorus, calcium and crude ash in broilers. *Journal of Animal Nutrition, 66:* 248 – 255.

SCHONER, B. R. J., HOPPE, P. P., SCHWARZ, G. and WIEESCHE, H. (1993). Effects of microbial phytase and inorganic phosphorus in broiler chickens: performance and mineral retention at various calcium levels. *Journal of Animal Nutrition. 69:* 235 – 244.

SIMONS, P. C. M. and VERSTEEGH, H. A. J. (1992). The effect of the addition of microbial phytase to layer feed on the technical result and skeleton and eggshell quality. *Spelderholt Publication, No. 568:* 223 – 231.

SIMONS, P. C. M., JONGBLOED, A. W., VERSTEEGH, H. A. J., SLUMP, P., BOSS, K. D., WOLTERS, M. G. E., BENDEKER, R. F. and VERSCHOOR, G. J. (1990). Improvement of phosphorus availability by microbial phytase in broiler and pigs. *British Journal of Nutrition, 64:* 525 – 540.

SWICK, R. A. and IVEY, F. J. (1990). Effect of dietary phytase addition on broiler performance in phosphorus deficient diets, *Poultry Science, 69:* 133 – 142.

TAMMINGA, S. and VERSTEGEN, M. W. A. (1992). Implications of nutrition of animals on environmental pollution. Pages 342 – 351. *In:* GARNSWORTHY, P. C., HARESIGN, W. and COLE, D. J .A. (Eds). *Recent Advances in Animal Nutrition*. Nottingham University Press. Nottingham.

TAYLOR, A. J., COLE, D. J. A. and LEWIS, D. (1979). The effect of crystalline lysine in the utilization of dietary protein by pigs. *Journal of Animal Science, 65:* 481 – 487.

VANKLOOSTER, C. E., van der PEET-SCHWERING, C. M. C., AARNINK, A. J. A. and LENIS, N. P. (1998). Pollution issues in pig operations and the influence of nutrition, housing and manure handling. Pages 507 – 518. *In:* WISEMAN, J., VARLEY, M. A and CHADWICK, J. P. (Eds). *Progress in Pig Science*. Nottingham University Press, Nottingham,

VAN DER PEET-SCHWERING, C. M. C., VERDOES, N., VOERMANS, M. P and BEELEN, G. M. (1996). Animal Nutrition and ammonia emission. Pages 618 – 626. *In:* VAN DER AAR, P. J., AARNINK, A. J. A., BLOK, M. C and VAN VUUREN, A. M. (Eds). *State of the art in research.* The Netherlands.

YI, Z., KORNEGAY, E. T. and DENBOW, D. M. (1996). Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poultry Science, 75:* 240 – 249.

CYTOGENETIC VARIATIONS IN *Clarias* species (CLARIIDAE: SURULIFROMIS) OF THE ANAMBRA RIVER USING LEUCOCYTES CULTURE TECHNIQUES

EYO, Joseph Effiong
Department of Zoology, University of Nigeria, Nsukka, Nigeria

ABSTRACT

Cytogenetic variations among four Clarias species from Anambra river, Nigeria were studied using leucocytes culture techniques. Heterogeneity in chromosome number (2n = 48 in Clarias ebriensis and C. albopunctatus to 2n = 56 in C. anguillaris and C. gariepinus) and Karyotype morphologies occurred among the clariids. The chromosomes were characterized by a high proportion of meta-submetacentric chromosomes and low proportion of acrocentric chromosomes. The females karyotype morphologies exhibited a heteromorphic pair suspected to be the sex chromosome complex. The following formulae were established for the male clariids; C. ebriensis 6m + 22sm + 20a FN =76; C. albopunctatus 4m ± 22sm + 22a FN = 74; C. gariepinus 8m + 24a FN = 88; and C. anguillaris 8m + 26m + 22a FN = 90. The female karyotype morphologies were C. ebriensis 6m + 23sm + 19a FN = 77, C. albopunctatus 4m ± 23sm + 21a FN = 75, C. gariepinus 8m + 25sm + 23a FN = 89 and C. anguillaris 8m + 27sm + 21a FN = 91. A generic chromosomal number of 2n = 54 ± 4 for the clariids was suggested. The almost uniform karyotype morphologies and the closeness of the chromosome numbers around the generic chromosome number may suggest success with which the clariids may hybridize in nature.

Keywords: Cytogenetics, Chromosomes, Karyotype, Idiogram, Clarias, Clariidae, Leucocyte culture

INTRODUCTION

Karyological evidences have often been utilized in solving problems relating to speciation, identity of sex determining chromosome, phyletic relationship, the taxonomic status and identity of both interspecific and even intergeneric hybrids among organisms. Concerning speciation, new evidence suggests that karyotype plays a primary role (White, 1978) contrary to Mayr (1963) original hypothesis that only polyploidy and geographical isolation lead to the formation of new species. Fish comprises polyphyletic group which has undergone an enormous expansion, the number of existing species totals about 20,000 (Lagler *et al.*, 1977). This is about 48.10 % of all the existing vertebrates. The capacity of this group to adapt has led to their colonization of extremely specialized niches. Pisces explosive expansion cannot be explained wholly in terms of geographical isolation since chromosome mechanism probably plays a role in speciation (Sola *et al.*, 1981).

About 1000 to 1500 species have been studied out to the 20,000 species (Lagler *et al.*, 1977). Evidence has been found of evolution by polyploidization in the Cypriniidae (Wolf *et al.,* 1969) and Salmoniidae (Ohno *et al.*, 1969) using karyological techniques. In salmonids currently undergoing expansion, chromosome polymorphisms were shown (Thorgaard, 1977). In the Poecilids, speciation may occur by interspecific hybridization. This apparently applies to *Poecilia formosa* Bloch, 1801; whose hybrid origin is widely accepted based on morphological and karyological characteristics (Prehn and Rasch, 1969).

The teleosts provide good materials for cytologists working on sex determination problems. In most species sex chromosome are absent or not morphologically identified. However, some species are known to exhibit male heterogamete whereas others have female heterogamete. Kallman (1973), reported that allopathic populations of the same species may display female or male heterogamete as shown in *Xiphophorus maculates* Hecke, 1848 in which the sex chromosomes were identified by means of pigment gene marker showing a sex-linked hereditary patterns. Feldber *et al.,* (1987) showed the occurrence of chromosome system for sex determination of ZZ/ZW type in *Semaprochilodus taeniurus* Newton, 1978 (Pisces: Prochilodontidae) which is lacking in *Semaprochilodus insignis* C. and V., 1929. Chromosome W found in the female was the largest in the complement. Chromosome Z was the next largest complement and tentatively considered as pair number one in males. Heaf and Schmid (1984) have shown sex-chromosome differentiation in another poecillid species, *Poecillia sphnops* var. *melonistica* Jordan and Snyder, 1906.

Karyotype evolution and geographical distribution among fishes have been studied. In the genus, *Oryzias;* Oyzidae, Uwa (1986), Uwa and Parenti (1988) and Uwa *et al*., (1988) divided the rice fish into three groups: monoarmed chromosome type (*O. melastigma* Jordan and Starks, 1906 (Uwa and Iwata, 1981) *O. javanicus* Jordan and Starks, 1906 (Uwa and Iwata, 1981), biarmed chromosome type *O. sp. (Oryzias* species) (Uwa and Magtoon, 1986) *O. curvinotus* Jordan and Starks, 1906 (Uwa*, et al.,* 1982), and fused chromosome type (*O. celebensis* Jordan and Seale, 1906 and *O. minutillus* Jordan Starks, 1906 in South and South Eat Asia to the Indian ocean. The biarmed chromosome types are distributed in East Asia and Luzon. The degree of the karyotype evolution by increased of biarmed chromosome number in this group seems to correlate with the geographical distribution from Indo-China to Japan and Luzon to China. Members of the fused arm chromosome types are found in Sulawesi and Thailand.

