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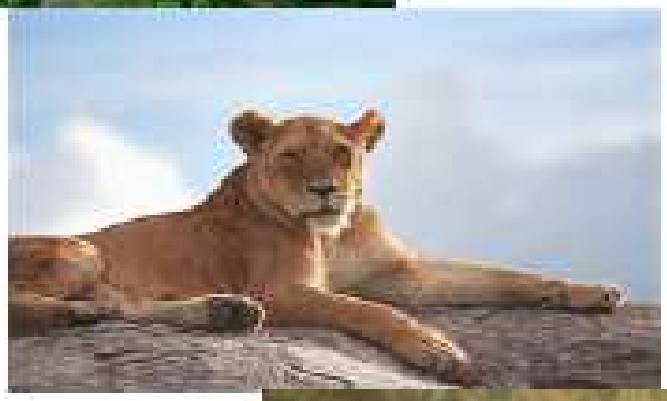

Volume 9 Number 1
April 2012

An International Multidisciplinary Peer Review Journal Publishing Original Research Involving the Use of Animals and Animal Products

ISSN: 5197- 3115 **Website: www.zoo-unn.org**

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ETHNOBOTANICAL STUDIES OF TRADITIONAL LEAFY VEGETABLES AND SPICES OF EBONYI STATE, NIGERIA: POTENTIALS FOR IMPROVED NUTRITION, FOOD SECURITY AND POVERTY REDUCTION

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ABSTRACT

Traditional leafy vegetables represent inexpensive but high quality nutritional sources, for the poor segment of the population especially where malnutrition is wide spread. The objectives of this research were a) to identify and document the traditional leafy vegetables and spices of Ebonyi State, and b) to assess their nutritional values with a view of enhancing their selection as components of cooked food. Market and field surveys were carried out for traditional leafy vegetables and spices in three major clans in Ebonyi North senatorial zone, Ebonyi State: Izzi, Izhia and Ngbo clans. Informal interviews were also conducted with some indigenes on the vegetables and spices identified, including where, when and how they were obtained for sale in the markets. Finally, proximate and Mineral content analysis of three of the traditional leafy vegetables was done to know their nutritional as well as mineral content. Results identified twenty-seven traditional leafy vegetables and five spices from 23 plant families. 46.7 % of the plant collections were seen and collected from the wild, while 40 % were cultivated. 33.3 % of the leafy vegetables were tree species, 30 % were herbaceous plants, and 23 % were climbers, while 13.3 % were shrubs. 60 % of the species were propagated by seed, while 36.7 % were propagated by vegetative means. The parts consumed were mainly the leaves (76.7 %), the stem, flower and the seeds. 40 % of the materials collected were major income earners for the rural populace, 36.7 % earned some income, although small, while 23.3 % had the potential of being transformed into large scale income earner. Results also indicated that three of the vegetables analysed were good sources of micro-nutrients. Their calcium content ranged between 54.06 - 90.10 mg/100 g, while zinc and lead which are antioxidants were absent. The ash content of the three plants ranged from 8.10 - 6.30 %, while protein ranged from 5 – 10 % of fresh weight or 13 - 30% for dry weight. Their fiber (roughage) content was high and will promote digestion and prevent constipation when consumed.

Keywords: Ethnobotanical, Leafy vegetables, Spices, Nutrition, Food security, Poverty reduction

INTRODUCTION

Vegetables are important protective foods, which are highly beneficial for the maintenance of good health and prevention of diseases. They contain valuable food nutrients, which can be successfully utilized to build up and repair the body. They are rich sources of carotene,

ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous (Sheela *et al.,* 2004; Nnamani *et al.,* 2010). In nature there are many underutilized traditional leafy vegetables of promising nutritive values, which can nourish the ever-increasing human population. Traditional leafy vegetables are vegetables of a locality which originated from an area and may or may not be confined to that particular region (Guarino, 1997). They account for about 10% of the world higher plants often regarded as weeds. Some of them grow in the wild and are readily available in the field as they do not require any formal cultivation (Nnamani *et al.,* 2008). Many of them are resilient, adaptive, and tolerate adverse climates (Raghuvanshi and Singh, 2001). Although, they can be raised comparatively at lower management cost even on poor marginal lands, they have remained underutilized due to lack of awareness and popularization of technologies for their proper utilization (Chweya and Eyzaguirre, 2002; Odhav, 2007).

Agriculture is a heritage occupation of the people of Ebonyi State, especially those of the Izzi and Ngbo/Izhia clan. Notwithstanding, stunting growth and deficiency of some micronutrient (vitamin A, iron, iodine and zinc) are prevalent. The World Health Organization (1992) reported that chronic under nutrition affects over 200 million people or 42% of the population in Sub-Sahara Africa. The long-term malnutrition problem of the poor nations cannot be solved by food aid or food trade with the affluent countries but rather by the adequate utilization of indigenous plant foods (Ihekoronye and Ngoddy, 1985). This is because traditional food resources can make substantial contribution in meeting the nutritional needs of the population, especially the low income group and particularly in times of seasonal scarcity (Okeke *et al.,* 2008).

Traditional leafy vegetables represent inexpensive but high quality nutritional sources, for the poor segment of the population especially where malnutrition is wide spread. Even though the bulk of their weight is water, leafy vegetables represent a veritable natural pharmacy of minerals, vitamins and phytochemicals (George, 2003). For example, the potassium content of leafy vegetables is good in the control of diuretic and hypertensive complications, because it lowers arterial blood pressure. The fiber content of vegetables contribute to the feeling of satisfaction and prevents constipation (Noonan and Savage, 1999), while the proteins in vegetables are superior to those found in fruits, although inferior to those found in grains and legumes (George, 2003). Characterization of the species is the first and most important step in understanding the entire food system of indigenous peoples (Okeke *et al.,* 2008). Increasing pressure caused by human activities is continually disrupting the existence, balance and natural regeneration of bioresources, with the result that some of the traditional leafy vegetables are already endangered. This gradual loss of genetic diversity may deprive future generations with useful resources for the enhancement of their health (Ayodele, 1996). Documentation of the wild and cultivated fruits and vegetables in some parts of Nigeria has been made (Okafor, 1975; Dania-Ogbe *et al.,* 2001), but none has been done for Ebonyi State, Nigeria.

The aims of this research were to: (i) identify and document the traditional leafy vegetables and spices of Ebonyi State and (ii) assess their nutritional values with a view of enhancing their selection as components of cooked food, thereby improving the nutritional statuses of both rural and urban dwellers.

MATERIALS AND METHODS

Plant Collection and Identification: Market surveys were carried out for traditional leafy vegetables and spices in two major clans in Ebonyi North senatorial zone, Ebonyi State: Izzi, Ezza and Ngbo/Izhia communities. Major markets in these clans were targeted: Nwakpu, Iboko, Iziogo, Nwaida and Nkwagu in Izzi clan and Eke Izhia, Okwor and Affia opfu (Odeatang Akpaka) in Ngbo/Izhia clan. The traditional leafy vegetables and spices on sale in these markets were recorded. Informal interviews were conducted with some of the marketers as to the variety of vegetables, where, when and how they are obtained for sale in the markets.

At other times, field surveys were carried out in at least three villages each in the three communities under study.

The researcher went into the farms and forests with at least two villagers for observations on the habits and forms of the plants. Identification of plants was done in the fields and markets. Plants that could not be readily identified were carried to the curator in the herbarium at the Department of Botany, University of Nigeria, Nsukka. Vouched specimens were deposited in the herbarium. Authorities for some of the species were sited from (Keay, 1989; Inyang, 2003).

Proximate analysis: Freshly harvested leaves of *Zanthoxylum zanthoyloides* Herms (Hercules club, 'Nka'), *Vitex doniana* Sweet (Black plum, 'Uchakuru') *and Adenia cissamploides* Zepernick, (Planch, 'Isororo') were collected from Izzi area of Ebonyi State and washed, cut and oven dried at 90°C for 6 h. The dried leaves were pulverized, packaged in airtight sterile bottles, labelled and stored in a refrigerator until used. The chemical analysis of percentage crude protein, crude fiber, moisture, ash, fat and carbohydrate were carried out using methods described by Pearson (1976). The crude protein was obtained by determining the organic nitrogen content of the sample using micro-Kjeldah method and multiplying the nitrogen by a protein conversion which is usually 6.25. The ash content of the leaves was estimated by igniting the weighed sample in the weighed crucible at a temperature of 500°C for about 3 h in a muffle furnace, while the moisture content was determined using oven method. The crude fiber and fat determination were done by hydrolyzing the sample with 0.128 ml of H2SO4 and 0.223 ml of KOH and Soxhlet extraction method, respectively. The carbohydrate content was determined by their difference.

Mineral Content Analysis: The mineral contents of the plant leaves, namely, Ca, Mg, Cu, Mn, Pb, P, Zn, were determined using dry ashing procedure as described by Association of Agricultural Chemists (AOAC, 1990). About 2 g of the sample was pre-ashed in a crucible for 1 - 2 h until the sample was completely charred on a hot plate. The pre-ashed sample was then placed on a muffle furnace and ashed at 500°C for about 3 h or until the ash was white.

After ashing the sample was cooled and weighed. This was transferred into a 50 ml volumetric flask by carefully washing the crucible with 5 ml of 30% HCl. The solution was diluted to volume with iodized water. The solution was then used for individual mineral determination using spectrophotometer and flame photometer.

RESULTS AND DISCUSSION

Traditional Leafy Vegetables and spices of Ebonyi State of Nigeria: Twenty-seven traditional leafy vegetables and five spices were identified and documented from the two clans studied. The scientific names, families, local and English names of the varieties, and their source localities were recorded (Table 1). The vegetables and spices belonged to 23 plant families including Pipilionaceae, Cucurbitaceae, Tiliaceae, Moraceae etc.

Leafy vegetables constituted 83.3 % of the plant collected, 10 % were spices, while 6.6 % could be used both as a vegetable or a spice. Out of these plants, 46.7 % are seen and collected from the wild, while 40 % are cultivated (Table 2). Among the cultivated species, 43.3 % are cultivated in compound farms (backyard gardens), 20 % in outlying farms, and 6.6 % still seen in the wild.

Leafy vegetables from tree species constituted 33.3 % of the plant collected, 30 % were herbaceous plants and 23 % were climbers, while 13.3 % were shrubs. 26 % of the plant materials identified are threatened, including *Newbualdia leavis* (P.Beauw) Seemann Bureau (Family Bignoniaceae), *Ipomoea aquatica* Forsk (Family Convolvulaceae), *Lecaniodiscus cupaniodes* (Family Sapindaceae), *Zanthoxylum zanthoxyloids* Lam (Family Rutaceae), *Occimum gratissimum* L. (Family Tiliaceae), *Piper guinensis* Schum and Thonn (Family Piperaceae), *Gongronema latifolia* Benth (Family Asclepiadaceae) and *Monodora myristica* (Family Annonaceae). Sixty (60) % of the plant species are propagated by seed, while 36.7 5 are propagated by vegetative means.

Table 1: Traditional Leafy Vegetables and spices of Ebonyi State of Nigeria

S/N	Scientific Name	Family	Local Name	English Name	Source
1	***Abelmoschus esculentus*(L)Monench**	Malvaceae	Okro, opfhuru	Ladies finger	Izzi, Ngbo/Izhia
2	***Adenia cissampeliodes***	Passifloraceae	Isororo	Planch,	Izzi
3	***Amaranthus spinosus* L.**	Amaranthaceae	Inene	Thorny pigweed, spiny amaranth	Izzi, Ngbo/Izhia
4	***Bombax bounopozense* L. Gaertn.**	Bombacaceae	Apkuto	White silk cotton	Izzi
5	***Capiscum frutescens* L.**	Solanaceae	Ekwuigbapu, akpoko	African pepper	Izzi, Ngbo/Izhia
6	***Citrullus vulgaris* Schrad var Colocynthis Lin O. Ktze**	Cucurbitaceae	Egusi, Ahu	Wild grourd Melon	Izzi, Ngbo/Izhia
7	***Colocasia exculentus* L.**	Araceae	Opoto nkashi	Cocoa yam	Izzi, Ngbo/Izhia
8	***Corchorus olitorius* L.**	Tiliaceae	Arira	Bush Okra	Izzi
9	***Cucurbita pepo* L.**	Cucurbitaceae	Ugboma	Pumpkin	Izzi, Ngbo/Izhia
10	***Ficus capensis* Thunb.**	Moraceae	Ekwuakpuru	Fig	Izzi, Ngbo/Izhia
11	***Ficus ottoniifolia***	Moraceae	Ekwuogbu	Hedge fig	Izzi, Ngbo/Izhia
12	***Gongronema latifolium* Benth**	Asclepiadaceae	Utamashi	Swallow apple, Sodom apple	Izzi
13	***Ipomoea aquatica Forsk***	Convolvulaceae	Ekwuuda	Swamp mor.glory	Izzi
14	***Ipomoea batatas (*L.) Lam.**	Convolvulaceae	Ekwoku	Sweet Potato	Izzi
16	***Lecaniodiscus cupaniodes***	Sapindaceae	Ukpocha	-	Izzi, Ngbo/Izhia
17	***Monodora myristica* Gaertn Dunal**	Annonaceae	Ehuru	Calabash Nutmeg	Izzi, Ngbo/Izhia
18	***Moringa olerifera* Lam**	Moringaceae	Ekwuesisa	Drum stick plant	Izzi, Ngbo/Izhia
19	***Newbualdia leavis* (P.Beauw) Seemann Bureau**	Bignoniaceae	Omirima	Boundary tree	Izzi
20	***Nuaclea diderrichii* (DeWild &Th. Due.) Merrill**	Rubiaceae	Uvuru	Opepe	Izzi
21	***Occimum gratissimum* L.**	Tiliaceae	Ahunji	Tea bush	Izzi, Ngbo/Izhia
22	***Piper guinensis* Schum &Thonn**	Piperaceae	Uzuza	Guinea blackpepp	Izzi, Ngbo/Izhia
24	***Pterocarpus santalinoides L, Herit ex D C***	Papilionaceae	Uturupka		Izzi, Ngbo/Izhia
25	***Pterocarpus soyauxii* Taub.**	Papilionaceae	Oha, Adudu	African padauk	Izzi
26	***Senna occidentalis* Linn**	Caesalpinaceae	Oshigbuomma	Negro Coffee	Ngbo/Izhia
27	***Solanum nigrum* Linn**	Solanaceae	Igbagba, anara	Black nightshade	Izzi, Ngbo/Izhia
28	***Talinum triangulare* Willd**	Portulacaceae	Ngbolodi	Water leaf	Izzi, Ngbo/Izhia
29	***Teliferia occidentalis* Hook**	Cucurbitaceae	Ugu	Fluted pumpkin	Izzi, Ngbo/Izhia
30	***Venonia amygdalina* Del.**	Asteraceae	Olubu	Bitter leaf	Izzi, Ngbo/Izhia
31	***Vitex doniana* Sweet**	Verbenaceae	Uchakuru	Black plum	Izzi, Ngbo/Izhia
32	***Zanthoxylum zanthoxyloids* Lam**	Rutaceae	Nkaa	Ata	Izzi, Ngbo/Izhia

Table 2: Characteristics of traditional leafy vegetables and spices of Ebonyi State Nigeria

S/N	Scientific Name	Local Name	Commodity Grouping	Status of domestication	Land Use Location	Life Form
1	***Abelmoschus esculentus***	Okoro	V	C	CF	S
2	***Adenia cissampeliodes***	Isororo	V	W	W	C
3	***Amaranthus spinosus* L.**	Inene	V	C	CF	H
4	***Bombax bounopozense* L. Gaertn.**	Apkuto	V	W	CF/W	T
5	***Capiscum frutescens* L.**	Ekwuigbapu, akpoko	V/SP	C	CF	H
6	***Citrullus vulgoris***	Egusi	v	C	OF	H
7	***Colocasia exculentus* L.**	Opoto nkashi	V	C	CF	H
8	***Corchorus olitorius* L.**	Arira	V	SW/C	OF	H
9	***Cucurbita pepo* L.**	Ugboma	V	C	OF	C
10	***Ficus sur* Thunb.**	Ekwuakpuru	V	W	W	T
11	***Ficus ottoniifolia***	Ekwuogbu	V	W	W	T
12	***Gongronema latifolia* Benth**	Utamashi	SP	C/W	OF	C
13	***Ipomoea aquatica Forsk***	Ekwuuda	V	W	W	C/H
14	***Ipomoea batatas* (L.) Lam.**	Ekwoku	V	C	CF	C/H
15	***Lecaniodiscus cupaniodes***	Ukpocha	V	W	W	T
16	***Monodora myristica***	Ehuru	SP	SW	W	T
17	***Moringa olerifera* Lam**	Ekwuesisa	V	C/SW	CF	T
18	***Newbualdia leavis* (P.Beauw) Seemann Bureau**	Omirima	V	W	CF/W	T
19	***Nuaclea diderrichii* (DeWild &Th. Due.) Merrill**	Uvuru	V	W	W	T
20	***Occimum gratissimum* L.**	Ahunji	V/Sp	C	CF	H
21	***Piper guinensis Schum &Thonn***	Uzuza	SP	W	CF	C
22	***Pterocarpus santalinoide***	Uturupka	V	W	CF	T
23	***Pterocarpus soyeaxii* Taub.**	Oha	V	W	CF	H
24	***Senna occidentalis***	oshigbuomma	*V*	W	*W*	S
25	***Solanum nigrum***	Igbagba, anara	V	C	CF	H
26	***Talinum triangulare* Willd**	Ngbolodi	V	C	CF	H
27	***Teliferia occidentalis* Hook**	Ugu	V	C	OF	C/H
28	***Vernonia amygdalina* Del.**	Olubu	V	C	CF	S
29	***Vitex doniana* Sweet**	Uchakuru	V	W	OF	T
30	***Zanthoxylum zanthoxyloids* Lam**	Nkaa	V	W	W	S

Table 2 continues

S/N	Scientific Name	Method of propagation	Status of the species	Part Consumed	Frequency (Occurrence)	Cash Income
1	***Abelmoschus esculentus***	SD	NT	L	F	M
2	***Adenia cissampeliodes***	VG	NT	L	O	P
3	***Amaranthus spinosus* L.**	SD	NT	L/ST	O	P
4	***Bombax bounopozense* L. Gaertn.**	SD	NT	L	O	S
5	***Capiscum frutescens* L.**	SD	NT	L	F	M
6	***Citrullus vulgoris***	SD	NT	L	O	P
7	***Colocasia exculentus* L.**	VG	NT	L	F	S
8	***Corchorus olitorius* L.**	SD	NT	L	O	S
9	***Cucurbita pepo* L.**	SD	NT	L	F	S
10	***Ficus sur* Thunb.**	SD	NT	L	O	S
11	***Ficus ottoniifolia***	SD	NT	L	F	S
12	***Gongronema latifolia* Benth**	VG	T	L/ST	F	P
13	***Ipomoea aquatica Forsk***	VG	T	L	O	S
14	***Ipomoea batatas (*L.) Lam.**	VG	NT	L	F	M
15	***Lecaniodiscus cupaniodes***	SD	T	L	O	S
16	***Monodora myristica***	SD	T	SD	F	M
17	***Moringa olerifera* Lam**	SD/VG	NT	L/ST/FL/SD	O	P
18	***Newbualdia leavis* (P.Beauw) Seemann Bureau**	VG	T	L	O	S
19	***Nuaclea diderrichii* (DeWild &Th. Due.) Merrill**	SD	NT	L	O	S
20	***Occimum gratissimum* L.**	SD	T	L	O	M
21	***Piper guinensis Schum &Thonn***	SD	T	SD/L	F	M
22	***Pterocarpus santalinoide***	VG	NT	L	F	P
23	***Pterocarpus soyeaxii* Taub.**	VG	NT	L	F	M
24	***Senna occidentalis***	SD	NT	L	O	S
25	***Solanum nigrum***	SD	NT	L /FT/ SD	F	M
26	***Talinum triangulare* Willd**	SD	NT	L	O	M
27	***Teliferia occidentalis* Hook**	SD	NT	L/SD	F	M
28	***Vernonia amygdalina* Del.**	VG	NT	L	F	M
29	***Vitex doniana* Sweet**	VG	NT	L	O	P
30	***Zanthoxylum zanthoxyloids* Lam**	VG	T	L	O	S

Key:

Land of location	***Commodity grouping***	***Part Consumed***	***Method of Propagation***	***Life form***	***Status of the species***	***Status of domestication***	***Frequency***	***Cash income***
CF = Compound farm	*V = Vegetable*	*L = Leaves*	*SD =Seed*	*T = Trees*	*T= Threat Slight*	*W = Wild*	*A= Abundant*	*M = Major*
OF = outlying farm	*SP = Spice*	*SD = Seed*	*VG=Vegetative*	*SH = Shrub*	*E = Endangered*	*SW = Semi wild*	*F = Frequent*	*S = Small*
W = Wild forest		*ST = Stem*	*NT= No Threat*	*C = Climber*	*2=Substantial*	*C = Cultivate*	*O = Occasional*	*P = Potential*
		Fl= Flower		*H= Herbs*	*3=Greatest*			

Table 3: Uses, income generation prospects and seasonality of the leafy vegetables and spices of Ebonyi State Nigeria

S/N	Scientific Name	Attributes: uses, medicinal values	Gender M, F	Unit of Sale	Unit price (N)*	Seasonality	
						R	D
1	***Abelnoschus esculentas***	Used for making soup	Female			R	D
2	***Adenia cissampeliodes***	Fresh fruit used in making soup	Female	bowl/a small heap	**350/50**	+	
3	***Amaranthus spinosus* L.**						D
4	***Bombax bounopozense* L. Gaertn.**	used for the preparation of yam	Female	Bundle	**20**	R	
5	***Capiscum frutescens* L.**	used for the preparation of yam and water yam	Female	Bundle	10	D	+
6	***Citrullus vulgoris***	Used for spicing food	female	Milk Cup	50	+	+
7	***Colocasia exculentus* L.**	Used as a major soup ingredient; moulded into lumps in special soups (a delicacy) during festivals. Egusi soup is highly valued in Ngbo/Izhia and used during major traditional functions and festivals	Female	Milk cup	**50**	R	
8	***Corchorus olitorius* L.**	Used for making soup					
9	***Cucurbita pepo* L.**	Used for soup and cooking yam pottage				R	
10	***Ficus capensis* Thunb.**	Leaves used as vegetables in soup. Also used to cook yam or cocoyam. It is soft when Cooked and high in B-carotene	Female	Bundle	20	+	
11	***Ficus ottoniifolia***	Used for making soup	Female	Bundle			+
12	***Gongronema latifolia* Benth**	Used for making soup	Female	Bundle			+
13	***Ipomoea aquatica Forsk***	Has a bitter after taste; Used for pregnant, lactating mothers and for sick people.	Female	Bundle	50	+	
14	***Ipomoea batatas (*L.) Lam.**	Also used to spice Goat/beef/chicken/fresh fish pepper soup					
16	***Lecaniodiscus cupaniodes***	Used as spice for making soup for new nursing mothers		Bundle		+	+
17	***Monodora myristica***	Tubers are eaten as staple food		Bundle		+	
18	***Moringa olerifera* Lam**	Used for making soup		Bundle			
19	***Newbualdia leavis* (P.Beauw) Seemann Bureau**	Used in making soup for pregnant and lactating mothers. Mixed with peanut butter and other traditional foods for flavour	Female	1 seed	20		
20	***Nuaclea diderrichii* (DeWild &Th. Due.) Merrill**	Used for making soup and for treating several ailments		Bundle/milk cup		+	+
21	***Occimum gratissimum* L.**	Used for making soup		Bundle		+	+

Table 3 continues

S/N	Scientific Name	Attributes: uses, medicinal values	Gender M, F	Unit of Sale	Unit price (N)*	Seasonality	
22	***Piper guinensis Schum &Thonn***						+
23	***Pterocapus mililbraedii ?***	Used as a spice in the preparation of pepper soup etc and as a medicinal plant	Female	Bundle	20	+	+
24	***Pterocarpus santalinoide***	Used to spice food especially for lactating mothers to clear womb and in pepper soups				+	
25	***Pterocarpus soyeaxii*** **Taub.**	Tender leaves used for preparing soups	Female	Bundle	20	+	+
26	***Senna occidentalis***	Tender leaves used for preparing soups	Female	Bundle	20		+
27	***Solanum nigrum***	Used for soup. Also acts as a laxative for children (Okeke *et al.,* 2008)	Female	1 bucket	200		R
28	***Talinum triangulare*** **Willd**	Leaves used as vegetable in soup. Fruit used for entertaining guest, eaten with spiced peanut butter. Smaller seeds from other species used in preparing yam pottage.	Female	Bundle	50	+	+
29	***Telifairia occidentalis*** **Hook**	Used for soup preparation.	Female	Bundle	20	+	
30	***Vernonia amygdalina*** **Del.**	Used for soup preparation. Washed extract is used in treating anaemia and	Female	Bundle	20	+	
31	***Vitex doniana*** **Sweet**	in building up blood for pregnant mothers					
32	***Zanthoxylum zanthoxyloids*** **Lam**	Used in cooking soups and other dishes. Some varieties can be chewed raw after washing. Used for the treatment of malaria and recommended for diabetics				+	+

* *Threshold prices for a bundle or cup of the item*

The parts consumed were mainly the leaves (76.7 %). Others include the stem, flower and the seeds. Majority of the plants collections have potential for generating income for the smallholder farmers, especially women

Uses, Income Generation Prospects and Seasonality of the Leafy Vegetables and Spices of Ebonyi State Nigeria: Table 3 indicates the uses of the traditional leafy vegetables/spices, including their medicinal values. In all the cases, it is women that either cultivates or collect the vegetables from the wild as an income generating commodity or to supplement Family's meal. At every season of the year, there are always some vegetables/spices for sale. However, most of them are abundant during the rainy season.

Proximate Composition of Three Traditional Leafy Vegetables: The result of the proximate analysis of three traditional leafy vegetables are presented in Figure 1 as culled from Nnamani *et al.* (2010). Their moisture contents were 9.6, 10.2 and 10.8 % in *Z. zanthoxyloides*, *V. doinana* and *A. cissampeloides*, respectively. This was low, but was attributed to the fact that the leaves were oven dried before analysis. Higher percentage moisture content is expected in freshly harvested leaves.

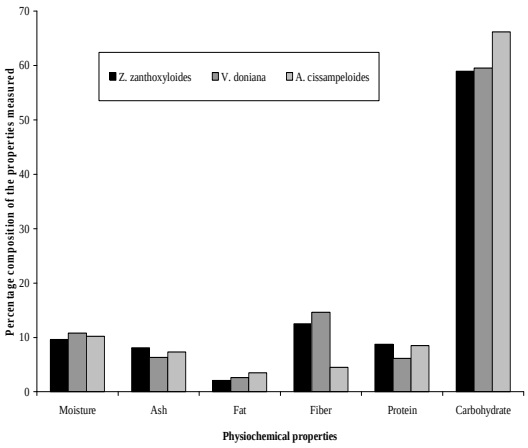

Figure 1: Proximate composition of three indigenous leafy vegetables

The ash content of the three plants, a measure of the mineral content of food ranged from 8.10 - 6.30% (Figure 1) with *Z. zanthoxyloides* having the highest and *V. doinana* having the least value. These results differed with the results of Ajayi *et al.* (2006) who reported an ash content of some leafy vegetables that ranged from 0.6 – 34%. Crude protein had values ranging from 8.74 - 5.12% (Figure 1) with *V. doinana* having the lowest and *Z. zanthoxyloides* the highest value. The amount of protein which is about 75 % (when converted) of the total nitrogen in the leafy vegetables was variable for the three plants, ranging from 5 – 10 % of fresh weight or 13 - 30% for dry weight. These percentages were higher than the 3 – 8% and 11 – 28% result reported by Oyenuga and Fetuga (1975), but lower than values reported for *Telfairia occidentalis* leaves (22.4%), *Tamarindus indica* (24.3%), *Hibiscus esculentus* (23%) and *Parkia biglobosa* (20.9%) reported for dry milled samples (Glew *et al.,* 1997; Akwawowo *et al.,* 2000; Igbal *et al.,* 2006). So consumption of 100 g of *V. doinana, Z. zanthoxyloides* and *A. cissompeloides* may not be capable of providing 27 g of protein which satisfies the recommended daily allowance of protein for children (FAO, 1986). The crude fat content of *A. cissompeloides*, *V. doinana* and *Z. zanthoxyloides* ranged from 3.50 to 2.10% (Figure 1) may not compare favourably with dry milled percentage values reported for *Brachystegia eurycoma* (5.78%) and *T. indica* 4.2% (Ajayi *et al.,* 2006). However, it is higher than the dry milled percentage values for other vegetables like *Celosia argentea* (0.7%), fluted pumpkin (1.8%), *Gnetum africanum* (1.2%) (Okafor, 1995). A child consuming 100 g of *V. doinana*, *A. cissompeloides*, *Z. zanthoxyloides* would be ingesting approximately 2.60, 3.5 and 2.10 % of fatty acid which translates to 22.2, 30.4 and 21.3 kcal of energy, and is approximately a high amount.

The fibre content of these leafy vegetables (Figure 1) ranged from 12.50 - 4.50%. These exceeded the fiber content of *T. triangulare* (2.0%) *T. occidentalis* (1.7%) and *C. argentea* (1.8%) (Akachukwu and Fawusi, 1995). This indicates that the fiber (roughage) content of these plants are high and will promote digestion and prevent constipation when consumed. The carbohydrate level of the

underutilized indigenous vegetable (Figure 1) ranged from 58.94 % in *Z. zanthoxyloides* to 66.20 % in *A. cissampeloides*. These values are high compared to the carbohydrate level of 8.0 g in *T. occidentalis* (FAO, 1986). This indicates that the indigenous vegetables can act as better food supplement in providing carbohydrate.

Mineral Content: The three vegetables studied are good sources of micro-nutrients. Results indicated that calcium content ranged between 54.06 - 90.100 mg/100 g (Figure 2). The highest value was obtained from *Z. zanthoxyloides,* followed by *V. doinana* and *A. cissompeloides*. Including these calcium rich vegetables in daily diet would ensure 20 – 25% of the daily requirement for calcium that aid strong bones and healthy teeth (Raghuvanshi and Singh, 2001). The result of the mineral analysis (Figure 2) also showed complete absence of zinc and lead which are antioxidants. This is of significant interest because it potentially indicates that the plants are endowed with essential nutrients good for human consumption.

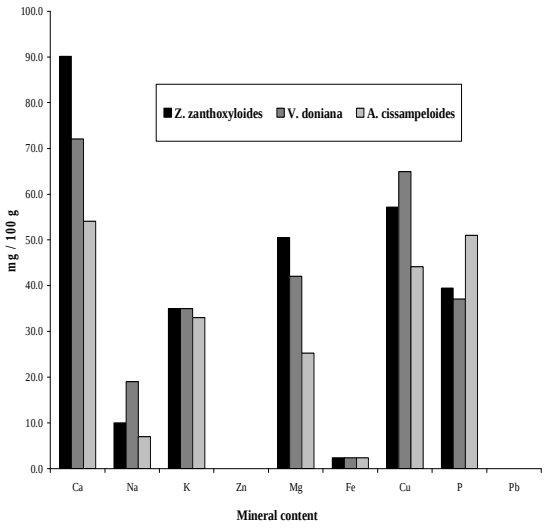

Figure 2: Physiochemical compositions of three indigenous leafy vegetables

Conclusion: Several traditional leafy vegetables and spices of Ebonyi State have been identified with potentials for income generation for the smallholders especially women. The plants were endowed with essential nutrients required for maintenance of good health. The presence of various phytochemicals in these plants has helped in meeting the nutritional needs of the rural farm families, thereby assisting in primary Health Care Delivery, since most of them cannot assess the general health care services beef up

The projection of Sub Saharan Africa for the next two decades, particularly as regards life expectancy and food security is rather bleak and challenging. Practical intervention in health and nutrition are needed. Identifying some of these underutilized traditional leafy vegetables and their inculcation into our diet could potentially address some of these challenges. The income generating potentials of the identified traditional vegetables is essential in alleviating the poverty level of rural farm families especially women on whose shoulders the responsibilities of caring for the Family rests.

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AGE AND GROWTH OF DOMINANT CICHLIDS IN GBEDIKERE LAKE, KOGI STATE, NIGERIA

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ABSTRACT

Age, growth and mortality of two dominant Cichlids collected from an Ox bow lake on the tributary of River Benue were studied between October 2006 and September 2008. Sixty samples of the fish species comprising thirty Tilapia zilli and thirty Oreochromis niloticus were obtained from the Artisanal fishers from the common landing site along the lake. Age was determined from Bhattacharya's length frequency assortment method using where applicable the scale of fish and opercula bones. Growth was found to be allometric among the species studied conforming to the growth factor $W = aL^b$. Four age groups were observed while averaged instantaneous total mortality for the species was 0.48/year and an exploitation rate of 0.41 while the longevity was 8 years.