Cytogenetical investigations among teleosts has revealed inter and intra-specific variations in nucleolar organizing region (NOR). Feldberg and Bertollo (1985) noted among the neo tropical cichlids that the NORs were located on the first chromosome pair in the complement and coincided with secondary constrictions observed there, whereas in *Cichlasoma facetum* Swainson, 1839 and *Geophagus brasilcensis* Heckel, 1840, the NOR were located on another chromosome pair. NORs have been investigated in several fish species, such as *Carassius sp.* Nilsson, 1832 (Funaki *et al.*, 1975; Ojima and Yamano, 1980), *Umbra limi* Gronow, 1763 (Kligerman and Bloom, 1977), cichlid fishes (Kornfield *et al*., 1979), *Fundulus diaphanous* Lacepode, 1803 (Howel and Black, 1979). In the fish species, the NORs were usually located near the telomeric regions of satellite chromosomes except for *F. diaphanous* where they were located on the secondary constriction of the sex chromosomes. The NORs are of obvious importance to geneticists. Kligerman and Bloom (1977) reported that the NORs are sites of prior genetic activities and where satellite chromosomes occur. This chromosome pair could serve as an adequate marker as in *Umbra limi.*

The interests of cytogeneticists and evolutionists are closely connected with that of ichthyologists in the study of hybrids. In the freshwater bony fish, evidences were frequently found in interspecific and even intergeneric hybrids where sympatric populations of different species exist. The development of fish culture has resulted in numerous experiments on hybridization to obtain new strains, which are more disease resistant, faster growing than their relative wild stock. In such case, karyological knowledge allows the parent species to be recognized and give some indication of the likelihood of success in the case of artificial crossbreeding. Furthermore, it may help to make predictions concerning hybrid fertility and the interactions of two parental stocks.

Although it is not possible to draw absolute conclusion about phyletic relationships from karyological comparison of different species, karyotype analyses have supplemented other taxonomic evaluating methods. The systematic definition of species depends on a careful morphological analysis, and the karyotype is a major morphological character among several others.

The phyletic relationship among the siluriform fishes based on karyotype and variation in chromosomal number revealed that, *Bagre marinus* Catesby, 1771 had 2n = 54 chromosomes composed of 12 metacentric, 8 submetacentric and the remainder with terminal or near terminal centromere (Fitzsimons *et al*., 1988). Furthermore, the karyotype of three species of ariid catfishes (*Arius dussumier* C and V, 1840, *Arius felis* C and V 1840 and *Bagre marinus)* indicated the same diploid chromosomal number, but each species had a different arm number. Data for 132 species in 14 families of catfishes indicated a predominance of 56 $\pm$ 2 chromosomes in the diploid set (Fitzsimons *et al*., 1988). According to them, the range in diploid number was most common among the Ariidae, Bagridae, Ictaluridae and Pimelodidae, which together have been suggested from osteological evidence as forming a group close to the ancestral stock from which present day catfish evolved. In a study of chromosomal evolution among the Ictalurid catfishes, LeGrande (1981) observed that a diploid chromosome number of 56 $\pm$ 2 was wide among 70 species of catfishes in 10 families and was especially more frequent in four families, the Ariidae, Bagridae, Ictaluridae and Pimelodidae. In addition, average diploid count of 56 modal count for catfishes were approximated from the averages and / or modal counts of ariids, (all 54), bagrids (mostly between 50 and 60, with a weak mode at 52), clariids (50, 52 and 56), ictalurids (mostly between 56 and 60 with exclusion of divergent karyotype in *Noturus*) and Primelodids (mostly 56). LeGrande (1981) hypothesized an ancestral karyotype of 2n = 56 for Ictalurids and pointed out that the closeness in this number to those reported for the Ariidae, Bagridae and Pimelodidae coincides with Gosline (1975 a, b) suggestion that these families plus Doradidae constitute a group near the ancestral stock from which living catfishes evolved. Considering the Clariids, Teugels *et al*. (1992) reported a standard karyotype of 2n = 56 for *Clarias gariepinus and* 2n = 52 for *Heterobranchus longifilis* Valenciennes, 1840. Their hybrids revealed an intermediate karyotype

of 2n = 54. Furthermore, sex chromosomes were observed as ZW heteromorphic pair in female hybrid karyotypes. The ZZ chromosome pair in male hybrids was similar to that found in male *C. gariepinus* and male *H. longifilis.*

Hartley (1987) divided the species of the sub-order: Salmonidea into two groups (categories A and B) based on chromosome number, chromosome arm number, and the distribution of one-armed and two-armed chromosomes. Category A karyotypes were widespread both in terms of geographic distribution and number of species, while category B karyotypes were restricted to member of the genera *Salmo* and *Oncorhynchus.* In category A, it was assumed that tetraploidy followed arrangement of the diploid ancestral karyotype of 2n = 50, NF = 60 (the lowest diploid and chromosome arm number found in the smolts) and that the resulting ancestral tetraploid salmon had 2n = 100, NF = 120 karyotype. Hartley (1987) observed that a series of pericentric inversion and centric fusions had reduced chromosome number and chromosome arm number through *Brachymystaz lenok* Gunter, 1866, (2n = 94, NF = 116) and *Hucho hucho* Gunter, 1866, (2n = 82, NF = 114) to the category A karyotypes (2n = 80, NF = 100) and finally to the category B karyotypes (2n = 60, NF = 104). He concluded that by the time the category B karyotypes were achieved, centric fusions had played a far greater role in the evolution of the vast majority o the salmonids karyotypes than pericentric inversions, although the latter had played important role in the evolution of the Atlantic salmon karyotype.

The karyotypic evolution of ten Neotropical cichlids showed that the chromosome evolution of the group was more conservative than divergent (Feldberg and Bertollo, 1985). All species had a chromosome number of 2n = 48, though with some differences in chromosome morphology. Pericentric inversions were probably the main event that led to the karyotypes more commonly found in this group. On the basis of fundamental number (FN), four general groups reflected the occurrence of a progressive number of chromosome rearrangements. Group one with FN = 48, (represented by *Chaetobranchopsis australis* Eigenmann and Ward 1907), group two with FN = 50 – 52 (comprising *Geophagus brasiliensis* Heckel, 1840; *G. surinamensis* Heckel, 1840 and *Gymnogeophagus balzanii* Ribeiro, 1918), group three with FN = 54 (represented by *Crenicichla lacustris* Ribeiro, 1918, *C. lepidota* Henkel, 1840 and *C. vittala* Heckel, 1840 and to *Batrachops semifasciatus* Heckel, 1840), group four with FN = 58 – 60 (represented by *Astronotus ocellatus* Swainson, 1839 and *Cichlasoma facetum* Swainson, 1839) (Feldberg and Bertollo, 1985). Despite the constancy of chromosome number, 2n = 48, a few distinctive characteristics which are products of structural rearrangements of chromosomes that occurred during their karyotypic evolution can be seen in some species or group of species.

Ichthyologists and fish taxonomists in the classification of related species have often employ knowledge from karyotype. For instance, the larval forms of most species are morphologically identical and quite different from the adult forms. Because of the resemblance among larval forms, their identification if often difficult. Morphological observations are inadequate *vis-à-vis* the identification of the species, except where the developmental stages can be followed, which is usually difficult and not documented for many fish species. In such cases, Sola *et al.* (1981) noted that the knowledge of the karyotype and their diversified forms might provide a precise diagnostic criterion. Oliveria *et al*. (1988) based the classification of Neotropic Ostrariophysi on karyotypic data and noted that 2n = 50 tend to prevail among Ostrariophysi. This character was also seen among the Otophysi (2n = 50). The Cypriniformes have a second modal number of 2n = 100, often seen among the families of Cyprinidae and Catostanidae. Yu *et al.* (1987) proposed that the cypriniformes might have originated from 2n = 50 ancestors. The characiphysi and characiformes differ from the cypriniforms by having almost 2n = 54. Among the characiformes, several groups may be characterized based on their chromosome number. The characiforms, erythrinidae and lebiasinidae are typified by chromosome number of less than 2n = 54 while the Serrasalminae are characterized by chromosome number of more than 2n = 54.