Keywords: Age, Cichlids Species, Instantaneous Growth, Exploitation, Gbedikere Lake, Nigeria

INTRODUCTION

Age and growth studies are very necessary to fishery science (Adeyemi *et al.,* 2009). Age, length and weight data are very important tools to fishery biologists since details of species growth, mortality rates, age at maturity and life span can be determined from such information (Ricker, 1975; Gulland, 1983).

Although numerous methods have been used to age fishes (Nielson and Johnson, 1983), three general methods predominate. The first is the mark and recapture method. The second is Peterson method which involves the comparisons of length frequency distribution of fish population samples (Ricker, 1975). This method requires measuring the lengths of a large number of fish in a population. The third method is to count growth marks that develop periodically in various hard parts of fishes. This is the most commonly used method. Several kinds of hard parts in fishes can be useful in determining age (Nielson and Johnson, 1983). Otoliths and scales are the hard parts most often used but in elasmobranchs and some other bony fishes, rings or bands in the vertebrae have been studied as well. Also bony fish opercula, fin rays and other calcified structures may show annual marks (Six and Horton, 1977). This work seeks to identify growth and mortality rate of *Oreochromis niloticus* and *Tilapia zilli* the two most dominant cichlids in Gbedikere Lake, Kogi State, Nigeria.

MATERIALS AND METHODS

Study Area: The study area was Gbedikere Lake; a natural lake located between Latitudes $7^0 25^N$ and Longitudes $7^0 30^E$ and is about 10km to the East of Oguma the Headquarter of Bassa Local Government Area of Kogi State. Water enters the Lake from tributaries that run from River Benue during rainy or flood season. When the season is over, the Lake separates out. The Lake is about 450m north of Gbedikere village. The water body covers an area of about 400m – 450m with a mean depth of 10 – 14m (UBRDA, 1985) depending on the season and is used for

domestic purposes and fishing; consequently most of the settlers around the Lake are fishermen (UBRDA, 1985).

Sampling: A total of 60 samples of fish were collected from the fishermen using gill nets, cast nets, hook and line and Malian traps between October 2006 and September 2008 and identified. The total length (TL) of the fish was measured from the tip of the anterior or part of the month to the caudal fin using meter rule calibrated in centimeters. Fish were measured to the nearest centimeter. Fish weight was measured after blot drying with a piece of clean hand towel. Weighing was done with a tabletop weighing balance, to the nearest gram. The length measurements were converted into length frequencies with constant class intervals of 2 cm. The mean lengths and weights of the classes were used for data analysis, the format accepted by FISAT (Gayanilo and Pauly, 1997). Age estimation were carried out, using where applicable, scale of fish and opercula bones as described in Hynes (1950), Bagenal (1978) in conjunction with von Bertallanffy growth model available in LFSA/FISAT computer programmes.

Age: Age estimation was also carried out, using where applicable, scales of fish and opercula bones (Hynes, 1950; Bagenal, 1978; Sparre and Venema, 1992) in conjunction with von Bertallanffy growth model (von Bertallanffy, 1938).

Growth: Estimation of growth parameters was done for length frequency, length-weight relationships and length at age using the Bhattacharya's, von Bertallanffy's, Powell-Wetherall and Ford-Walford methods.

Analysis: Length and weight data collected were analysis using the length frequency based fish stock assessment computer programme (Gayanilo and Pauly, 1997). Fish growth was described by the equation: $Lt = L\infty\ (1\text{-}e^{-k\ (t\text{-}to)})$ (von Bertallanffy, 1938), where: Lt = predicted length at time t, L∞ = asymptotic length or maximum attainable length, e = base of the natural log t, T = time, t_o = the size at which organism would theoretically have being age 0 and K = instantaneous growth rate or growth coefficient. Values of length at infinity (L∞ parameter of the von Bertallanffy growth formula (VBGF) expressing asymptotic length i.e. the mean length the fish in a population would reach if they were to grow indefinitely), K (growth curvature factor), t_o (size at which organism would theoretically have been at age 0) and Ø'= Phi – Prime (i.e. length based index of growth performance ($Ø' = \text{Log}_{10}\ K + 2\ \text{Log}_{10}\ L\infty$) were estimated from the von Bertallanffy growth equation thus:

$L(t) = L\infty \times \{1 - \exp(-k \times (t - t_o)\}$ ..... (1)

A series of algebraic manipulations gave:

$L(t + \Delta t) = a + b \times L(t)$ ..... (2)

(Sparre and Venema, 1992).

$a = L\infty \times (1 - b)$ and $b = \exp(-k \times \Delta t)$ ..... (3)

Since k and L∞ are constants, a and b also become constants if Δt is a constant. The growth parameters k and L∞ were then derived from $k = 1/\Delta t \times \text{Lnb}$ and $\Delta t = a/1\text{-}b$. a and b were obtained from carrying out the regression analysis of the Ford-Walford plot (Figure 1 and 2). The Ford-Walford plot was used to estimate L∞ graphically from the intersection sample point of the 45^0 diagonal where L (t) = L (t+Δt).

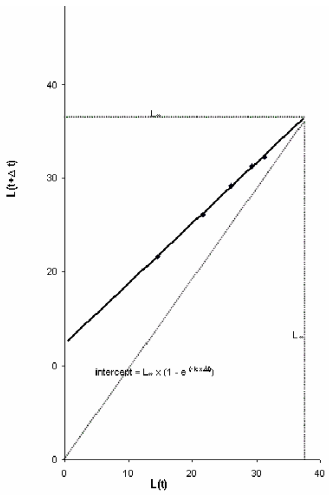

Fig 1: Ford-Walford plot for estimating L∞ for *Tilipia zilli* at Gbedikere Lake, Kogi State.

RESULTS

An examination of the relationship that exists between the lengths and weights of the cichlid species was undertaken using the ICLARM length-weight programme (Table 1). For growth parameters, the asymptotic length, L∞ was 34.52cm in *T. zilli* and 43.82 in *O. niloticus,* respectively.

The instantaneous growth rate and growth coefficient or growth curvature factor (K) were 0.44 Yr^{-1} and 0.32, respectively.

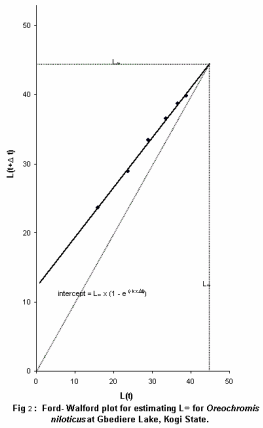

Fig 2: Ford- Walford plot for estimating L∞ for *Oreochromis niloticus* at Gbediere Lake, Kogi State.

Table 1: Length-weight relationships of *T. zilli* and *O. niloticus* in Gbedikere Lake, Kogi State, Nigeria

Species	a	b	r	n	p
Tilapia zilli	0.00452	3.496	0.9548	30	< 5%
Oreochromis niloticus	0.01768	3.047	0.9361	30	< 5%

The highest age at t_o were -0.222 and -0.350 for *T. zilli* and *O. niloticus,* respectively. Furthermore, the growth performance indices (Ø') were 2.72 and 2.79 for *Tilapia zilli* and *Oreochromis niloticus,* respectively (Table 2).

Table 2: Growth parameters of *Tilapia zilli* and *Oreochromis niloticus* in Gbedikere Lake, Kogi State, Nigeria

Species	K (yr^{-1})	t_o	L∞	Ø'
Tilapia zilli	0.44	-0.222	34.52	2.72
Oreochromis niloticus	0.32	-0.350	43.82	2.79

The species (*T. zilli* and *O. niloticus*) caught fall within 1+ and 2+ age brackets (Table 3).

DISCUSSION

Growth in Gbedikere Lake cichlid fish species could be related to the availability of food as reported by Adeyemi *et al.* (2009).

Table 3: Length at age of *Tilapia zilli* and *Oreochromis niloticus* in Gbedikere Lake, Kogi State, Nigeria

Species	Total Length at Age (cm)				
	Age	L1	L2	L3	L4
Tilapia zilli	1+	12.4			
	2+		14.0		
	3+			17.1	
	4+				
Mean					**14.5**
Oreochromis niloticus	1+	11.1			
	2+		12.7		
	3+			15.8	
	4+				19.7
Mean					**14.8**

The rate at which they grow could be related to the rate at which they are harvested. Rikhter and Efanov (1976) demonstrated that fish with a high natural mortality mature early in life, compensating for the high mortality by starting to reproduce early. This also is supported by the small sizes at which the species reach maturity. The length-weight relationships obtained for the species compared favourably with those obtained elsewhere. Bongoyinge (1984) obtained Log W = -4.4656 + 3.21 Log L, recorded for *Tilapia mariae* in Port Harcourt. Log W = -1.5273 + 3.1014 Log L (r = 0.9923) was obtained by Bankole (1989) in Tiga Lake. Furthermore, Olatunde (1983) obtained Log W = -4.1352 + 2.880 Log L (r = 0.974) in Zaria, while Kings (1996) obtained Log W = 5.2591 + 3.25 Log L (r = 0.949) for *Clarias gariepinus* and Adeyemi *et al.* (2009) obtained b value of 3.496 for *Tilapia zilli* in Gbedikere lake respectively. The attempt at ageing the fish based on scale and length frequencies was a difficult one because identification of the year classes was difficult. Programmes in the FiSAT were very helpful especially, in the estimation of the length at infinity (L∞), K and t_0.

Although drastic changes that lead to ring formation in the hard parts of temperate fish do not occur that sharply in the tropics (Sparre and Venema, 1992), it was still possible to deduce some reasonable conclusions with the aid of the hard parts used, such as the opercula bones and scales. The bulk of the fish species (*O. niloticus and T. zilli*) caught from the Gbedikere Lake fall within 1+ and 2+ age brackets. The implication for the fishery is that many of the fish would enter the fishery at an early age and this could lead to the present experience of the fish reproducing early (Adeyemi, 2011). However, Landau (1979) found similar length at age in *Tilapia galilaea* of Lake Kinneret where the length at age distribution ranged from 1+ to 4+ in the fish caught over four seasons.

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DIET AND DIETARY HABITS OF *Labeo senegalensis* IN A TROPICAL FRESHWATER ECOSYSTEM

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ABSTRACT

The food composition and dietary habit of Labeo senegalensis at Idah area of river Niger was carried out between January and February, 2010. 155 samples were obtained from artisanal fishermen and brought to biological sciences laboratory for analysis. Fish samples were dissected and the stomach contents emptied into Petri-dishes containing 10% saline solution and observed under a dissecting microscope. The food items were counted and the stomachs were scored according to its fullness. The result shows that the fish feed mainly on mud (4.31%), sand particles (6.00%), plant materials (80.65%), fish remains (5.81%), insect parts (2.58%) and small gravel (0.65%) respectively. L. senegalensis in Idah area of River Niger is a herbivorous detrivore feeding more on plant materials.

Keywords: Food items, Feeding habits, Stomach contents, Herbivorous detrivore

INTRODUCTION

Life processes includes reproduction, growth and development are done at the expense of food eaten by living organisms including fish. In various studies aimed at understanding the feeding regimes, food preference, migrations, growth and breeding patterns of fish, diet has been found to be an important factor, especially in governing their growth, condition factor, fecundity and migration patterns (Rao, 1974; Adeyemi *et al.,* 2009a).

Feeding habits of fish provide essential information of bionomic studies of single species; the analysis of stomach contents in fish is a common method for investigating the diet of fish, and thus describing food chains and webs shared by different species. Such studies also reveal interactions among species (Kenneth *et al.,* 2004). Accurate quantification of fish diets is an important aspect of fisheries management (Quinton *et al.,* 2007).

Studies on the aspect of biology of fishes such as growth pattern, reproduction and nutrition are necessary as they would furnish relevant information for the formulation of fisheries management policies (Adedolapo *et al.,* 2008). Recent studies carried out on the feeding habits of fish species in Nigerian aquatic ecosystem include that of Adedolapo *et al.* (2008) and Adeyemi *et al.* (2009b).

Labeo is the largest genus of carp fishes (Order: Cypriniformes) of the family Cyprinidae. The families Cyprinidae are fusiform fishes which are slightly compressed. They have an inner surface of lips with transverse folds; snout with discrete tubercles short barbell at the corners of mouth; they

have naked head with body covered by cycloid scales (Balogun, 2006).

MATERIALS AND METHODS

Study Area: The tropical freshwater ecosystem studied was Idah area of River Niger in Kogi State, Nigeria (Figure 1). The area is located on latitude 7 06'N and longitude 6 43'E of the Greenwich meridian in the Guinea Savannah vegetation zone of Nigeria. The study area experienced two weather conditions, dry season which starts from November to April and wet season which starts from April to October (Adeyemi *et al.,* 2009a).

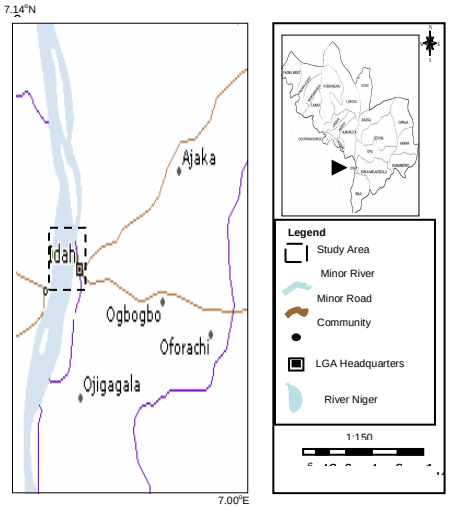

Figure 1: The map of Idah showing the study area

Sampling: A total of one hundred and fifty five (155) samples of *Labeo senegalensis* were purchased from fishermen at Idah area of River Niger between January and February, 2010. The fish sampled were transported in plastic buckets to the Biological Sciences Laboratory, Kogi State University, Anyigba for analysis those that could not be treated were preserved in a freezer until the next day. They were sorted and identified up to species level using the guides of Teugels *et al.* (1992), Olaosebikan and Raji (1998), Idodo-Umeh (2003) and Paugy *et al.* (2004).

Stomach Content Analysis: Each stomach was dissected and split open and the contents emptied into Petri-dishes containing 10% saline solution and observed under a dissecting microscope.

The food items were counted and the stomachs were scored 0, 25, 50, 70 and 100% according to its fullness as described by Olatunde (1978).

Analysis: The stomach contents were analyzed by frequency of occurrence as described by Hynes (1950) and Bagenal (1978). In the frequency of occurrence method each food item was identified and number of stomach in which each food occurred was counted and expressed as a percentage of stomach containing food. The method showed the proportion of individuals eating a particular food item in a species. The occurrence of each food item was expressed as a percentage of all stomach with food. That is, P = (b/a) x 100 where a = Total number of fish examined with food in the stomach; b = number of fish containing a particular food; P = percentage of occurrence of each food item.

RESULTS

The six food items obtained from the stomach of the fish were; fish remains (5.81%), mud (4.31%), sand particles (6.00%), plant materials (80.65%), insect parts (2.58%) and small gravel (0.65%) (Table 1).

The stomach fullness classification of *Labeo senegalensis* based on the degree of fullness indicated that eighteen males (11.61%) had food in their stomach while 2 (1.29%) had no food in their stomach. There was food in 127 (81.94%) stomachs of female while 8 (5.16%) had no food. The males had 1(0.65%) full stomach, 8(5.16%) almost full, 6 (3.86%) half full, 3 (1.93%) almost empty and 2 (1.30%) empty. The female had 11 (7.10%) full stomach, 50 (32.30%) almost full, 43 (21.94%) half full, 32 (20.65%) almost empty and 8 (5.16%) were empty. There was no significant difference ($p>0.05$) in the degree of stomach fullness of the fish. Overall, 145 (93.55%) of the sample had food in their stomach while 10 (6.45%) were

empty stomach. This indicated a high feeding intensity (Table 2).

Table 1: Percentage frequency of food items in the stomach of *Labeo senegalensis* from Idah area of River Niger

Items	Frequency of Occurrence (%)
Fish remains (bones, flesh, particles)	5.81
Mud	4.31
Sand particles	6.00
Plant materials	80.65
Insect parts	2.58
Small gravel	0.65

Table 2: Stomach contents classification of *Labeo senegalensis* based on degree of fullness at Idah area of River Niger

Sex	Male n = 20	Female n = 135	Combined n = 155
% Stomach with food	18 (11.61)	127 (81.94)	145 (93.55)
% Stomach without food	2 (1.29)	8 (5.16)	10 (6.45)
% Degree of fullness			
Full (4/4)	1 (0.65)	11 (7.10)	12 (7.75)
Almost full (3/4)	8 (5.16)	50 (32.30)	58 (37.46)
Half full (1/2)	6 (3.86)	43 (21.94)	49 (25.80)
Almost empty (1/4)	3 (1.93)	32 (20.65)	35 (22.58)
Empty (0/4)	2 (1.30)	8 (5.16)	10 (6.46)

DISCUSSION

Qualitative and quantitative composition of fish diets is important to growth, maturity and fecundity changes in fish amongst other factors. Study of dietary habits of fish, based on stomach content analysis, is widely used in fish ecology as an important means of investigating tropic relationship in the aquatic communities (Fagbenro *et al*., 2000). This study showed that the items found in the diet of this species include fish remains (bones, flesh, particles), mud, sand particles, plant parts and insect parts. Ayotunde *et al*. (2007) reported whole worm, worm part, nematode, mud, plant part, unidentified items, and detritus in the gut of *Labeo coubie*, another member of the family Cyprinidae. Other food items present in small quantities includes Rotifera (*Kerattela* sp., *Polyarthera* sp. and *Philodina* sp.) and Crustacean (*Copepod* sp., *Decapods* sp. and *Daphnia* sp.). The presence of plant tissues (68.7% occurrence) according to their findings showed that *Labeo coubie* is able to digested plant matter, making it also herbivorous. This is in line with the findings of this study where 80.65% of items were plant materials.

The worms were the single most prominent food group. It is likely that being a detrivorous or herbivore it could also be a benthic feeder. *Labeo senegalensis* forages the river bottom. In the process it catches worm and this may account for the prominence of worm and worm parts in its food.

Conclusion: The findings of this study showed that 80.65% of items in the diet of *Labeo senegalensis* were plant materials with other materials forming 19.35% of the components. *Labeo senegalensis* in Idah area of River Niger of Kogi State could therefore termed a herbivorous detritivore feeding more on plant materials.

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INVESTIGATION OF TRACE METALS IN THE TISSUES OF A FRESHWATER FISH (*Crysichthys nigrodigitatus*) FROM THE IKPOBA RIVER DAM, BENIN CITY NIGERIA

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ABSTRACT

The concentrations of zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb) and chromium (Cr) were evaluated in whole fish, muscle, liver and gills of the benthic fresh water fish, Crysichthys nigrodigitatus, from Ikpoba River Dam in Benin City, Nigeria, by atomic absorption spectrometric technique. The concentrations of the aforementioned trace metals were also determined in water and sediment. The concentration profile of Zn in fish tissue in descending order was liver > muscle > whole fish > gills while for Cd the profile in descending order was liver > whole fish > gills > muscle. The concentration profile in descending order for Cr, Pb and Cu was liver > whole fish > muscle > gills, liver > gills >whole fish > muscle and muscle > whole fish > liver > gills respectively. The concentration profile of the metals in descending order in water was Zn > Pb > Cd > Cr > Cu while in sediment the concentration profile was Zn > Cu > Pb > Cd > Cr. The mean concentration of Cr in the examined fish tissues exceeded the WHO maximum allowable limit for food and fish while the mean concentration of Pb in water exceeded the WHO maximum allowable limit for drinking water. It was advocated that regulatory agencies should further monitor the Dam in other to produce sufficient data to implement better management strategies which would alleviate negative impacts.

Keywords: Trace Metals, Zinc, Cadmium, Copper, Lead, Chromium, *Crysichthys nigrodigitatus,* Ikpoba Dam, Benin City, Nigeria

INTRODUCTION

The Committee for Inland Fisheries of Africa (CIFA, 1989) reported that freshwater fishes are vital to Africa's proteinous food security and should be protected from damage by wastes arising from the ever increasing urbanization, industrialization, and agricultural and forestry activities. These activities already resulted in increased contamination and in some cases pollution of surface waters. It is well documented that trace metals and organic compounds can be accumulated by aquatic biota (GESAMP, 1993; Calamari and Naeve, 1994).

Furthermore, the toxic effect of hazardous waste generated by industrial and domestic activities on aquatic animals has been documented (Corrales and Horton, 1995). The pollution of the aquatic environment with trace metals has become a global problem during recent years owing to the fact that they are indestructible and majority of them have toxic effects on organisms (Macfarlane and Burchett, 2000). The concentration of a metal in an organism has been described as the product of equilibrium between the concentration of the metal in the organism's environment and its rate of ingestion and excretion (Idodo-Umeh, 2002).

Previous studies have revealed that various aquatic bodies and their resources in Nigeria contain varying levels of trace metals (Ezemonye, 1992; Fufeyin, 1994; Wangboje and Oronsaye, 2001; Ogri *et al.,* 2003). Trace metals may be introduced into the aquatic environment as a result of natural processes or through anthropogenic processes (Calamari and Naeve 1994). There are five potential routes for a pollutant to enter fish and these include food, non-food particles, gills, oral consumption of water and the skin (Heath, 1991). According to Villareal-Trevino *et al.* (1986), trace metals such as copper, zinc, lead and chromium are present in domestic drainage pipes. Furthermore lead and cadmium have not been shown to play any beneficial roles, but rather they inhibit biological systems in fish. Lead produces changes in the pigmentation patterns of certain fish species and modifies the physiochemical changes of the cellular pigment by direct interference with the germinal plasma. The Ikpoba River Dam has been reported to receive effluents containing a wide range of pollutants including trace metals (Fufeyin, 1994). This study has been undertaken specifically to determine the concentrations of zinc, cadmium, chromium, lead and copper in whole fish, muscle, liver and gills of a benthic fresh water fish, *Crysichthys nigrodigitatus* from the Ikpoba River Dam, which is widely consumed by inhabitants of the city.

MATERIALS AND METHODS

Study Area: The Ikpoba River flows through Benin City, Edo State, Nigeria and lies within Latitude 6.5°N and Longitude 5.8°E. The river is dendrite in the upper reaches and its head waters originate from the Ishan Plateau. The river was impounded in 1977, forming the Ikpoba Dam. The dam is situated some 3.75km South-East of the University of Benin. The dam at full capacity is 3.25km long and 0.6km wide with a mean crest level of 36.8 metres. The storage capacity of the dam is l.5 million m^3. Some activities in the area include fishing, farming, cattle grazing, fetching of water and car washing. There is a sand excavation site within the catchment area.

The study area is surrounded by arable farm land. Samples of water, fish and sediments were collected from the Okhoro site and the low lift pump station site of the dam in January, March, July and September 2007. The study area is about 1.2km in length within the dam.

Sampling and Analysis

Water: Duplicate water samples were collected at random in one litre plastic bottles with screw caps at 30cm depth. The water samples were fixed with 5ml of concentrated nitric acid and transported to the laboratory within 24 hours in an ice chest. In the laboratory, the samples were stored at -5°C in a Sonoko deep freezer (Ademoroti, 1996).

Bioindicators: The fish samples were captured using baited hooks and set gill nets while operating from a dug-out canoe. The fish were washed in flowing water to remove adhering debris and transported to the laboratory within 24 hours in an ice chest. In the laboratory, the samples were stored at -5°C prior to further analysis.

Sediment: Duplicate sediment samples were collected using an Eckman grab apparatus. Samples were placed in black polythene bags that had previously been soaked overnight in 5% nitric acid and rinsed with distilled water. Samples were thereafter transported to the laboratory within 24 hours in an ice chest. In the laboratory, the samples were stored at -5°C.

Analyses: On the day of analyses, all frozen samples were allowed to thaw at room temperature (28°C). The water samples were vigorously shaken and aspirated into the flames of a Varian Techtron Spectra B atomic absorption spectrophotometer for trace metal determination (APHA, 1989). The fish samples after defrosting were identified (Idodo-Umeh, 2002), dissected to separate the muscle, liver and gills. All sampled sections of the fish was oven dried to constant weight at 105 ± 2°C and then ground to powder. One gram of ground fish each section sampled was digested using a 1:5:1 ratio mixture of 70% perchloric acid,

concentrated nitric acid and concentrated tetraoxosulphate (VI) acid at 80 ± 5°C until colourless solutions were obtained (Streedevi *et al.,* 1992). The final volume was made up to 20ml with deionised water. The sediment samples were oven dried to constant weight at 105 ± 2°C, ground to powder and sieved through a 200 mm grid mesh to remove ungrounded materials. One gram of each ground sample was digested using a 1:5:1 ratio mixture of 70% perchloric acid, concentrated nitric acid and concentrated tetraoxosulphate (VI) acid (Streedevi *et al.,* 1992). Blank solutions were handled as detailed for the samples. Digested fish and sediment samples were analysed read using a Varian Techtron Spectra B atomic absorption spectrophotometer. The concentration of trace metals in water were expressed in mg/l while the trace metal concentrations in fish and sediment were expressed in mg/kg. Statistically, all data were presented as means of triplicate determinations.

RESULTS

The concentration of Zn in water ranged from 0.07 - 0.96 mg/I while the concentration of Pb in water ranged from 0.05 - 0.08 mg/l. The concentration of Cd in sediments ranged from 0.46 - 0.75 mg/kg while the concentration of Cu in sediments ranged from 8.14 - 9.25 mg/kg. The trace metal profile in water in descending order was Zn > Pb > Cd > Cr > Cu, while the trace metal profile in sediments was Zn > Cu > Pb > Cd > Cr (Table 1). The highest concentration of Zn recorded in whole fish was 14.95 mg/kg while to lowest concentration of zinc was recorded in the gills with a value of 10.52 mg/kg. Generally, the concentration of Zn in descending order was liver > muscle > whole fish> gills. The highest concentration of Cd was recorded in the liver with a value of 25.42 mg/kg, while the lowest concentration of the metal was recorded in the muscle with a value of 0.14 mg/kg. The concentration profile of Cd in descending order was liver > whole fish > gills > muscle (Table 2). The highest concentration of Cr recorded in the gills was 2.66mg/kg while the lowest concentration recorded in the liver was 10.13 mg/g. The Cr profile in descending order was liver> whole fish > muscle > gills (Table 2). The highest concentration of Pb recorded in the liver 9.86mg/kg, while the lowest concentration recorded in whole fish was 3.76 mg/kg. The Pb profile in descending order was liver > gills > whole fish > muscle (Table 2).

The highest concentration of Cu recorded in the whole fish was 12.98 mg/kg while the lowest concentration recorded in the muscle was 14.45 mg/kg. The Cu profile in descending order was muscle > whole fish > liver > gills (Table 2). The distribution co-efficient (DC) values for trace metals in the Ikpoba River Dam expressed the solubility of trace metals in aquatic systems. The highest DC value of 0.069 was computed for Cd, closely followed by a value of 0.068 recorded for Cr. The Lowest DC value of 0.003 was computed for Cu (Table 3). Comparison of trace metal concentrations in water with some other studies indicated that the dam water had save levels of Zn, Pb, Cd, Cr and Cu (Table 4). The comparison of trace metal concentrations in the fish with some other fishes from earlier studies indicated that the trace metal concentrations in *Crysichthys nigrodigitatus* from the Ikpoba River Dam were higher than recommended limits (Table 5). Similarly, trace metals concentration in the sediment from the Ikpoba River Dam were higher than recommended limits (Table 6).

DISCUSSION

Aquatic biota including fish absorb and accumulate trace metals from water and the levels of metals accumulated vary from organ to organ (Gerhadt, 1992), Fish can regulate metal concentrations to a certain extent, where after bioaccumulation will occur (Heath, 1991). According to Kotze (1997), the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue, furthermore physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal. In this study, Zn, Cd, Cr, Pb and Cu were detected in all the fish tissues analysed. Zn, Cd, Cr and Pb, had the highest concentration in the

Table 1: Mean concentrations of trace metals in water and sediment, from the ikpoba River Dam

Survey Period	Sample	Zn	Cd	Cr	Pb	Cu
January	Water (mg/l)	0.96	0.05	0.02	0.06	0.02
2007	Sediment (mg/kg)	11.02	0.49	0.53	1.92	9.25
March	Water (mg/l)	0.07	0.03	0.10	0.05	0.01
2007	sediment(mg/kg)	10.19	0.46	0.56	1.72	8.98
July	Water (mg/l)	0.62	0.06	0.06	0.06	0.03
2007	Sediment (mg/kg)	13.14	0.75	0.36	2.42	8.52
September	Water (mg/l)	0.45	0.04	0.03	0.08	0.04
2007	Sediment (mg/kg)	15.25	0.64	0.33	3.15	8.14

Table 2: Mean concentration of trace metals in the tissues of *Crysichthys nigrodigitatus* from the Ikpoba River Dam

Survey Period	Whole fish	Muscle	Liver	Gills
		Zinc (mg/kg)		
January 2007	14.28	14.05	25.05	14.19
March 2007	14.95	15.18	11.54	11.75
July 2007	13.25	16.33	20.92	10.52
September 2007	13.07	19.23	22.50	12.25
		Cadmium (mg/kg)		
January 2007	0.50	0.20	15.45	0.14
March 2007	0.34	0.21	20.13	0.25
July 2007	0.16	0.19	21.23	0.15
September 2007	0.09	0.14	25.42	0.28
		Chromium (mg/kg)		
January 2007	2.94	3.98	11.25	2.66
March 2007	0.45	1.06	11.04	1.75
July 2007	4.75	3.97	10.13	1.84
September 2007	4.77	2.85	12.23	1.51
		Lead (mg/kg)		
January 2007	5.24	4.27	4.35	4.48
March 2007	3.76	3.65	6.46	3.75
July 2007	4.15	4.52	7.49	2.35
September 2007	5.44	5.47	9.86	8.44
		Copper (mg/kg)		
January 2007	12.15	15.53	5.62	4.02
March 2007	11.56	14.45	7.45	6.28
July 2007	12.98	15.57	6.32	2.65
September 2007	9.66	16.44	4.65	9.18

Table 3: The distribution co-efficient values for trace metals from the Ikpoba River Dam

Trace metal	Mean level in water	Mean level in sediment	DC value
Zn	0.53	12.40	0.043
Cd	0.04	0.58	0.069
Cr	0.03	0.44	0.068
Pb	0.06	2.30	0.026
Cu	0.025	8.72	0.003

Source: Booth (1976)

Table 4: Comparison of trace metal concentrations in water of Ikpoba River Dam with some other studies

Water Body	Zn	Cd	Cr	Pb	Cu	Reference
Ikpoba River Dam	0.53	0.04	0.03	0.06	0.025	This study
Alaro River	ND	0.004	0.003	0.023	0.0025	Fakayode (2005)
Lower Ikpoba River	0.127	ND	0.059	0.082	0.129	Oguzie (2003)
Delimi River	8.5-18.0	1.5-2.1	ND	0.8-1.25	2.6-3.8	Njoku and Keke (2003)
Buguma Creek	0.010-0.43	0.01-0.11	0.01-1.49	0.01-0.61	ND	Ogbeibu and Oribhabor (2009)
Warri River	0.0-0.63	0.0-0.05	0.0-0.06	0.0-0.001	0.0-0.26	Okaka and Wogu (2011)
WHO Limit	5.0	0.05	0.05	0.05	1.0	WHO (1984)

ND = Not determined

Table 5: Comparison of trace metal concentrations (mg/kg) in fish with some other studies

Water body /fish species	Zn	Cd	Cr	Pb	Cr	Reference
Ikpoba River Dam *(Crysichthys nigrodigitatus*)	Whole fish: 13.88 Muscle: 16.41 Liver: 20.00 Gills: 12:18	Whole fish: 0.27 Muscle: 0.19 Liver: 20.55 Gills: 0.21	Whole fish: 3.22 Muscle: 2.97 Liver: 11.16 Gills: 1.94	Whole fish: 4.64 Muscle: 4.47 Liver: 7.04 Gills: 4.76	Whole fish: 11.59 Muscle: 15.49 Liver: 6.01 Gills: 5.53	This study
Ikpoba River (*Clarias gariepinus*)	Whole fish: 6.22 Muscle: ND Liver: ND Gills: ND	Whole fish: ND Muscle: ND Liver: ND Gills: ND	Whole fish: 0.88 Muscle: ND Liver: ND Gills: ND	Whole fish: 2.22 Muscle: ND Liver: ND Gills: ND	Whole fish: 4.93 Muscle: ND Liver: ND Gills: ND	Obasohan *et al.* (2007)
Ogba River (*Erpetoichthys calabaricus)*	Whole fish: 6.26 Muscle: ND Liver: ND Gills: ND	Whole fish: 0.14 Muscle: ND Liver: ND Gills: ND	Whole fish: 4.79 Muscle: ND Liver: ND Gills: ND	Whole fish: 0.95 Muscle: ND Liver: ND Gills: ND	Whole fish: 4.79 Muscle: ND Liver: ND Gills: ND	Obasohan *et al.* (2005)
Okumeshi River (*Tilapia nilotica*)	Whole fish: ND Muscle: ND Liver: ND Gills: ND	Whole fish: ND Muscle: 0.62 Liver: 0.31 Gill: 0.21 Bone: 0.04	Whole fish: ND Muscle: 0.06 Liver: 0.17 Gill: 0.06 Bone: 0.04	Whole fish: ND Muscle: <0.01 Liver: 0.01 Gill: <0.01 Bone: <0.01	Whole fish: ND Muscle: ND Liver: ND Gills: ND	Ekeanyanwu *et al.* (2011)
WHO Limit	10-75	2.0	0.15	2.0	30	WHO (1994)