From the foregoing review, karyotypic information appears to be very important in discriminating species. The technical and interpretation problems often encountered by fish cytologists should not be employed as a base for judging the validity of karyotypic information *vis-à-vis* classification of related and unrelated species. The identification of artificial (diploidy and/or polyploidy) and natural hybrids, and of sex determining chromosomes is currently cytogenetically accomplished. For a proper discrimination of the species, information from fish molecular biology, anatomy, anatomy, physiology ethology and ecology is highly desirable. This study further enriches the information on fish cytogenetic by investigating into the karyotypic variations among members of the Pisces family Clariidae of Anambra river, Nigeria using leucocytes culture techniques.

MATERIALS AND METHODS

Collection and Care of Catfish: Catfish specimens were collected from Anambra river, Nigeria. The fish were captured using set nets (mesh size 70 mm – 120 mm) and long line baited with earthworm and palm fruits. Specimens were also bought from the fishers at the landing site. All catfishes were transported to Fisheries and Hydrobiology wet laboratory, Department of Zoology, University of Nigeria, Nsukka. Identification and classification of fish were done with Lowe-McConnell (1972), Sydenham (1983) and Ezenwaji (1989) (see Eyo, 1997). Live specimens were given 1 ppm potassium permanganate ($KMnO_4$) flush prophylactic treatment for 10 minutes. This was to avoid the introduction of wild ectoparasites and pathogens. Catfishes were acclimatized in nine aquaria tanks (80 x 40 x 40 cm) in groups of eight fish per tank for 14 days. All fishes were fed with 5 % body weight daily with 40 % formulated fish feed (Eyo, 2004a). All other culture conditions were as explained in Eyo and Ezechie (2004).

Phytohaemaglutinin Extraction: Phyto-hemaglutinin (PHA) used for this study was extracted from kidney bean, *Phosealus vulgaris* using a modified method of Rigas and Osgood (1954). Finely ground and sieved red kidney bean seed (250 grams) was digested with one litre of 1 % NaCl for twenty-four hours at 4 ^{0}C refrigeration. The extract was centrifuged at 1400 rpm for 15 minutes using Multex centrifuge. The light yellow supernatant was recovered and the precipitate discarded. The supernatant was adjusted to pH 5.7 and cold ethanol added to final concentration of 30 % ethanol, and centrifuged at 1400 rpm for 15 minutes. The precipitate was rejected and 400 ml of 10 % ethyl ether, absolute ethanol and 75 % ethanol in the ratio (1:1:1) was added to the yellow supernatant. A milky white colour developed upon shaking. The milky white supernatant was re-incubated at 4 ^{0}C refrigeration for 24 hours.

After the re-incubation, the extract was centrifuged at 1400 rpm for 15 minutes. The resulting yellow supernatant was discarded. The milky white precipitate was dissolved in 150 ml of 1 % NaCl. The pH was re-adjusted to 5.7 and the ethanol fractionation repeated by adding 300 ml of 1:1:1 volume of absolute ethanol, 10% ethyl ether and 75% ethanol. The resulting milky white extract was shaken vigorously and re-incubated at 4 ^{0}C for 24 hours. The extract was centrifuged at 1400 rpm for 15 minutes. The resulting light yellow supernatant discarded and the milky white precipitate dissolved in 150 ml of 1% sodium chloride. The ethanol fractionation was repeated by adding 200 ml of 1:1:1 volume of ethanol, 10% ether and 75% ethanol. The extract was vigorously shaken and centrifuged at 2000 pm for 15 minutes. The milky white precipitate was dissolved in 100 ml of 0.1M phosphate buffer pH 8.0 and 40 ml of saturated ammonium sulphate solution added. A light pink colour developed. The light pink extract was centrifuged at 2000 rpm for 15 minutes and the precipitate discarded, 105 ml of saturated ammonium sulphate solution was added to 150 ml of the supernatant, shaken vigorously and centrifuged at 2000 rpm for 15 minutes. The precipitate was dissolved in 100 ml deionized water, shaken vigorously and centrifuged.

The procedure was repeated by adding 50 ml of deionized water and 17.5 ml of saturated ammonium solution was added. The extracted was vigorously shaken and centrifuged at 2000 rpm for 15 minutes. The phytohaemagglutinin (PHA) was freed from the ammonium sulphate by dissolving the light pink precipitate in 50 ml of deionized water, vigorously shaken and centrifuged. This procedure was repeated twice, and the resulting phytohaemagglutinin (2 grams) was freeze-dried. A stock solution of 1 mg ml^{-1} phytohaemagglutinin was made by dissolving 0.1 gram of PHA in 100 ml of 0.85 % sodium chloride solution. The stock solution was stored at 10 $\pm$ 5 ^{0}C.

Blood Sampling: Blood was collected by heart puncture after anaesthezing the catfishes in L/1500 solution of MS-222 (Methanesulfonate salt) for 3 minutes. The abdomen wiped with absolute ethanol and the visceral cavity opened to expose the heart. One ml of blood was obtained using a sterile 2 ml plastic syringe containing 0.2 ml of 0.1 M sodium citrate (an anticoagulant) with a 21 gauge, 1-inch needle. The sampled blood from each catfish was stored in a labeled sterile capped 5 ml plastic tube at 10 $\pm$ 5 ^{o}C until used. Contamination of sampled blood was avoided by dealing with only one sex of a fish species per day. All the equipments were heat sterilized before and after use. Catfishes sampled for blood were 20 males and 18 females of *Clarias gariepinus,* 21 males and 24 females of *Clarias anguillaris,* 30 males and 21 females of *Clarias ebriensis* and 26 males and 30 females of *Clarias albopunctatus.*

Blood Leucocytes Separation: Blood leucocytes were separated by three simple sedimentation methods modified from Blaxhall (1981) and the leucocytes-rich supernatant used as inoculums. The methods were:

1. **Hank's balance salt solution (HBSS) sedimentation:** 1 ml of anticoagulated blood was diluted with 1 ml of HBSS and mixed by gentle inversion in 5 ml sterile

caped plastic test tube. The content was centrifuged at 2,700 rpm for 3 minutes using micro angle centrifuge.

2. **Hank's balance salt solution plus phytohaemagglutinin separation:** 1 ml of HBSS and 0.2 ml of 1 mg ml^{-1} PHA were added to 1 ml of anticoagulated blood. The contents were mixed and centrifuged at 2, 700 rpm for 3 minutes to ensure proper settling out of erythrocytes.

3. **Phytohaemagglutinin separation:** 1 ml of anticoagulated blood was diluted with 0.2 ml of 1 mg ml^{-1} PHA. This was mixed by gentle inversion in a sterile 5 ml capped plastic test tube. The content was centrifuged at 2,700 rpm for 3 minutes. In each case, the leucocyte rich supernatant was used as inoculum.

Blood Leucoyte Culture Techniques: The complete culture medium used composed of 20 ml of HBSS, 2 ml of Eargle's Essential Culture Medium. Laboratory prepared phytohaemagglutinin was added to make up either 5 % of the entire culture medium. Furthermore, freshly prepared rabbit sera were added to make up 20 % of the entire culture medium. The complete medium was aseptically filtered using membrane filter. The complete culture medium (5 ml) was placed in sterile tube. Various glass and plastic sterile test tubes were tried for culturing of fish leuocytes. About 0.05 ml of leucocyte rich supernatant was aseptically placed on the bottom of the culture tube containing 5 ml of the complete culture medium using a sterile 1 ml syringe fitted with 26-gauge 1¼-inch needle. Culture vials were incubated at $36 \pm 1\ ^0C$ for 72 hours.