Table 6: Comparison of trace metal concentration (mg/kg) in sediment with some other studies

Water body	Zn	Cd	Cr	Pb	Cr	Reference
Ogba River	12.40	0.58	0.44	2.30	8.72	This study
Ikpoba Reservoir	28.48	ND	0.60	8.88	21.50	Fufeyin, 1998
Ikpoba River	1.475-1.663	ND	0.024-0.033	0.660-12.98	0.283-4.755	Obasohan *et al.* 2007
Madivala lake	ND	1.52-5.42	0.75-2.455	1.10-7.9	ND	Begum *et al.* 2009
Unpolluted sediment for African inland waters	0.095	0.011	ND	0.019	0.033	GESAMP 1982

liver, with mean concentration values of 20.00 mg/kg, 20.56 mg/kg, 11.16 mg/kg and 7.04 mg/kg, respectively. This observation was in agreement with Kotze *et al.* (1999) and Nussey *et al.* (2000) who indicated that the liver is associated with storage and detoxification functions. The highest concentration of Cu, was recorded in the muscle tissue, with a mean concentration of 15.49 mg/kg. According to Seymore *et al.* (1996), Cu and Zn accumulate mainly in the muscle, kidney, heart, liver, bone, skin and gills of fish. Furthermore, fish in an ecosystem contaminated by trace metals are known to accumulate significantly more metals in edible muscle tissues than do fish in an uncontaminated ecosystem (Du Preez *et al.,* 1993). With regard to health risk to man, the concentration of Zn in the tissue of *Crysichthys nigrodigitatus,* fell below the World Health Organisation (WHO) maximum allowable limit of 10 – 75 mg/kg for food and fish. The concentration of Cu in the fish tissues were also below the WHO limit of 30 mg/kg for food and fish. Zn and Cu may therefore not pose immediate health hazard to man The Cr concentrations in the fish tissues exceeded the WHO limits of 0.15 mg/g. The concentration of Cd in the fish tissues were generally below the WHO limit of 2.0 mg/g. The only exception was in the liver, with a mean value of 20.56 mg/kg and this could be attributable to the fact that Cd had the highest distribution co-efficient value of 0.069 compared to the other trace metals investigated. The presence of Cr, Cd and Pb at low concentrations, inhibit photosynthesis and phytoplankton growth (Florence and Stauber, 1986). At higher concentrations of these elements, delayed embryo development, tissue damage, reduced growth and fish kill have been reported (Siem *et al.,* 1984; Oronsaye, 2001). The Zn, Cd, Cr and Cu concentrations in water did not exceed the WHO maximum allowable limits for drinking water. The only exception was Pb (mean value of 0.06 mg/l) which was above the 0.05 mg/l WHO limit. The mean concentrations of the investigated metals in sediment were all above the benchmark for unpolluted sediment for African inland waters. In the sediments, Zn had the highest concentration of 12.40 mg/kg. This value was exceeded by values for Zn from the sediments of the Lagos Lagoon (Okoye, 1991) and the Ikpoba reservoir (Fufeyin, 1994), with values at 147 mg/kg and 193.08mg/kg respectively. It has been reported that sediments are sinks for both inorganic and organic pollutants and are thus a potential source of toxicity to aquatic biota (Fernandes, 1997; Long *et al.,* 1998).

Conclusion: This study has revealed that Zn, Cd, Cr, Pb and Cu were present in the tissues of the fish, *Crysichthys nigrodigitatus,* water and sediment of the Ikpoba River Dam. In other to produce sufficient information to implement better management strategies which would alleviate negative impacts, there is the need for regulatory agencies and other concerned parties to further monitor the Ikpoba River Dam. This study is considered to be a part of such monitoring efforts and will contribute to the data base regarding heavy metal pollution in the Ikpoba River Dam.

ACKNOWLEDGMENT

We are grateful to the Professor Solomon Wangboje Foundation for funding. We wish to thank Engineer Osawe Omozusi of the Edo State Urban Water Board for assisting with technical information on the Ikpoba River Dam. We are also grateful to Messrs Ernest Glory and Sunday Ikwedu for their assistance in fish capture.

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DETECTION AND DIAGNOSIS OF PRIONS, THE CAUSATIVE AGENT FOR THE NEURODEGENERATIVE TRANSMISSIBLE SPONGIFORM ENCEPHALOPATIES (TSEs) IN SHEEP, CATTLE AND HUMANS – A REVIEW

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ABSTRACT

The technique to detect and diagnose infectious prions, the causative agent of transmissible spongiform encephalopaties (TSEs) at very early stages of infection where the disease can be controlled and even eliminated has been developed. The technique now in the field is being applied worldwide for the screening of cattle, sheep, rodents, humans and other animals. It has not only saved lives but also billions of dollars in agriculture by preventing the spread of infection in livestock. In the United Kingdom, over 2 million cattle have been destroyed due to prion infection. Prion has also been identified in the United States, and more recently in South Korea. While in Nigeria, many of our cattle and sheep have not been screened and no awareness exist for TSEs. Preliminary work is already underway. Also screening studies are just commencing at a very slow pace in Jos. Given the globalized nature of the present world, where infections move across continents at a rapid pace, it is very disturbing that the nation has no policy and is ill-prepared to respond to it should an epidemic of TSEs break out among its livestock or population. It is necessary to apply this ultrasensitive technique for detection and diagnosis of infectious prions to the screening of livestock, and the protection of Nigerian citizens from TSEs. The technique is also a sensitive tool for studying and uncovering the mechanism of potential drugs that can inhibit or slow down the spread of TSE infection. It will advance the frontiers on the study of the mode of infection and conversion of prions and has significantly already contributed to the new paradigm that has changed our knowledge on infections.

Keywords: Detection, Prions diagnosis, Neurodegenerative, Transmissible spongiform encephalopaties, Sheep, Cattle, Humans

INTRODUCTION

TSEs have been observed in humans, sheep and cattle (Figure 1). It has also been noted in deer, elk, mink, cats, rodents, exotic ungulates, other mammals, but not in dogs, rabbit, horses or birds. In humans it exists as CJD or vCJD, its variant form which resulted from humans infected by infected cattle. Infections can occur from ingestion or inoculation, or contact with infected surgical materials, and incubation time before the manifestation of symptoms can be from months to decades. TSEs derive their name due to the malfunction and death of brain neurons which exhibit spongiform pathology. Accumulation of plaques or amyloid (Figure 2).

But what is the causative agent? After eliminating bacteria, viruses or other microorganisms, Prusiner in his Nobel work purified the protein components from infected scrapie fibrils and cloned the gene (Prusiner *et al.,* 1984). Furthermore, others workers had cloned the mice and hamster prion gene (Chesebro *et al.,* 1985; Oesh *et al.,* 1985) and found no differences between the normal prion gene and the diseased prion gene. Thus the

gene responsible for TSEs was the host gene. A most convincing proof came with the cloning of the knock-out mice (mice which lack the prion gene) and the discovery that they did not develop TSEs (Chesebro, 1999).

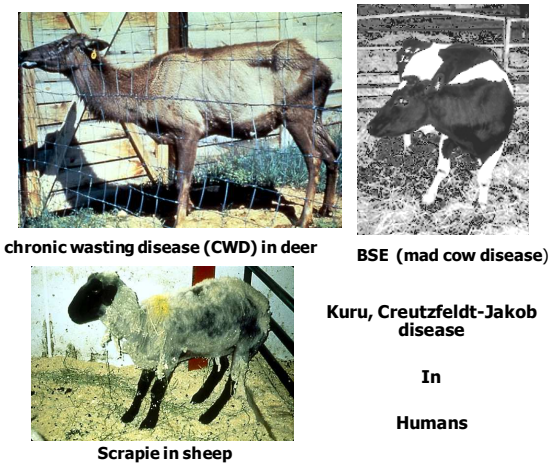

Figure 1: TSE (prion) diseases in animals

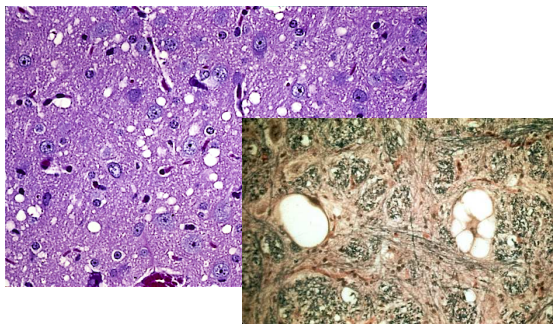

Figure 2: Malfunction and death of neurons in brain leads to spongiform pathology

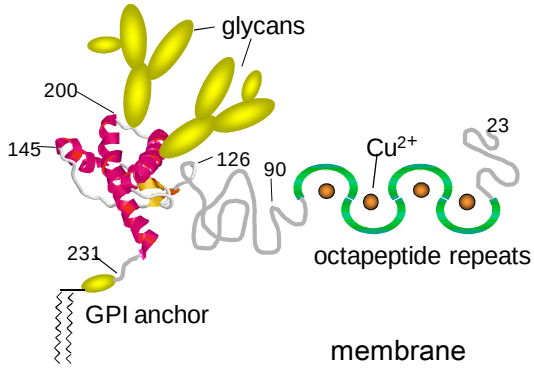

Figure 3: Normal prion protein: (normal; PrP-sen or PrP^C). *Sensitive to proteases; Soluble in detergents; In diverse tissues, cell types; Apparent cellular roles; Adhesion; Differentiation; Neuritogenesis; Synaptogenesis; Cell survival; Resistance to oxidative stress; Essential for TSE diseases.*

MATERIALS AND METHODS

A comprehensive search was made from the internet, various journal articles and textbooks reports on the detection and diagnosis of prions, the causative agent for the neurodegenerative transmissible spongiform encephalopaties (TSEs) in sheep, cattle and humans in various parts of the world. Such articles were assembled, studied and synthesized into this review.

RESULTS AND DISCUSSION

Normal Role of Prions (PrP-sen): How then does the normal prion protein differ from the diseased prion protein, since they are the same protein from the same host gene? The normal prion protein is highly sensitive to protease digestion, and is therefore designated as PrP-sen. It is soluble in detergents and present in diverse tissues and cell types. Its apparent cellular roles include adhesion, differentiation of cells, neuritogenesis, synaptogenesis as well as cell survival. It is also known to play key roles in the homeostasis of metals most especially copper in the central nervous system. Also altered sleep patterns and circadian rhythm activity regulated by the penal gland has been observed in mice that lack prions such as the knock-out mice (Tobler *et al.,* 1996; 1997). Normal PrP has also been shown to have super-oxide dismutase activity, thus making it a powerful anti-oxidant for the central nervous system (Brown *et al.,* 1999). Other roles in which PrP has been implicated includes signal transduction in neuronal cells (Mouillet-Richard *et al.,* 2000), and the activation of lymphocytes (Li *et al.,* 2001) (Figure 3).

Infectious and Diseased Prion (PrP-res): However, diseased prions associated with TSEs are generally designated as PrP-res, because they are highly resistant to protease digestion. Their capacity to form insoluble aggregates and polymers enable them to form amyloids, spongiforms and plaques in the brain. They are always found within the region of neuropathology and are always associated with infectivity.

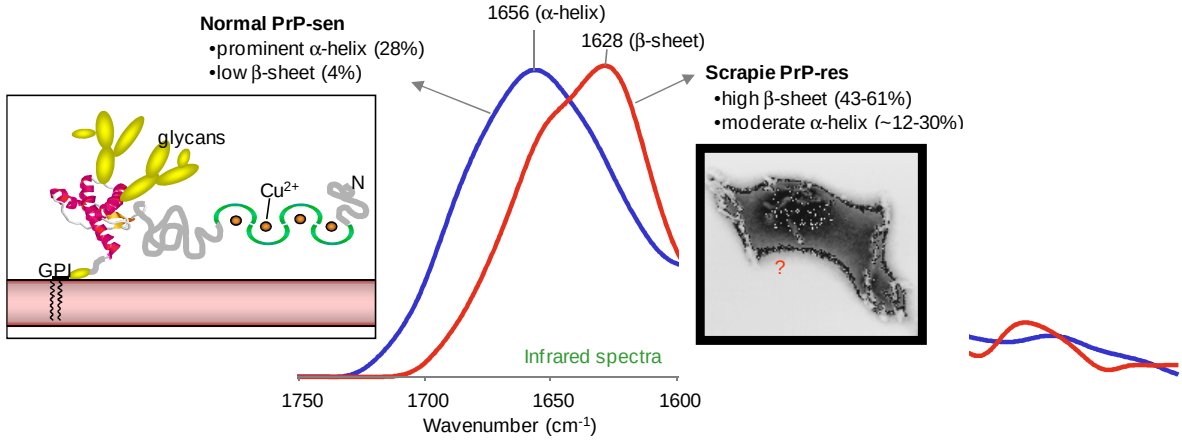

Figure 4: Conformational difference between normal and TSE-associated PrPs with no known chemical modification; conversion involves refolding from α-helix + random coil to β-sheet

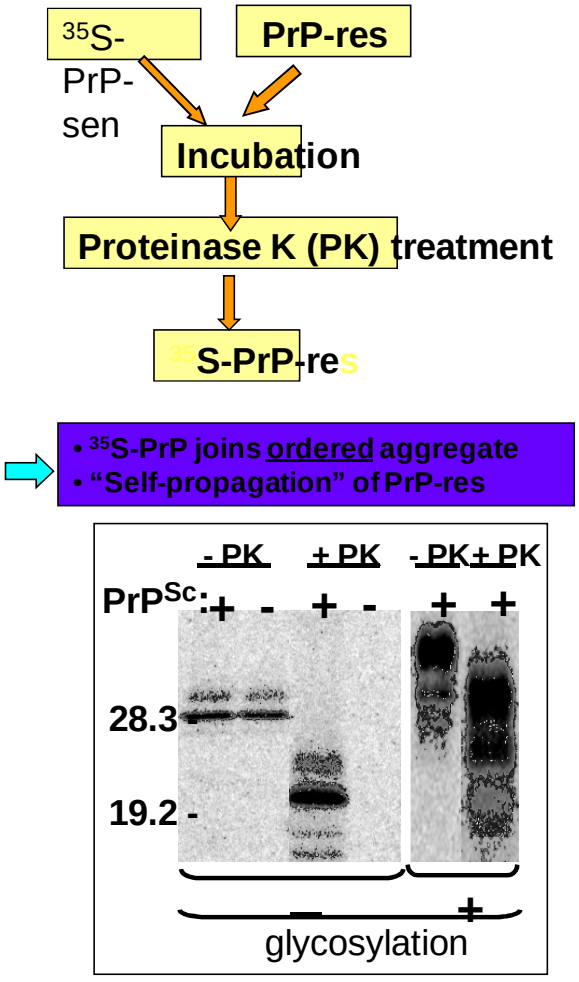

Figure 5: PrP-res-induced conversion of PrP-sen into a PrP-res-like protease-resistant form (Kocisko *et al.*, 1994)

They are not only located in the brain but are also found in lymphoid tissue. Yet, their covalent structure is indistinguishable from normal PrP-sen. PrPres isoforms are co-noted with subscripts to indicate the infected specie: PrP-sc for scrapie or sheep, PrP-CJD for humans, PrP-BSE for cattle, etc. PrP-res differ mainly from the normal PrP-sen by conformational differences (Figure 4).

Normal PrP-sen contains 28% alpha helix, and 4% beta-pleated sheet, while the abnormal and infectious PrP-res contain 43 – 61 % beta pleated sheet. PrP-sen therefore absorbs at 1656 cm^{-1} wavelength while PrP-res can be identified at 1628 cm^{-1} due to its higher beta sheet content.

PrP Conversion: Mode and Mechanism of Infection: One of the intriguing problems of molecular biology and biochemistry is to show how PrP-sen is converted to PrP-res. When 35-S-PrP-sen is incubated with PrP-res and treated with proteinase K, the product is 35-S-PrP-res, indicating the conversion of PrP-sen to PrPres (Kocisko *et al.,* 1994). Also continuous amplification of infectivity when crude brain haemogenates is used as a source of PrP-res has also been observed (Saborio *et al.,* 1999; 2001) (Figure 5). Furthermore, structural assessments of the various domains of PrP-sen upon conversion to PrP-res oligomers are well known and have been illustrated in figure 6. It can be seen that the octarepeat region which normally binds four copper atoms remain exposed to proteases and can easily undergo alteration. Independent deletion mutagenesis studies have shown that the N-terminus of PrP facilitates prion propagation and PrP-res formation (Flechsig *et al.,* 2000; Supatta *et al.,* 2001).

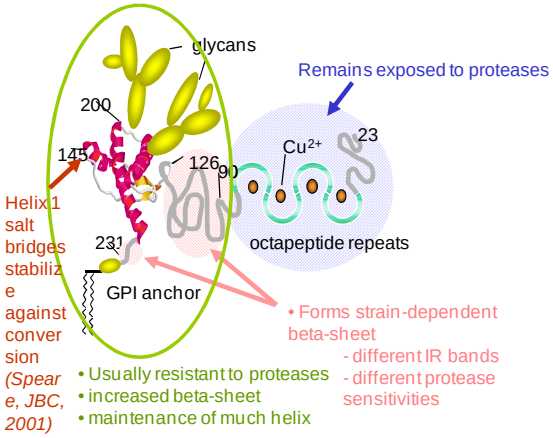

Figure 6: Fates of domains of PrP-sen upon conversion to PrP-res oligomers

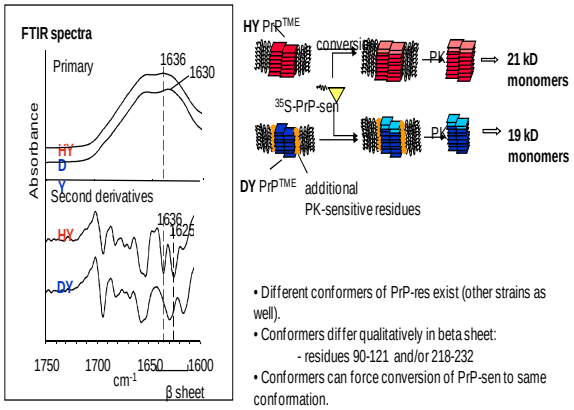

Figure 7: Hyper and Drowsy strain-dependent conformers of PrP-res in hamsters

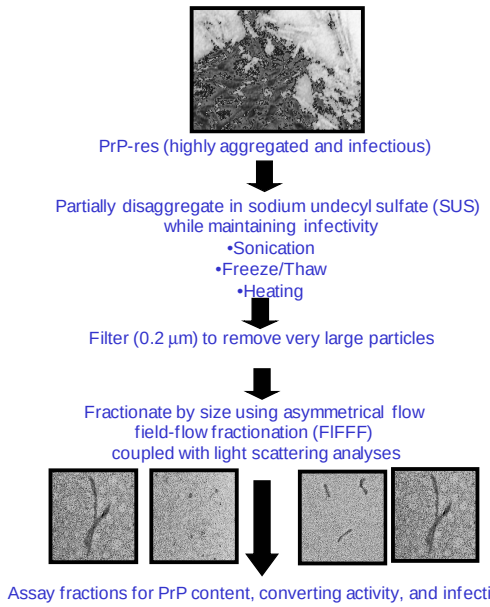

Figure 8: Fragmentation & fractionation of infectious PrP-res preps (Jaysilveira *et al.,* 2005)

However, residues 90 to 231 which include the Helix 1 salt bridges are unaltered and stabilized against conversion. This region is also known to form strain dependent beta sheets identified by various infra-red bands to reflect strain specific conformations.

In general, PrP-sen conversion reaction to PrP-res showed that it can be stimulated by sulfated glycans, chaperone proteins, partially unfolding detergents and temperature increase to 65^0C (Wong *et al.,* 2000). It is inhibited by disulfide bond reduction and requires PrP-res as multimers for seeding the process. Additionally, there appears to be specific bonding of PrP-sen to the PrP-res polymer (DeBurman *et al.,* 1997). This specificity is thought to be responsible for the strain specificity observed in infected animals. In addition, the binding of PrP-sen to PrP-res precedes the conversion to the proteinase K resistant state because the product remains associated with pre-existing PrP-res (Baron *et al.,* 2002; Baron *et al.,* 2003). The conversion process is virtually irreversible without denaturants in vitro and is consistent with an autocatalytic polymerization mechanism (Vorberg and Priola, 2002).

Several other factors have been observed to influence the rate of conversion of PrPsen to PrP-res (Korth *et al.,* 2000; Saborio *et al.,* 2001). Correlations between PrP sequences show that the closer the sequences between PrPsen and PrP-res, the higher the conversion rate. Thus the more the similarities in structure between species, the higher the rate of conversion and inter-species transmissibilities of TSEs. It is therefore reasonable that the highest rate of conversion is obtainable within the same species (Prusiner *et al.,* 1990).

One of the most fascinating aspects of TSEs is the existence of different strains in the same species. In hamsters, PrP-res have two or more strains which manifest with different symptoms of TSE. These strains are distinguished by intro-red spectroscopy. The 1636 cm-1 conformer is referred to as PrP-Hyper because these TSE infected hamsters are hyperactive before their death. Another hamster strain has a slightly different conformation at 1630 cm-1 wavelength and is noted as PrP-

drowsy because these TSE infected hamsters are usually drowsy before their death (Figure 7).

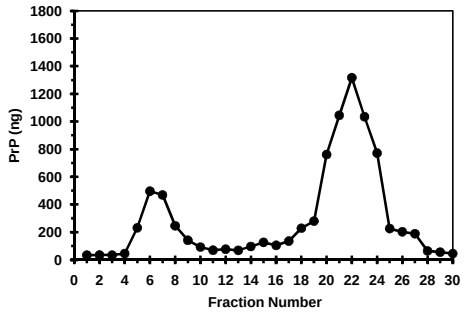

Figure 9: Infectivity in flow field flow fractionation of partially disaggregated PrP-res

There have been concerns as to whether PrP-res infect PrP-sen as a monomer and whether particle size affects infectivity. Studies applying asymmetrical flow field-flow fractionation (FIFF) coupled with light scattering analysis led to generation of fractions of PrP-res which were in turn used to assay for converting activity and infectivity (Silveira *et al.,* 2004). These results indicated that particle size was important in conversion and infectivity. While large fibrils were not the most infectious particles, surprisingly smaller fibrils from monomers to pentamers had the lowest capacity to induce infectivity (Figure 8). The most infectious particles were 17 to 27nm in diameter; they were 500 - 600 Daltons which indicates a mass equivalent of 25 molecules of PrP, although part of this mass could be due to bound detergent molecules. These most infectious particles were found to be roughly spherical and slightly elongated in structure. They were associated with the highest level of specific converting activity. Therefore large amyloid fibrils or spongiform plaques are much less infectious per unit protein than the smaller but most infectious particles (Figure 9).

Prions and Protein Folding Diseases: The transmissible spongiform encephalopathies (prion diseases) are not the only protein folding diseases, and are not the only neurodegenerative diseases. They are however unique because they are the only ones that are transmissible.

Their infectious causative agent is a non-living protein, and is the first known particle that can transmit infection without recourse to DNA or RNA. They have therefore changed our conventional understanding of infectious diseases. Other protein misfolding diseases that are not transmissible include Alzheimer's disease, type II diabetes, arnyotropic lateral sclerosis, Parkinson's disease, sickle cell anaemia, cystic fibrosis, Huntington's disease, spinocerebellar ataxis, etc. However while some of this protein misfolding diseases are neurodegenerative, non are transmissible as is noted for the prion diseases. Despite their low rate of propagation interspecies, that they are transmissible at all from one species to another is a challenging problem confronting the world. The interspecies rate of transmission is as illustrated significantly threatening mankind.

Death through Prion Infection: Besides infecting cattle, sheep, rodents and humans, death through TSEs has been described as the worst way of dying. Young women once infected, go into menopause despite their age (Max, 2006). Also infected individuals are unable to sleep, and at best go into a state of stupor, where they still retain full consciousness. There is a gradual decay of muscular coordination, which affects both sight and speech. Max who interviewed patients with CJD in Europe also noted a rapid loss of weight that resembles those of AIDS patients. Young infected males are also known to become sterile with the progression of the disease. It is possible that these changes may be associated with a dysfunctional circadian rhythm where normal PrP function is lost.

Detection and Diagnosis: By the application of an ultra-sensitive means of detecting infectious prions at ag and fg levels, infections can be controlled at a much earlier and less threatening stage, thereby intervening with drug inhibitors to prolong the life of patients, as well as prevent the spread of infection to healthy life stock, thereby saving the world agricultural industry billions of dollars (Atarash *et al.,* 2007;

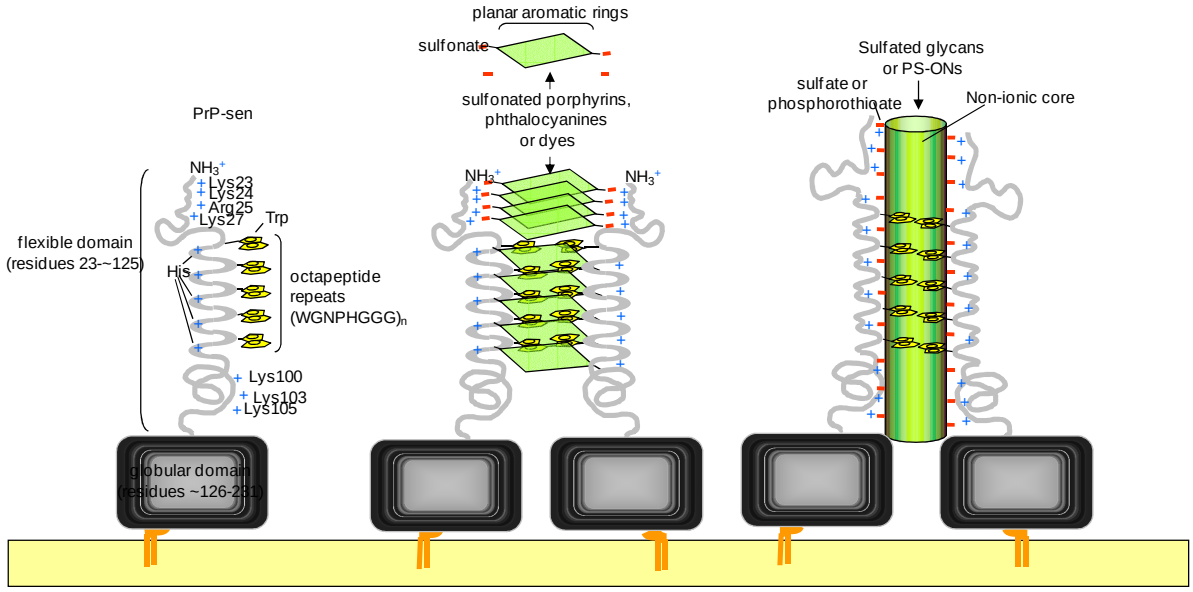

Figure 10: Stacked amphipath model of inhibitor interactions with PrP-sen

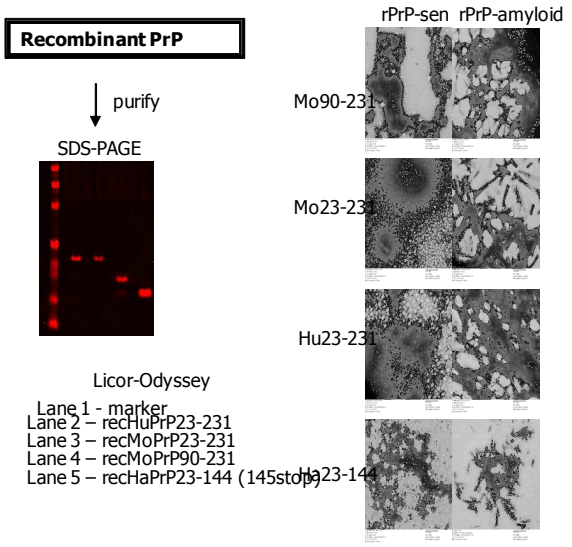

Figure 11: electrophoresis of recombinant prion isoforms

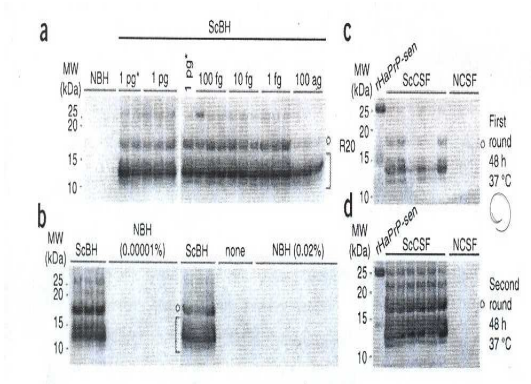

Figure 12: QUIC reactions seeded with brain homogenates and CSF samples from normal or scrapie-affected hamsters (Atarashi *et al.*, 2007; 2008).

2008). Nigeria requires this ultra-sensitive technique to ensure that its cattle and sheep which serve as the key source of protein for its population ore completely screened. It need not wait until it experiences an epidemic the method is also being applied at hospitals in the developed world to prevent infected surgical tools from being used inadvertently on healthy people. PrP-res are very insoluble and sticky and are not inactivated by radiation or by conventional heating. It also is known to bond to metals and is extremely stubborn to destroy. Detecting PrP-res on such tools will require their immediate disposal for new and uninfected ones. We have commenced experiments with rodents at the University of Nigeria Nsukka, as work with cattle and sheep would require more hands and capital.

As there are no practical treatment known, this technique is based on the conversion process, and can be applied to screen drugs and inhibitors of conversion. Hundreds of compounds that inhibit the conversion of PrP-sen to PrP-res, that can serve as potential therapeutic agents for TSE treatment are regularly being identified using aspects of an ultra-sensitive technique on prion detection. Once to be useful potential inhibitors, they are then tested in TSE infected deer cattle, hamsters, sheep, and other lower animals before clinical trials in CJD infected humans. So far at least two inhibitors of conversion are

being tested in CJD patients — pentosan polysulfate and quinacrine. Quinacrine is used also as an anti-malarial drug and is not expected to have life threatening side effects. A stacked amphipathic model has been proposed as a mechanism for inhibitor binding and action on the PrP-res molecule (Figure 10). The compounds are thought to stack at the N-terminal octarepeat region between residues 23-1 25, the same site necessary for PrP-sen conversion to PrPres (Supattapone *et al.,* 2001).

Conclusion: Finally, to develop an ultra-sensitive means of detecting infectious prions and diagnosis of TSEs requires a reasonable understanding of the cell-free conversion process. A clean and steady supply of PrP-sen is obtained by generating recombinant forms of the protein from bacteria. To assure their purity they are subjected through SDS-PAGE electrophoresis. Figure 11 shows the various recombinant PrP proteins when subjected to electrophoresis, and their purity. Their capacity to generate amyloid was also tested as is evident in figure 11. RecHuPrP231 refers to human recombinant Prp-sen full length 23 – 231. Mo and Ha refers to mouse and hamster recombinant PrP segments, respectively. Satisfied with the purity and spectroscopic characterization of the recombinant PrP segments its effect on the cell free conversion reaction with Pr Pres from brain haemogenates of sheep, as well as cerebrospinal fluid of sheep is tested (Figure 12). ScBH represents scrapie infected sheep brain homogenate, while NBH designates normal brain hemogenate used here as control. Also ScCSF represents scrapie sheep cerebrospinal fluid, while NCSF stands for the Normal Cerebrospinal fluid used here as control. MW indicates the molecular weight markers which helped to identify the molecular weight of the various PrP fragments present after the reaction have been subjected to proteinase K digestion, and Western blotting. Of significant note is the complete absence of the 17kd band in either the normal brain homogenate control or the normal cerebrospinal fluid control. However the 17kd band is clearly evident even at 1fg and 20ag levels of infected cerebrospinal fluid (ScCSF) or infected scrapie brain homogenate (SCBH) (Atarashi *et al.,* 2007; 2008). These results showed possibility of detecting infectious prions at minute quantities, and that TSEs can be diagnosed at the very onset of infection, when conversion from PrP-sen to Pr P-res is still very low. Under such intervention, drug inhibitors could be used to shoal an otherwise infectious and presently incurable disease. Also billions of Naira could be saved from preventing the spread of infection to yet to be infected livestock. It should be noted that since PrP-res and PrP-sen have the same primary and covalent structure, the body's immune system does not recognize the infectious PrP-res as foreign. Therefore PrP-res is able to evade the body's cellular and humoral immune response. However monoclonal antibodies directed to various sites of conversion have become useful tools for western blotting as well as obtaining information on drug design and action against TSEs as mankind is confronted without question by the most challenging paradigm which has changed the way we see infectious diseases.