Chromosome Harvesting and Slide Preparation Techniques: The cultured leucocyte cells were arrested 3 – 6 hours with 0.3 $\mu g\ ml^{-1}$ of colchicine solution before the end of the 72 hours culture period to give good mitotic chromosome spread for karyotyping (Eyo, 1997). At the end of incubation and metaphase arresting period, the cells were harvested by centrifuging at 1000 rpm for 5 minutes. The supernatants were discarded and the white button-like residue given 5 ml of 0.075 M KCl hypotonic treatment for 10 minutes and expirated using a micropipette. The residues were obtained through centrifugation and the supernatants discarded. 2 ml of freshly prepared cold Carnony's fixative (absolute ethanol and glacial acetic acid (3:1)) was added to the residue cells, expirated to disperse the cell pellets and left standing for 15 minutes. The fixation process was repeated twice at 15 minutes intervals. After the final centrifugation, the fixative was decanted leaving about 3 drops in which to re-suspend the cells through expiration. The chromosome spread was accomplished using a modification of Mellman (1965) air-dried technique.

Thoroughly clean grease free slides (commercially pre-cleaned slides) were dipped into deionized water and frozen. The chilled slides were observed for cleanliness by adherence of water film. Excess fluid was shaken off and one drop of cells suspension was placed on the center of the wet slide inclined at an angle of 45^0. A blotter placed underneath the slides removed excess fluid. Drying was completed immediately by blowing the slides to promote evaporation and by gentle warming under 80 watts electric bulb.

The quality of slides was checked using a hand lens. Inadequate spreading was corrected by re-fixation of the cells. Slides were stained in 10 % buffered Giemsa stain for 5 minutes, and dehydrated for 1 min each in two jars of acetone, one jar of acetone and xylene solution (1:1) and finally one jar of xylene. Slides were observed to destain in acetone when shaken and or agitated. Thus, shaking and agitation was avoided. Slides were scanned for metaphase chromosomes using a binocular light microscope at X 1500 magnification. Slides with good metaphase chromosomes were processed into permanent mounts. The chromosomal arm lengths were measured to the nearest micron using ocular micrometer.

Chromosome Analysis Nomenclature: Ten slides per species showing good metaphase chromosome were analysed. The frequency of diploid chromosomes number per species was recorded. The maximum or minimum mean and modal chromosome number were computed for each species. F-LSD was employed to separate the differences in modes. The arm ratio r, (long arm L/ short arm S) and the centromeric index i (100 x short arm/total length of chromosome) were calculated. Furthermore, each chromosome was classified into any of the six groups; M, m, sm, st, t, and T based on Levan *et al.* (1964) classification (Table 1).

Table 1: Chromosome arm nomenclature relevant to Clariid cytotaxonomy

Term	Location	d value	r value
M	Median point	M 00	1.0
m	median region	m 0.0 – 2.5	1.01 -1.7
Sm	Submedian region	Sm 2.5-5.0	1.7-3.0
St	subterminal region	st 5.0-7.5	3.0-7.0
t(a)	terminal region	t 7.5-10	7.0-00
T(a)	Terminal point	T 10-0	00

Based on the nomenclature, the karyotypic difference within and between the *Clarias* species were ascertained.

RESULTS

Cytogenetic Variations among four *Clarias* Species from Anambra River, Nigeria: Tables 2 and 3 illustrates the modal chromosomes and karyotype morphology distribution respectively among the *Clarias* species of Anambra river. The modal metaphase chromosome numbers were 2n = 48 in *C. ebriensis,* 2n = 48 in *C. albopunctatus,* 2n = 56 in *C. gariepinus* and 2n = 56 in *C. anguillaris*. They were characterized by a high proportion of meta-submetacentric chromosomes and low proportion of acrocentric chromosomes. All female karyotypes exhibited a heteromorphic pair suspected to be sex chromosome complex. The following formulae were established for the male clariids; *C. ebriensis* 6m + 22sm + 20a FN =76; *C. albopunctatus* 4m ± 22sm + 22a FN = 74; *C. gariepinus* 8m + 24a FN = 88; and *C. anguillaris* 8m + 26m + 22a FN = 90. The female karyotype morphologies were *C. ebriensis* 6m + 23sm + 19a FN = 77, *C. albopunctatus* 4m ± 23sm + 21a FN = 75, C*. gariepinus* 8m + 25sm + 23a FN = 89 and *C. anguillaris* 8m + 27sm + 21a FN = 91. Furthermore, figures 1 – 4 displays representative metaphase chromosomes of males and females *Clarias* species from Anambra River, Nigeria.

The karyotype distributions and idiograms were developed based on chromosome nomenclature of *Clarias* species using their centromeric indices and presented on figures 5 and 6, 7 and 8, 9 and 10 and 11 and 12 for males *C. ebriensis, C. albopunctatus, C. gariepinus* and *C. anguillaris* respectively. In *C. ebriensis,* six (6) chromosomal length distributions occurred with modal chromosome percentage total length of 7.00 %. Seventy five percent of the modal chromosomes were submedian chromosomes while 12.50 % were both median and terminal chromosomes (Figure 5 and 6).

In *C. albopunctatus,* the chromosome total length percentage distributions exhibiting bimodal length peaks of 9.01 % made up of 50.00 % submedian and 50.00 % terminal chromosomes; and 7.50 % made up of 100% submedian chromosomes (Figures 7 and 8). *C. gariepinus* exhibited chromosome total length percentage distributions of 8.37 % and 3.90 % with modal chromosome percentage length of 5.58 %. Fifty-seven percentage (57.00 %) of the modal chromosome percentage total length distribution were submedian chromosomes while 29.00 % were terminal chromosomes with only one (14.00 %) median chromosome (Figure 9 and 10). Similarly, *C. anguillaris* exhibits chromosome total length percentage distribution of 8.80 % to 4.40 % with bimodal chromosome percentage total length of 6.60 %, made up of 40.00 % median

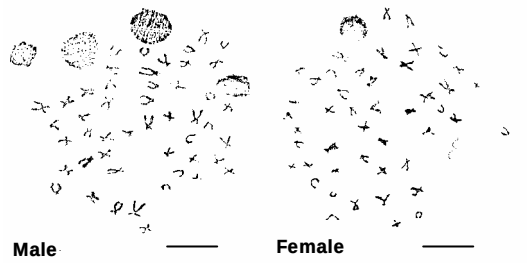

Figure 1: Metaphase chromosomes of *Clarias ebriensis* from Anambra river, Nigeria (bar = 10μ)

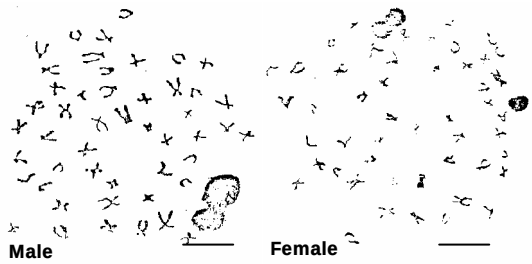

Figure 2: Metaphase chromosomes of *Clarias albopunctatus* from Anambra river, Nigeria (bar = 10μ)

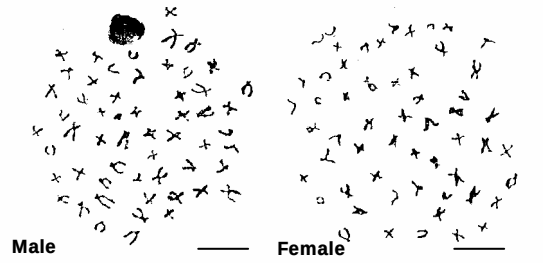

Figure 3: Metaphase chromosomes of *Clarias gariepinus* from Anambra river, Nigeria (bar = 10μ)

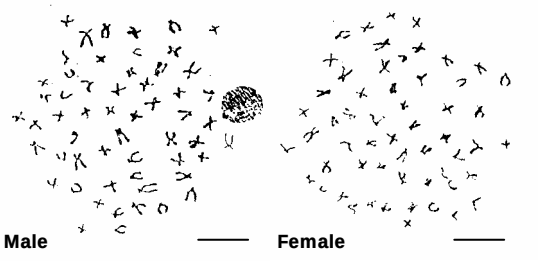

Figure 4: Metaphase chromosomes of *Clarias anguillaris* from Anambra river, Nigeria (bar = 10μ)

chromosomes and 60.00 % terminal chromosomes; and 5.52 %, made up of 100 % submedian chromosomes. The chromosome total length has been categorized into 13 length groups (Figures 11 and 12).

Table 2: Frequency distribution of the diploid chromosome number among *Clarias* species from Anambra River, Nigeria

Species	No. of slides scanned			Diploid chromosome number									
	Male	Female	Total	48	49	50	51	52	53	54	55	56	57	58
C. ebriensis	4	3	7	17	6	-	1	5	8	8	-	10	1	-
C. albopunctatus	2	3	5	15	2	3	2	8	-	14	8	2	-	-
C. gariepinus	4	3	7	7	-	3	3	2	-	10	-	11	-	3
C. anguillaris	2	4	6	6	3	3	-	4	4	6	-	8	-	3

Table 3: Karyotype distribution among *Clarias* species from Anambra River, Nigeria

Species	Karyotype			
	Male	FN	Female	FN
C. ebriensis	6m + 22sm + 20a	76	6m + 23sm + 19a	77
C. albopunctatus	4m + 22sm + 22a	74	4m + 23sm + 21a	75
C. gariepinus	8m + 24sm + 24a	88	8m + 25sm + 23a	89
C. anguillaris	8m + 26sm + 22a	90	8m + 27sm + 21a	19

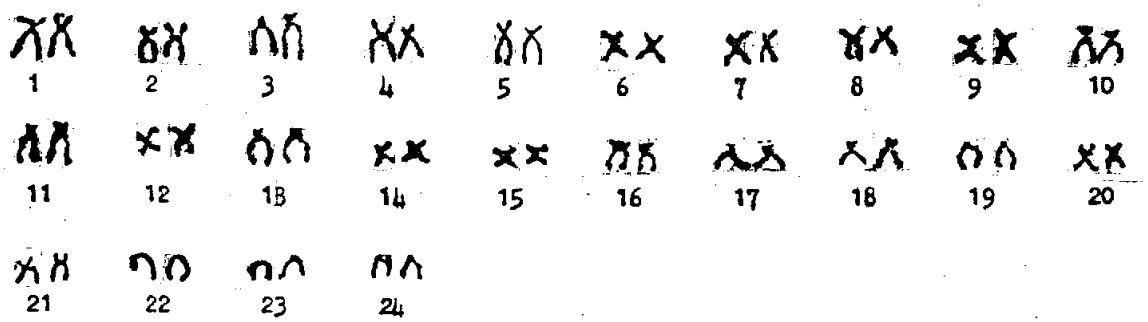

Figure 5: Karyotype of *Clarias ebriensis* from Anambra river, Nigeria

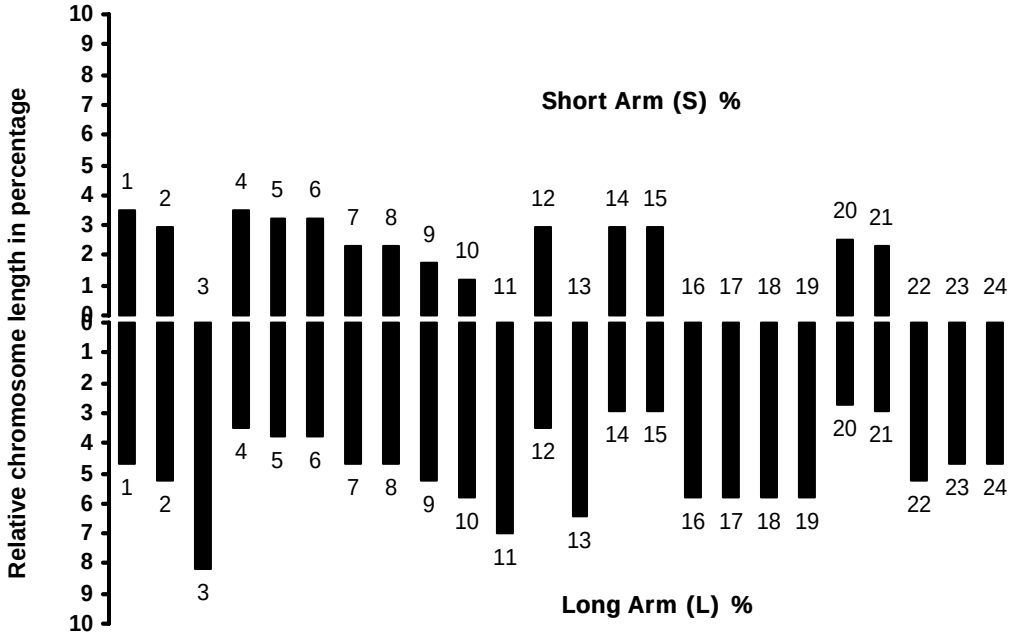

Figure 6: An idiogram of the karyotype of male *Clarias ebriensis* showing the morphology of the chromosomes. The 0 represents the position of the centromere

DISCUSSION

The chromosome number of *Clarias* species of Anambra river, varied from 2n = 48 to 2n = 56 with modal chromosome number of 2n = 56 in *C. gariepinus* and *C. anguillaris.* The observed range and modal chromosome number is in agreement with Ozouf-Costaz *et al.* (1990) for the clariids.