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EFFECT OF HERBICIDE (PRIMEXTRA) ON TISSUE CHOLESTEROL LEVEL IN *CLARIAS GARIEPINUS* JUVENILE

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ABSTRACT

Juvenile Clarias gariepinus were exposed to sub lethal concentrations (0.04, 0.06 and 0.10µg/L) of primextra for 21 days in a static renewal bioassay system. The changes in the tissue cholesterol concentrations were determined every seven days. The result showed that primextra had adverse effect on the tissue cholesterol levels in C. gariepinus. When compared with the control, the liver and muscle cholesterol concentrations were significantly (P>0.05) elevated due to primextra exposure. However, the kidney cholesterol levels in the primextra-exposed fish were lower (P > 0.05) than the control. The cholesterol concentrations in the treatment groups were also different (P<0.05).Generally, the liver and muscle cholesterol concentrations increased with duration of exposure. The induction of hypercholesterolemia in both the muscle and the liver and hypocholesterolemia in the kidney of the treated fish are indications of dysfunctional lipid physiological processes occurring in the fish due primextra exposure.

Keyword: Primextra, *Clarias gariepinus*, Cholesterol, Kidney, Liver

INTRODUCTION

There is a growing awareness of the effect of herbicides on aquatic organisms particularly the fish. Many studies have shown that most chemicals including agrochemicals affect several physiological and biochemical functions in an animal (Maduka, 2002). Primextra is a pre-emergent broad spectrum herbicide for weed control in maize and sorghum farmlands. Primextra can be toxic if inhaled, swallowed or absorbed through the skin. It has been found that empty containers of this herbicide retains product residue for a long time and those applied in the weed control programmes accidentally leach into the aquatic environment either through runoff and/or as aerosol carried by wind (Syngenta, 2007).

Cholesterol is a chemical that is naturally produced by the body and is a combination of lipid and steroid. It is a basic material needed in the construction of animal cell wall/ cell membrane. Tissue cholesterol is produced by the liver and is used as starting point for the synthesis of other steroid molecules. Excess cholesterol is stored in the muscle as fat and this provides energy during period of extensive exercise or during period of food deprivation. The liver manufactures and secretes LDL cholesterol into the blood. High levels of cholesterol in the blood stream, depending on how it is transported, are strongly associated with progression of arthrosclerosis in man. Biosynthesis of cholesterol is directly regulated by the cholesterol level present and

can be turned off when cholesterol level is high (Castell, 1979).

This study was therefore aimed at investigating the sublethal effect of primextra on the tissue cholesterol level of *Clarias gariepinus under* laboratory conditions.

MATERIALS AND METHODS

Eighty one (81) healthy juveniles of *Clarias gariepinus* with mean body weight 20.38 ± 1.25g and length 14.32 ± 0.50 cm were obtained from Freedom Fish Farm in Nsukka, Enugu State, Nigeria. They were transported to the Fisheries and Hydrobiology Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka in a plastic fish transport container. The fish were acclimatized for three weeks before the commencement of the study. During the period of acclimatization and the experiment, the fish was fed *ad libitum* on 45% crude protein diet.

The fish were randomly divided into three replicate groups of nine fishes per replicate. The fish in groups 1, 2 and 3 were treated with 0.04 µg/L, 0 .066 µg/L, and 0.092 µg/L of primextra respectively. The fourth group was exposed to tap water as the control experiment. The sublethal primextra concentrations were prepared from the commercial preparation containing 290g of metalachlor and 370g of atrazine as the stock. The primextra and water were changed daily in a static renewal bioassay system. The temperature and pH value of the tap water used in this study were 24.50 ± 2.0^{o}C and 7.4 pH, respectively.

The fish were killed and the tissues (kidney, liver and muscle) were extracted and rinsed with physiology saline. One gram of the excised tissues was weighed, homogenized, centrifuged and the supernatant was used for the assay of cholesterol (Zlatkis *et al.,* 1967) using Randox kit. The data obtained were analyzed statistically with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The result showed that primextra caused significant increase in the liver cholesterol concentration in *C. gariepinus* when compared with the control (Table 1). The magnitude of increase was 2.26, 2.56 and 1.79 folds in the fish exposed to 0.04, 0.06 and 0.09µg/l primextra, respectively.

The cholesterol concentration in the control group did not change throughout the study. The liver cholesterol decreased from 8.51 ± 2.1mg/g and 9.69 ± 5.23mg/g on the 7th day to 7.73 ± 3.82 mg/g and 7.19 ± 6.3 mg/g in the group treated with 0.04 and 0.06µg/l primextra, respectively at the end of the study. When the fish was exposed to 0.09µg/l primextra the liver cholesterol concentration slightly increased from 6.78 ± 4.7mg/g on the 7th to 7.03 ± 6.76mg/g liver on the last day. The percentage change in the liver cholesterol concentration is shown in Figure 1. The change was highest on day 7 being 126, 156 and 79.98 % in the fish treated with 0.04, 0.06 and 0.09 µg/l, respectively. At the end of the study the percentage change were 105.6 %, 60% and 87% in the groups treated with 0.04, 0.06 and 0.09µg/l, respectively. The observed increase in hepatic cholesterol in this study was in agreement with the work of Garthoff *et al.* (1977) that liver cholesterol level increased in rats fed with PCB. However, Maduka (2002) reported that lindane caused decreased liver cholesterol in *Clarias gariepinus* after 21 days. Similarly, Kling and Gamble (1982) had earlier reported decreased hepatic cholesterol levels in rats treated with PCB.

The muscle cholesterol concentration increased with increasing duration of exposure (Table 2). The cholesterol concentration was significantly different within the treatment groups ($P \geq 0.05$). The percentage change in the muscle cholesterol indicated that on the 7th day, the percentage increase over the control were 118, 164 and 66% in the fish exposed to 0.04, 0.06 and 0.09µg/l, respectively. By the end of the study the changes were 208, 170 and 202% increase over the control in the fish treated with 0.04, 0.06 and 0.09µg/l, respectively.

Table 1: Liver cholesterol concentration of *Clarias gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups (µg/L)	Duration of exposure (days)		
	7	14	21
Control (0.0)	3.77±0.47[a]	3.77±0.45[a]	3.76±0.56[a]
Group A (0.04)	8.51± 2.1[b]	6.98±4.29[b]	7.734±3.82[b]
Group B (0.06)	9.67± 4.56[c]	6.17±2.6[d]	7.19±6.3[d]
Group C (0.09)	6.78±4.7[d]	6.03±6.2[d]	7.03±6.76[d]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 2: Muscle cholesterol concentration of *C. gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups(µg/L)	Duration of exposure (days)		
	7	14	21
Control	1.09±1.94[a]	1.09±1.93[a]	1.09±1.94[a]
Group A (0.04)	2.38±10.77[b]	2.54±14.55[b]	3.37±25.61[b]
Group B (0.06)	2.78±1.05[c]	1.29±10.47[c]	2.95±11.14[c]
Group C (0.09)	1.82±1.09[d]	2.51±16.78[b]	3.28±8.51[b]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 3: Kidney cholesterol concentration of *C. gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups (µg/L)	Duration of Exposure (days)		
	7	14	21
Control (0.0µg/L)	3.95±7.57[a]	3.95±7.6[a]	3.92±3.8[a]
Group A (0.04)	3.66±0.53[b]	3.14±2.73[b]	2.80±2.16[b]
Group B (0.06)	3.66±0.52[b]	2.22±1.89[c]	3.76±3.90[a]
Group C (0.09)	3.66±0.49[b]	3.53±2.78[d]	3.86±1.22[a]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 4: Regression functions of the percentage changes in the tissue cholesterol levels in *C. gariepinus* exposed to primextra for 21 days

Tissue	Primextra concentration(µg/l)	Regression function		
		Intercept	Slope	R^2
Liver	0.04	68.64	0.5	0.63
	0.06	125.9	-1.46	0.23
	0.09	189.2	-6.85	0.78
Kidney	0.04	2.24	-1.49	0.98
	0.06	-12.18	0.41	0.37
	0.09	-21.9	0.27	0.01
Muscle	0.04	-3.67	9.71	0.99
	0.06	112.13	0.39	0.01
	0.09	62.67	6.43	0.86

The percentage increase in the muscle cholesterol was highest on day 21 in all the treatment irrespective of the concentration. In both the liver and the muscles, it likely that primextra may have promoted the activities of HMG-CoA reductase to increase mevalonate production that favours the cholesterol biosynthesis.

The result of this study is consistent with earlier reports with mammalian subjects (Hafeiz and Bartke, 1972; Nagaoka *et al.* 1986) that xenobiotic intoxication results in elevated tissue cholesterol level.

The changes in the kidney cholesterol concentration are shown in Table 3. The cholesterol concentration in the control did not

change during the exposure period and did not differ from the values in the treatment groups on day 7 of the study (P< 0.05). Thereafter, the cholesterol concentrations in the treatment groups remained generally lower than the control (P≤0.05).

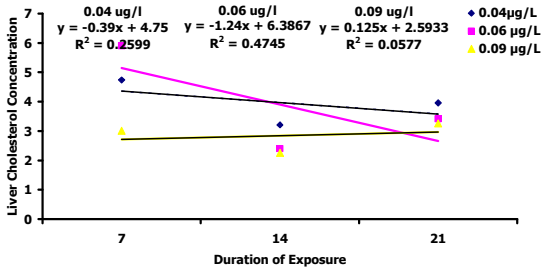

Figure 1: Percentage changes in liver cholesterol concentration of *Clarias gariepinus* exposed to sublethal concentrations of primextra for 21 days

The percentage decrease was the same on day 7 (7.2%) in all the treatment groups and thereafter, it fluctuated in the groups. The decrease was highest (43.7%) in the fish treated with 0.06 µg/l primextra on day 14. This was followed by 28% decrease observed in the fish exposed to 0.04µg/l on day 14. At the end of the study the percentage decrease in the kidney cholesterol was highest (28%) in the fish treated with 0.04µg/l and least (1.54%) in the group exposed to 0.09µg/l**.**

The observed decrease in the kidney cholesterol concentration in this study is in agreement with the report of Venkataramana *et al.* (2006) that cholesterol concentration was decreased in the heart muscles of gobiid fish *Glossogobius giuris* exposed to varying concentrations of malathion for 96h.The result of this study was consistent with the reports of Ghosh and Chatterjee (1989) and Piska *et al.* (1992), that agrochemicals induced decreased tissue cholesterol in fish. Similarly, Jyothi and Narayan (2001) reported decreased serum cholesterol level in the Indian catfish *Clarias batrachus* following exposure to pesticides carbaryl and phorate.

This trend according to Brycesmith and Waddson (1974) could be due to impaired pyruvate metabolism in the kidney, resulting in low production of acetyl-CoA. According to Kling *et al.* (1978) the observed decrease in the kidney cholesterol level could be due to inhibited conversion of acetate and mevalonate to cholesterol following the exposure to primextra. The regression functions (Table 4) showed that the percentage change was not concentration dependent in all the treatment groups. According to earlier reports (Kim *et al.,* 2004; Singh *et al.*, 2009) HMG-CoA reductase is a primary rate-determining enzyme in the tissue cholesterol biosynthesis whose inhibition would result in decreased cholesterol synthesis and *vice-versa*. Thus, it is likely that the reported decrease in the kidney cholesterol concentration may be due to inhibited HMG-CoA reductase activity in the organ. Thus, in conclusion, the induction of hypercholesterolemia in the liver and muscle and reduced renal cholesterol concentration could be part of the mechanism of primextra exerting its effect in the fish and could be of immense value in policy formulation regarding safe levels of the compound in the aquatic environment.

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PRODUCTION TRAITS OF BROILER CHICKEN STRAINS FED *AD LIBITUM* AND RAISED ON DEEP LITTER SYSTEM IN THE HUMID TROPICS

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ABSTRACT

This study was conducted to evaluate the effects of strain and age on production traits of commercial broiler chickens reared on full-feeding, and under the same housing, feeding regime, agro-climatic zone and management practices. A total number of 150 broiler day-old chicks, that, is 50 chicks each of Arbor Acres (strain A), Hubbard (strain B) and Marshall (strain C) were purchased from local hatcheries, and raised on deep litter in separate pens for 49 days (7 weeks). Data collected include live body weight, body length, breast girth, shank length and thigh length. In addition, data on feed intake, feed conversion ratio and feed efficiency at two weeks interval beginning from 1st week were taken. Analyzed results showed that genotype and age of birds had highly significant (P<0.01) effects on all the performance traits of broiler chickens. Strains A and B appeared superior to strain C in body weight, but the latter was better (P<0.01) in shank length and breast girth than the former. As regards feed conversion, strain C was the poorest, intermediate in strain B, and strain A the best. The feed efficiency and feed conversion ratio were related but in a reverse manner. Strain A was adjudged good and profitable because the strain had the highest mean values in body weight and feed efficiency coupled with the lowest feed conversion ratio at maturity, and could be recommended to poultry farmers in this zone for increased productivity, income generation and maximum profit.

Keywords: Poultry, Broiler, Strain, Traits, Body weight, Feed efficiency, Feed conversion, Feed efficiency

INTRODUCTION

Poultry products such as meat and eggs are excellent sources of animal proteins necessary to meet protein requirements of both infants and vulnerable people. Globally, in order to meet the increasing demand for poultry products, new strains of both broilers and layers are being bred and developed with fastest growth rate and improved carcass quality with less abdominal fat. For breeding programmes to be successful, a breeder must take into consideration the interrelationships between body weight and other body conformation traits. Such conformation traits include shank length, thigh length, breast girth and body length, and according to Ibe (1989), some of these conformation traits are good indicators of body weight and market value in broilers. Previous study had reported that the relationships between body weight and conformation traits are direct and positive (Okon *et al.,* 1997). In addition, Kabir *et al.* (2008) found that the exact time to slaughter a mature broiler

depends on its body weight and general development. It was reported in the literature that researchers and local farmers make use of body weight and body dimensions as parameters for selection in order to improve the productivity of their breeds (Fitzburgh, 1976). In most places, and especially in the villages where scales are not readily available, prediction equations according to Ozoge and Herbert (1997) and Nesamvuni *et al.* (2000) may be derived from body measurements, and used to predict body weight of animals. There are genetic differences in growth rate between strains, and the changes in weight ranking may be critical in the age range between 8 – 12 weeks (Deeb and Lamont, 2002). Body weight according to Chambers (1990) is the most frequently used indicator of growth. Body weight is a qualitative trait, controlled by few pairs of genes, highly heritable and influenced also by the environment. Previous investigators had posited that differences in growth pattern are under genetic control, and that variations exist within species (Lilja *et al.,* 1985; Carborg *et al.,* 2003). Growth rates in birds have been categorized into two levels, that is, low and high growth rates (Lilja 1983). The researcher indicated that a high growth capacity is characterized by a rapid early development of the digestive organs and the liver whereas; a low growth rate is characterized by a rapid early development of the pectorals and feathers. In a study, Reddish and Lilburn (2004) observed that selection for breast muscle yield and body weight in commercial broilers has resulted in genotypes far different from broilers processed in the past. Furthermore, Ajayi and Ejiofor (2009) found significant differences between strains and sexes in body weight and body dimensions. The authors' findings are consistent with other studies reported in literature (Razuki, 2002; Razuki *et al.,* 2007; Razuki *et al.,* 2011). Significant strain effects on feed consumption and feed conversion have been reported (Berrong and Washburn, 1998; Razuki, 2002; Razuki and Al-Rawi, 2007). Furthermore, Adebambo *et al.* (2008), Olawumi (2011) and Olawumi and Dudusola (2011) observed significant effect of breed on feed efficiency in commercial layer strains. In view of the importance of broiler chickens to the socio-economic wellbeing of our people, and the desire to guide local farmers on the choice of broiler strain to procure for increased meat production and optimum profit, this present investigation was conducted to assess the genetic differences in production traits of three strains of commercial broilers. The objectives of this study include: (a) determination of strain(s) with superior growth rates, and recommending same to local farmers and (b) identification of feed efficient strain(s) to be recommended to poultry farmers.

MATERIALS AND METHODS

Study Location: The study was carried out at the Animal Breeding Unit, Teaching and Research Farm, Ekiti State University, Ado-Ekiti, between September, 2010 and December, 2010. Ado-Ekiti is situated along latitude $7^{0}31^{1}$ and $7^{0}49^{1}$ North of the Equator and longitude $5^{0}71^{1}$and $5^{0}27^{1}$East of the Greenwich Meridian. The city falls under Derived Savannah zone. The city enjoys two separate seasonal periods namely, Rainy (May-October) and Dry (November-April) seasons.