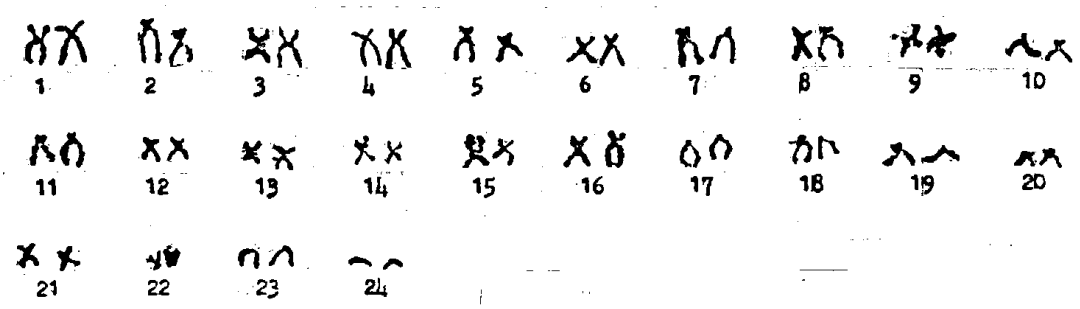

Figure 7: Karyotype of *Clarias albopunctatus* from Anambra river, Nigeria

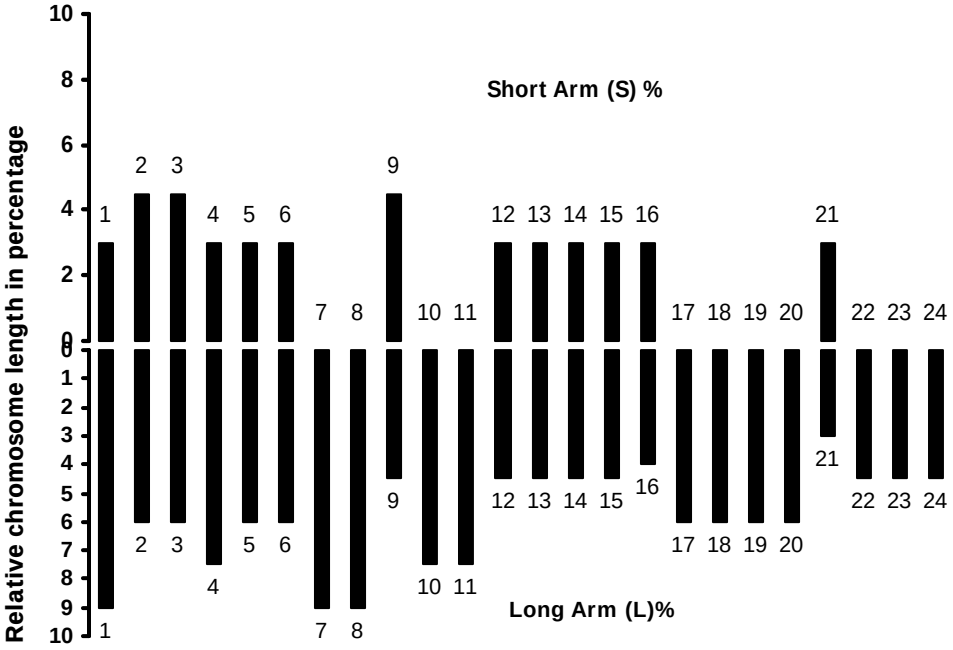

Figure 8: An idiogram of the karyotype of male *Clarias albopunctatus* showing the morphology of the chromosomes. The 0 represents the position of the centromere

Teugels *et al.* (1992) reported 2n = 52 for *Heterobranchus longifilis* Valenciennes, 1840 and 2n = 54 in the hybrid of *C. gariepinus* vs. *H. longifillis.* The result of the present studies indicated that the karyotypes consisted more of submedian chromosomes in *C. ebriensis* and *C. anguillaris* and almost equal number of submedian and terminal chromosomes in *C. gariepinus* and *C. albopunctatus* in which the centromeres were clearly defined. Differences in chromosome sizes were observed, but were very gradual and at no point were the variations greater than error involved in the method of measurement. Similar karyotypes were described for *C. gariepinus* (Ozouf-Costaz *et al.,* 1990) and *H. longifilis* (Teugels *et al.,* 1992) strains used for aquaculture. The observed chromosomal divergence between these clariids species was consistent with their morphological differences (Eyo, 2003). The almost uniformity of chromosome number and morphology within the clariids may also suggest success with which many species will hybridize. Crosses between clariids (*C. gariepinus* vs *H longifilis*) using artificial fertilization method have been reported (Teugels *et al.*, 1992). In nature, the occurrence of chromosome number around modal values among the clariids may suggest that chromosomal changes may be associated with the process of speciation within the group, possibly through high rate of hybridization resulting from communal spawning. Information on the chromosome number among siluriform fishes indicated that diploid chromosome number for catfishes range from the mid-20's to well over 100 with most species having a diploid set in the mid-40's to upper 100's, but no clear modal number have been suggested. An increase in diploid chromosome number is associated with a concomitant change in arm number. In the study of chromosome number among the siluriform fishes, Fitzsimons *et al.* (1988) noted that a diploid chromosome number of 56 ± 2 was widespread

Figure 9: Karyotype of *Clarias gariepinus* from Anambra river, Nigeria

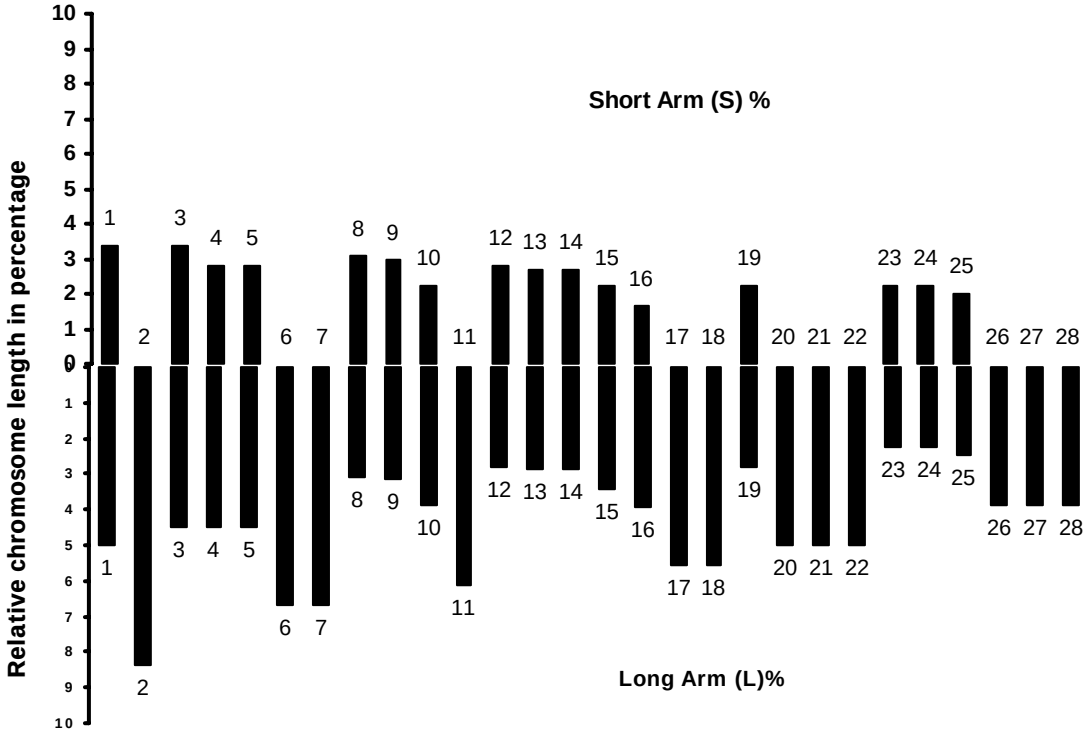

Figure 10: An idiogram of the karyotype of male *Clarias gariepinus* showing the morphology of the chromosomes. The 0 represents the position of the centromere

among 70 species of catfishes in 10 families and was especially frequent in four families, the Ariidae, Bagridae, Ictaluridae and Pimelodidae. The observed range for the clariids 2n = 48 to 2n = 56 fall below the speculated 2n = 56 ± 2 range of Fitzsimons *et al.* (1988). Thus the range for the clariids may be put at 2n = 52 ± 4. The observed similarities in chromosome number although with almost similar morphometric and meristic characters may explain the placement of both *C. anguillaris* and *C. gariepinus* in the *subgenus Clarias (Clarias*) by Teugels (1982). The disparity in chromosome number (Eyo, 1997) along with dissimilar morphometric (Eyo, 2002; 2003) and meristic (Eyo, 2004b) characters of *C. ebriensis* and *C. albopunctatus* from *C. gariepinus* and *C. anguillaris* may equally explain the placement of *C. ebriensis* and *C. albopunctatus* in the subgenera *Clarias (Anguilloclarias)* and *Clarias (Clarioides)* respectively.

Karyological evidences have been employed in solving problems relating to chromosome number, functional arm, phyletic relationship, the taxonomic status as well as possibility of speciation among the studied *Clarias* species. For instance the wide dispersal of chromosome number around modal value (2n = 56) among the clariids suggested possibilities of the species undergoing speciation.

Conclusively, the grouping of *Clarias* either as macroclarias or as megaclarias based on similarities in size and morphological feature should be adopted as a stock management tool. The key diagnostic characters (Eyo, 2003) as well as the relationship between standard length and morphometric characters (Eyo and Inyang, 2003) may be adopted by clariid taxonomist.

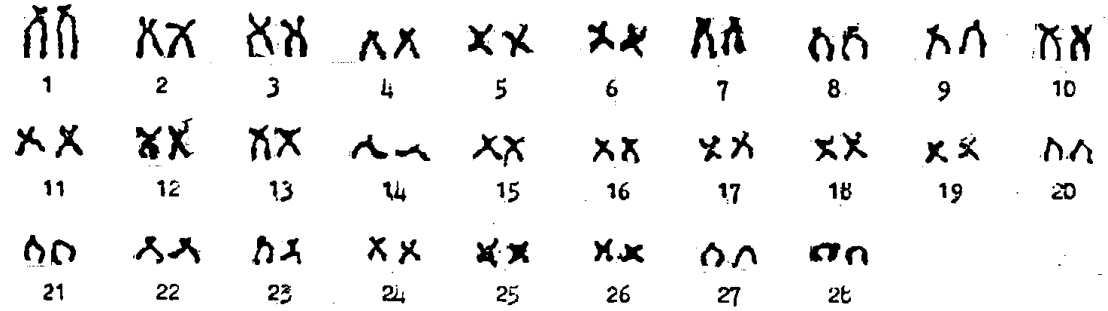

Figure 11: Karyotype of *Clarias anguillaris* from Anambra river, Nigeria

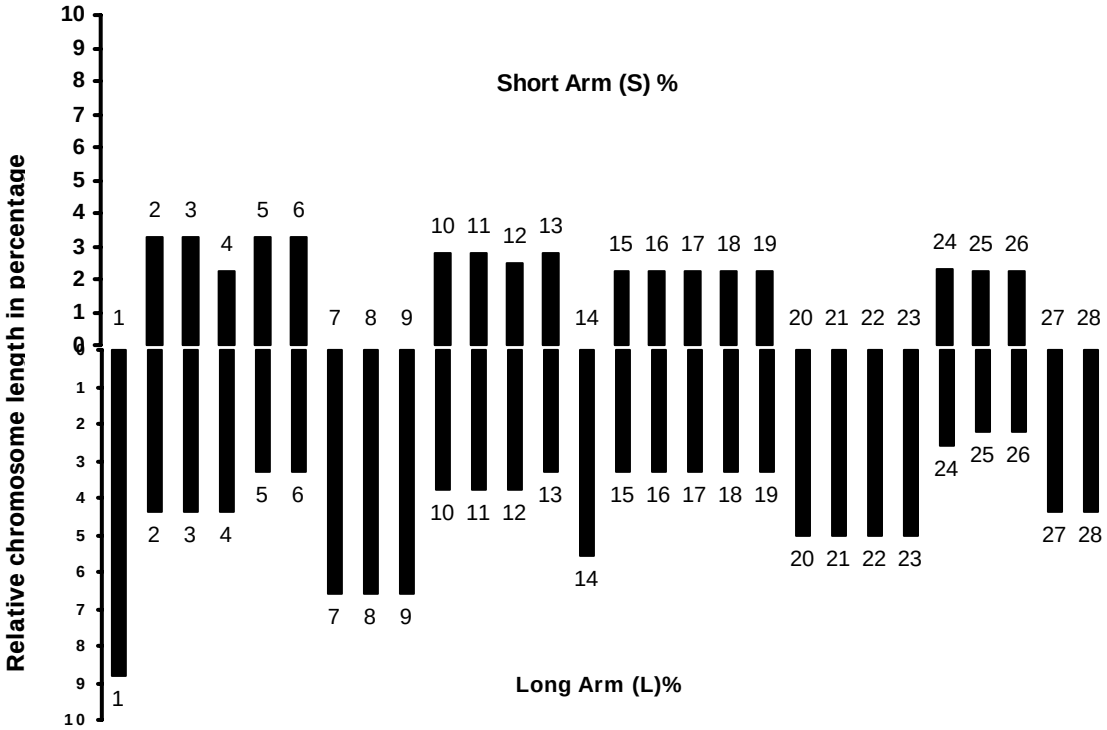

Figure 12: An idiogram of the karyotype of male Clarias anguillaris showing the morphology of the chromosomes. The 0 represents the position of the centromere

The cytotaxonomy of the clariids places them into two chromosomal number types; 2n = 56 represented by C*. anguillaris* and *C. gariepinus,* and 2n < 56 as in *C. ebriensis* and *C. albopunctatus* justifies the managerial designation of the clariids into megaclarias and macroclarias.

ACKNOWLEDGEMENT

The author acknowledges Drs. N. M. Inyang and B. O. Mgbenka for supervising the PhD thesis from which this paper was extracted. Furthermore, greatly acknowledged are, the Nigerian Academy of Science for their Postgraduate fellowship award in 1993; and Prof. G. G. Teugels (late), Drs. D. H. J. Sydenham and H. M. G. Ezenwaji for identifying the species and provision of relevant research documents. Finally, I am thankful to Nkiruka Eze for typesetting the manuscript.

REFERENCES

BLAXHALL, P. C. (1981). A comparison of methods used for the separation of fish lymphocytes. *Journal of Fish Biology, 18:* 177 – 181.

EYO, J. E. (1997). *Morphometric and cytogenetic variations among Clarias species (Clariidae) in Anambra river, Nigeria.* PhD Thesis, Department of Zoology, University of Nigeria, Nsukka. 267 pp.

EYO, J. E. (2002). Conspecific discrimination in ratio morphometric characters among members of the Pisces Genus*: Clarias*

Scopoli, 1777. *The Zoologist, 1(2):* 23 – 34.

EYO, J. E. (2003). Congeneric discrimination of morphometric characters among members of the Pisces Genus *Clarias* (Clariidae) in Anambra River, Nigeria. *The Zoologist, 2(1):* 86 – 103.

EYO, J. E. (2004a). The Effects of Substituting Soyabean Meal for Breadfruit Meal on Diets Acceptability, Growth Response and Cost of Diets Fed to *Heterobranchus bidorsalis* (♂) X *Clarias gariepinus* (♀) Hybrid. *Bio-Research*, *2(2):* 27 – 33.

EYO, J. E. (2004b). Differences in meristic counts of the genus *Clarias* (Pisces: Clariidae) in Anambra river, Nigeria. *Animal Research International, 1(1):* 31 – 35.

EYO, J. E. and EZECHIE, C. U. (2004). The Effects of Rubber (*Havea brasiliensis*) Seed Meal Based Diets on Diet Acceptability and Growth Performance of *Heterobranchus bidorsalis* (♂) *X Clarias gariepinus (♀)* Hybrid. *Journal of Sustainable Tropical Agriculture Research*, *10:*

EYO, J. E. and INYANG, N. M. (2003). Regression Model and *Clarias* species (Clariidae – Scopoli 1777) Taxonomy. *Journal of Science and Technology Research, 2(3): 40 – 49.*

EZENWAJI, H. M. G. (1989). Aspects *of the Biology of some "small" Clarias species in Anambra River Basin, Nigeria.* PhD Thesis, University of Nigeria, Nsukka. 244 pp.

FELDBERG, E. and BERTOLLO, L. A. C. (1985). Karyotypes of 10 species of Neotropical cichlids (Pisces: Perciformes). *Caryologia, 38 (3 - 4):* 257 - 268.

FELBERG, E., BERTOLLO, L. A. C., TOLEDO, L. F. A., FORESTI, F., FICHO, O. M. and SANTOS, A. F. (1987). Biological aspects of Amazonian fish IX. Cytogenetic studies in two species of the genus *Semaprochilodous* (Pisces, Prochilodon-tidae). *Genome, 29:* 1 – 4.

FITZSIMONS, J. M., LEGRANDE, W. H. and KERTH, J. W. (1988). Karyology of the marine catfish Bagre marinus (Ariidae) with an analysis of chromosome numbers among Siluriforms fishes. *Japanese Journal of Ichthyology, 35(2):* 189 – 193.

FUNAKI, K., MATSAI, S. and SASAKI, M. (1975). Location of nucleolar organizers in animal and plant chromosomes by means of an improved N-banding technique. *Chromosoma, 22(4):* 223 – 236.

GOSLINE, W. A. (1975). The palatine-maxillary mechanisms in catfishes, with comments on the evolution and zoogeography of modern Siluriods. *Occasional Papers, California Academy of Science, 120:* 1 – 31.

HAAF, T. and SCHMID, M. (1984). An early stage of ZW/ZZ sex chromosomes differentiation in *Poecilia sphenops* var *melanistica* (Poeciliidae, Cyprinodontiformes). *Chromosoma, 89:* 37 – 41.

HARTHEY, S. E. (1987). The chromosomes of salmonid fishes. *Biological Reviews, 62:* 197 – 214.

HOWEL, W. M. and BLACK, D. A. (1979). Location of the nucleolus organizer regions on the sex chromosomes of the banded killifish, *Fundulus diaphanus. Copeia, 1979:* 544 – 546.

KALLMAN, K. D. (1973). The sex-determining mechanism of the platyfish *Xyphophorus maculatus.* Pages 19 – 28. *In:* SCHRODER, J. H. (Ed). Genetics and mutagenesis of fish. Springer-Verlag, Berlin.

KLIGERMAN, A. D. and BLOOM, S. E. (1977). Rapid chromosome preparations from solid tissues of fishes. *Journal of Fishery Research Board of Canada, 34:* 266 – 269.

KORNFIELD, I, L., RICHLER, U. R. and WAHRMAN, J. (1979). Biochemical and cytogenetical differentiation among cichlid fishes of the sea of Galilee. *Evolution, 33:* 1 – 14.

LAGLER, F. K., BARDACH, I. E., MILLER, R. R. and MAY PASSINO, D. R. (1977). *Ichthyology.* Second Edition, John Wiley and Sons, New York. 506 pp.

LEGRANDE, W. H. (1981). Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtones, *Nototus*. *Copeia, 1:* 33 – 52.

LEVAN, A., FREGDA, K. and SANDBURG, A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas, 52:* 201 – 220.

LOWE-MCCONNELL, R. H. (1972). *Freshwater Fishes of the Volta and Kainji lakes.* Ghana University Press, Accra.

MAYR, E. (1969). *Principles of systematic zoology.* THM Edition, McGraw-Hill Coy, Ltd. Bombay. 428 pp.

MELLMAN, W. J. (1965). Human peripheral blood leucocyte cultures. Pages 22 – 56. *In:* YUNIS, J. J. (Ed). *Human Chromosomes Methodology.* Academic Press, New York.

OHNO, S., MURAMOTO, J., KLEIN, and ATKIN, N. B. (1969). Diploid tetraploid relationship in clupeoid and salmonoid fish. Pages 139 – 147. *In:* DARLINGTON, C. D. and LEWIS, K. R. (Eds.). *Chromosomes Today,* Volume 2, Oliver and Boyd, Edinburgh.

OJIMA, Y. and YAMANO, T. (1980). The assignment of the nuclear organizer in the chromosomes of the fauna (Carassius,

Cyprinidae, Pisces). *Proceedings of the Japanese Academic of Sciences, 56B:* 551 – 556.

OLIVERIA, C., ALMEIDA TOLEDO, L. F., FOREST BRISKI, H. A. and TOLEDO FILHO, S. A. (1988). Chromosome formulae of Neotropical freshwater fishes. *Review of Brazilian Geneticist, 11(3):* 577 – 624.

OZOUF-COSTAZ, C., TEUGELS, G. G. and LEGENDRE, M. (1990). Karyological analysis of three stains of the African catfish *Clarias gariepinus* Clariidae used in aquaculture. *Aquaculture, 87:* 271 – 277.

PREHN. L. M. and RASCH, E. M. (1969). Cytogenetic studies of *Poecilia* (Pisces). 1. Chromosome numbers of naturally occurring poeciliid species and their hybrids from eastern Mexico. *Canadian Journal of Genetics and Cytology, 11:* 880 – 895.

RIGAS, D. A. and OSGOOD, E. E. (1954). Purification and properties of the phytohemagglutinin of *Phaseolus vulgaris*. *Journal of Biological Chemistry, 212:* 607 – 609.

SOLA, L., CATAUDELLA, S. and CAPANNA, E. (1981). New developments in vertebrate cytotaxonomy III. Karyology of bony fishes: A Review. *Genetica, 54:* 285 – 328.

SYDENHAM, D. H. J. (1983). A key to the genus *Clarias* in Nigeria. *Memograph of the Department of Zoology, University of Ibadan, Nigeria.* 2 pp.

TEUGELS, G. G. (1982). A systematic outline of the African species of the genus *Clarias* (Pisces, Clariidae) with annotated biography. *Annales du musee Royal de L' Afrique Centrale, Tervuren, Sciences Zoologyques.* 230 pp.

TEUGELS, G. G., OZOUF-COSTAZ, C., LEGENDRE, M. and PARENT, M. (1992). A karyological analysis of the artificial hybridization between *Clarias gariepinus* Burchell, 1822 and *Heterobranchus longifilis* Valenciennes. 1840 (Pisces: Clariidae). *Journal of Fish Biology, 40:* 81 – 86.

THORGAARD, G. H. (1977). Heteromorphic sex chromosomes in male rainbow trout. *Science, 196:* 900 – 902.

UWA, H. (1986). Karyotype evolution and geographical distribution in the ricefish, genus Oryzias (Oryziidae). Pages 867 - 876. *In:* UYENO, T., ARAI, R., TANIUCHI T. and MATSUURA, K. (eds.) *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes.* Ichthyological Society of Japan, Tokyo.

UWA, H. and IWATA, A. (1981). Karyotype and cellular DNA content of *Oryzias javanicus* (Oryziidae, Pisces). *Chromosomes Information Service (CIS), 31:* 24 – 26.

UWA, H. and MAGTOON, W. (1986). Description and karyotype of a new rice fish, *Oryzias mekongensis,* from Thailand. *Copeia, 1986(2):* 473 – 478.

UWA, H. and PARENTI, L. R. (1988). Morphometric and meristic variation in ricefish, genus Oryzias: a comparison with cytogenetic data. *Japanese Journal of Ichthyology, 35(2):* 159 – 166.

UWA, H., TANAKA, K. and FORMACION, M. J. (1982). Karyotype and banding analyses of the Hawaiian medaka *Oryzias curvinotus* (Pisces). *Chromosome Information Service (CIS), 33:* 15 – 17.

UWA, H., WANG, R. and CHEN, Y. (1988). Karyotypes and geographical distribution of ricefishes from Yunnan, South Western China. *Japanese Journal of Ichthyology, 35(3):* 332 – 340.

WHITE, M. J. D. (1978). *Modes of speciation.* Freeman and Company, San Francisco. 187 pp.

WOLF, U., RITTER, H., AKIN, N. and OHNO, S. (1969). Polyploidization in the fish family Cyprinidae, order Cypriniformes. I. DNA content and chromosome set in various species of Cyprinidae. *Humangenetik, 7:* 240 – 244.

YU, X., ZHOU, T., LI. K. and ZHOU, M. (1987). On the karyosystematics of cyprinid fishes and a summary of fish chromosomal studies in China. *Genetica, 72:* 225 – 236.

ANIMAL RESEARCH INTERNATIONAL
VOLUME 2, NUMBER 1, MARCH 2005

CONTENTS **PAGES**