Management and Experimental Birds: A total number of 150 broiler day-old chicks, that, is 50 chicks each of Arbor Acres (strain A), Hubbard (strain B) and Marshall (strain C) were purchased from local hatcheries, and raised on deep litter in separate pens for 49 days (7 weeks). The chicks were brooded using coal pot to supply heat for the first three weeks of life. Antibiotics and vitamins were administered as and when due. Also, vaccines against infectious Bursae and Newcastle diseases were given at specified age intervals. Their beddings are made up of dry wood shavings to prevent coccidiosis outbreak, and high level of hygiene was maintained throughout the experimental period to ensure favourable and conducive environment for growth, and to prevent disease outbreak. The birds were fed *ad libitum* with starter mash (1 – 4 weeks) containing 3000Kcal/KgME, 22% CP and finisher feed (5 – 8 weeks) containing 3100Kcal//KgMe, 21% CP.

Data Collection: Ten birds per strain taken at random were starved overnight and weighed from the pen each time the exercise was carried out. The birds were weighed at 7th day (week 1), and subsequently at two weeks interval up to 7 weeks of age, that is, 1st, 3rd, 5th and 7th week. Other linear measurements taken were body length, breast girth, shank length and thigh length. In addition, data were taken on feed intake, feed conversion ratio and feed efficiency at two weeks interval beginning from 1st week. Live body weights were weighed using sensitive scale (gm), while other parts were measured with tailor's tape rule in centimetre (cm).

Feed conversion ratio was computed for each breed. It refers to the ratio of feed (g) consumed/bird to average body weight on strain basis. Feed conversion ratio (FCR) = feeds (g)/bird/week ÷ average body weight (g)/bird/week. Feed efficiency also refers to the ratio of average body weight to feed (g) consumed, was calculated thus: Feed efficiency (FE) = average body weight (g)/bird/week ÷ feeds (g)/bird/week

Statistical Analysis: Data collected were subjected to analysis of variance, and the differences between means for breed and age were separated using Duncan new multiple range test (SAS, 2001). The appropriate statistical model used was: $Y_{ijk} = \mu + G_j + A_i + (GA)_{ij} + \varepsilon_{ijk,}$ Y_{ijk}= observation of the k^{th} population, of the j^{th} genotype and i^{th} age, μ= common mean, G_j= fixed effect of j^{th} genotype (j=3), A_i = fixed effect of i^{th} age (i=4), $(GS)_{ij=}$ fixed genotype x age interaction effects and ε_{ijk}= random error.

RESULTS

Strain Effects on Body Weight and Linear Measurements: Body weights of broilers at the 3rd week had significant (P<0.01) differences among the strains. Strains A and B recorded the highest mean values, while the lowest body weight was recorded for strain C. There was significant (P<0.05) strain differences in body weight and thigh length at the 3rd week of age. Strains A and B still maintained the lead, and recorded the highest mean values, while the lowest body weight was recorded for strain C at the 3rd week (Table 1).

There was no significant (P>0.05) effect of strain on body weight, body length and thigh length at the 5th and 7th week. The only two traits that were significantly (P<0.01) affected by genotype were shank length and breast girth. For shank length, strain C recorded highest mean value, while strains A and B were similar. As regards breast girth, strain C had the highest mean value, and the lowest was recorded for strains A and B in the 7th week (Table 1).

Strain Effects on Feed Intake and Feed Conversion Ratio: Strain A recorded the highest mean values at 1st and 3rd week of age. In the 1st week, strain A had the highest mean value of feed intake, followed by strains B and C. A similar pattern occurred for feed intake in 3rd week; however, strain C was superior to other strains in feed intake at 5th and 7th week of age. In the 5th week, strain C recorded the highest mean value of feed intake, followed by strains A and B, respectively. The 7th week mean values of feed intake showed the superiority of strain C followed by strains B and A, respectively (Table 1).

There were significant (P<0.01) differences among different strains for feed conversion ratio. Strain C recorded the highest mean values in almost all the age sub-divisions, intermediate in strain B, and strain A had the lowest (Table 1).

Strain Effects on Feed Efficiency: There were significant (P<0.01) effect of strains on feed efficiency. Strain A recorded highest mean values in all the weeks, followed by strains B and C, respectively (Table 2).

DISCUSSION

The significant differences observed in growth rate in these strains at two weeks interval was an indication that these strains have different genetic potentials for growth, and that the three strains studied have different ancestors.

Table 1: Breed differences in body weight and linear measurements of varied strains of broiler chicks raised in deep litre at 1, 3, 5 and 7 weeks

Traits	Week 1			Week 3		
	Strain A	Strain B	Strain C	Strain A	Strain B	Strain C
Body weight (g)	122.60±4.42^{a}	114.80±4.42^{a}	97.60±4.42^{b}	530±17.8^{a}	500±17.8^{ab}	460±17.8^{b}
Body length (cm)	13.44±0.28	12.8±0.28	13.12±0.28	19.28±0.31	18.92±0.31	18.52±0.31
Thigh length (cm)	5.60±0.12^{a}	4.54±0.12^{b}	5.46±0.12^{a}	7.14±0.31^{a}	6.06±0.31^{b}	6.06±0.31^{b}
Shank length (cm)	2.72±0.13	2.54±0.13	2.50±0.13	3.56±0.13	3.64±0.13	3.74±0.13
Breast girth (cm)	6.32±0.22	6.52±0.22	6.16±0.22	8.20±0.13	8.46±13	8.32±0.13
Traits	**Week 5**			**Week 7**		
Body weight (g)	1104±69.25	1060±69.25	1110±69.25	1775±22.17	1732±22.17	1732±22.17
Body length (cm)	26.04±0.62	26.14±0.62	24.68±0.62	29.15±1.10	29.62±1.10	27.49±1.10
Thigh length (cm)	10.54±0.31	9.68±0.31	9.84±0.31	13.47±0.17	13.17±0.17	13.55±0.17
Shank length (cm)	5.32±0.16^{b}	5.38±0.16^{b}	5.92±0.16^{a}	5.87±0.10	5.87±0.10	5.89±0.10
Breast girth (cm)	12.04±0.20^{b}	12.12±0.20^{b}	16.46±0.20^{a}	13.70±1.52	17.37±1.52	13.69±1.52

^{ab} means along rows with different superscripts are significantly different; Strain A: Arbor Acre Strain B: Hubbard, Strain C: Marshall

Table 3: Breed differences in feed intake, feed conversion and feed efficiency of varied strains of broiler chicks raised in deep litre at 1, 3, 5 and 7 weeks

Week	Strains	Production Parameters		
		Feed intake	Feed conversion	Feed efficiency
1.	A	290^{a} ± 0.00	2.36^{c}±0.00	0.42^{a} ± 0.00
	B	278.6^{b} ± 0.00	2.43^{b}±0.00	0.41^{b} ± 0.00
	C	278.5^{c} ± 0.00	2.85^{a}±0.00	0.36^{c} ± 0.00
3.	A	1150^{a} ± 0.00	2.17^{c}±0.00	0.46^{a} ± 0.00
	B	1148^{b} ± 0.00	2.30^{b}±0.00	0.44^{b} ± 0.00
	C	1144^{c} ± 0.00	2.49^{a}±0.00	0.42^{c} ± 0.00
5.	A	1721^{b} ± 0.00	1.56^{b}±0.00	0.64^{a} ± 0.00
	B	1696^{c} ± 0.00	1.61^{a}±0.00	0.63^{b} ± 0.00
	C	1729^{a} ± 0.00	1.56^{b}±0.00	0.64^{a} ± 0.00
7	A	2200^{c} ± 0.00	1.24^{b}±0.00	0.807^{a} ± 0.00
	B	2201^{b} ± 0.00	1.27^{a}±0.00	0.787^{b} ± 0.00
	C	2203^{a} ± 0.00	1.27^{a}±0.00	0.786^{c} ± 0.00

^{abc} means along columns with different superscripts are significantly different, Strain A: Arbor Acre, Strain B: Hubbard, Strain C: Marshall

This result agreed with those obtained in the previous studies (Leeson *et al.,* 1997; Faran *et al.,* 2000a; Faran *et al.,* 2000b) who reported marked strain differences for body weight in chickens. There were also significant (P<0.01) strain differences in thigh length of broiler chickens in the present study. Strains A and C were similar in thigh length, but higher than strain B. Strain differences in body length, shank length and breast girth were however not significant (P>0.05) in the first week of birds' age. At 3rd week, strains A and C still maintained their superiority in body weight despite the fact

that they were all given the same treatment in terms of feed quality and quantity. In agreement with the present study, Deep and Lamont (2002), Rondelli *et al.* (2003), Zhao *et al.* (2009) and Taha *et al.* (2010) observed that strains differed in growth rate and weight gain at different ages. Their findings also corroborate those of Pingel *et al.* (1990) who reported that age was the major determinant of growth and physiological development in chicks. The other traits, that is, body length, shank length and breast girth measured at 3rd week were not significantly (P>0.05) affected by genotype.

The raised chicken, strain C, at maturity had broader chest and longer shank than strains A and B. This singular attribute could be used to differentiate between the various strains at matured weight. This result was comparable to the findings of Ajayi and Ejiofor (2009) who reported significant effect of genotype on breast girth and shank length. The non-significant effect of strain on other traits at 5th and 7th week of age implies that the three strains were at par in terms of body weight, body length and thigh length. Our data on body weight at maturity were inconsistent with those of Ajayi and Ejiofor (2009) and Razuki *et al.* (2011). Significant effect of strains on body weight was observed between 6th and 7th week of age. The observed differences between the present study and those of Ajayi and Ejiofor (2009) and Razuki *et al.* (2011) might be as a result of differences in genetic constitution of the birds used, health status and management practices. The differences in genetic make-up coupled with the bird's inherent abilities to adjust and adapt to fluctuating weather conditions are the major factors determining the reproductive performance of any breed of chickens reared in any production environment. Regardless of strain, there was an increase in body weight and all other linear measurements as the birds advanced in age.

With regard to feed intake, there was an increase in feed consumption as the birds advanced in age, and this increment occurred across the strains. Between 1st and 3rd week, strain A consumed more feed than strains B and C, and this might be the reason why the former recorded superior body weight during this period. The result agreed with those of Leeson *et al.* (1997), Rondelli *et al.* (2003) and Taha *et al.* (2010) who found significant differences in feed intake among strains of chickens. Feed intake between 5th and 7th week showed that strain C had the highest mean values, intermediate in strain B, and strain A, being the lowest. This implies that at maturity, strain C consumed more feed than the rest, but the excess feed consumed was not translated to more meat because the strain performed poorly in feed efficiency. It was this same strain C that had the highest feed conversion ratio. This is an indication that the strain was a poor converter of feed to flesh, and at maturity will not generate good returns or dividends when compared with strains A and B. Generally, Strain A was superior in terms of feed efficiency from 1st to 7th week, strain B has intermediate mean values, and strain C the least.

Our data on feed conversion were consistent with previous studies in literature (Rondelli *et al*., 2003; Taha *et al.,* 2010). The researchers found significant strain differences in feed conversion among chicken breeds. Strain A with the highest feed efficiency and lowest feed conversion ratio at maturity (7th week) will be preferable to other strains for increased meat production and maximum profit. Regardless of strain, it was indicated in this study that feed conversion ratios decreased with advancing age of the birds. On the contrary however, mean values for feed efficiency increased as the birds grew in age across the strains.

In this study, it was observed that the strain with the highest feed conversion ratio recorded the lowest mean value in feed efficiency while, the strain with the lowest feed conversion had the superior mean value for feed efficiency. Strain C was a poor feed converter, and the least in feed efficiency, while strain A with lowest feed conversion ratio recorded the highest mean value in feed efficiency, and therefore, the preferred one among the three genotypes. It infers that a good and feed efficient strain must have the least feed conversion ratio for the sustainability of poultry farming. The result of this study corroborates the findings of Adebambo *et al.* (2008) and Olawumi and Dudusola (2011) who reported

significant breed differences in feed efficiency among different strains of chickens. In general terms, there was consistent increase in body weight and linear measurements with advancing age of the birds regardless of bird's genotype. In addition, feed conversion ratio and feed efficiency were related but in a reverse manner. As one increases with the advancing age of the birds, the other one decreases in mean value under normal circumstances.

Conclusions: (i) Genotype of birds had significant ($P<0.01$) effect on all the performance traits of broiler chicken breeds. (ii) Strains A and B appeared superior to strain C in body weight, but the latter was better ($P < 0.01$) in shank length and breast girth than the former. (iii) As regards feed conversion, strain C was the poorest while strain A was the best. (iv) The feed efficiency and feed conversion ratio are related but in a reverse manner, that is, one (feed efficiency) increases with advancing age of the birds while the other (feed conversion) decreases in value simultaneously. (v) Therefore, strain A with the highest mean values for body weight and feed efficiency coupled with the lowest feed conversion ratio is recommended to researchers and poultry farmers in this zone for increased productivity and maximum profit.

ACKNOWLEDGEMENTS

The authors are indebted to the Staff of Teaching and Research Farm, Ekiti State University, Ado-Ekiti for their support and assistance during the period of data collection.

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EFFECT OF *Prosopis africana* ETHANOLIC LEAF EXTRACTS ON PACKED CELL VOLUME OF *Rattus norvegicus*

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ABSTRACT

This research sort to find out if Prosopis africana ethanolic leaf extract can help in fighting against anaemia in animals which is among the major causes of death in animals in developing countries. Seventy-five albino rats of 90 days old and approximately 200g were used. The rats were randomly selected and placed into five cages representing five different treatments. The extract of Prosopis africana was given to the rats in solution as daily water. Group A rats (cage A) received 0 ml of the extract per 100 ml of distilled water and served as control group. Group B rats (cage B) received 1 ml of extract in 100 ml of distilled water, group C rats (cage C) received 2 ml of extract in 100 ml of distilled water, and group D rats (cage D) received 3 ml of extract in 100ml of distilled water, while group E rats (cage E) received 4ml of the extract in 100 ml of distilled water. Blood samples were collected weekly for eight weeks using capillary tubes and centrifuged with haematocrit centrifuge at 10,000 rpm for 5 minutes. At the end of the experiment, it was discovered that rats in cage E had the highest level of PCV (45.70%), followed by rats in cage D (42.93%) and those in cage C (41.97%). Rats in cage B had the lowest PCV among the treated groups, while rats in cage A had the lowest PCV among the treated and untreated groups.

Keywords: *Prosopis africana, Rattus norvegicus,* Packed cell volume, Anaemia

INTRODUCTION

Prosopis africana is a genus of flowering plant in pea family Fabaceae. It contains about forty-five species of spiny trees and shrubs. It is found in sub tropical and tropical regions of America, Africa, West Asia and South Asia. It is known to contain a myriad of complex chemical compounds which is health wise beneficial to humans and animals (Edeoga *et al.,* 2005). This plant has been used traditional in the treatment of various types of ailments (Adewumi *et al*., 2001; Tagboto and Townson, 2001; Aderbauer *et al*., 2008). The tree, *Prosopis africana* is commonly called iron wood. It provides nutritional service whereby people use it as special spices in local relishes. The seed is used as food condiments. *Prosopis africana* has been reported to be of medicinal value, its gum is used in pharmaceutical industries as gel in tablets formation (Attama *et al*., 2000; Adikwu *et al*., 2001). It is also used as an anti-tyrosine and because of that, the plant may be useful in preventing skin whitening or as anti-browning agent (Baurin *et al*., 2002). This plant is listed among the plants used by local farmers in the treatment of trypanosomiasis in Northern Nigeria (Atawodi *et al*., 2002). Almost all the parts of *Prosopis africana* are used in medicine; the leaves are used for treatment of headache and toothache; the back is used for eyewashes; the roots are used for treatment of gonorrhea,

tooth and stomach ache (Tagboto and Townson, 2001).

Thus, this study sort to find out if *Prosopis africana* leaf extract can help in building up the packed cell volume of rats and as such an agent in fighting against anaemia in animals which is among the major causes of death in animals in developing countries.

MATERIALS AND METHODS

Extract: The leaves of *Prosopis africana* plant were collected and sun dried for seven days. The dried leaves were blended into powder. 70% ethanol was added to the power and mechanically shake for five minutes. The alcoholic content of the extract was evaporated using a vacuum evaporator. The extract was bottled and kept in refrigerator pending use.

Animal: Seventy-five albino rats of 90 days old and approximately 200 g were used. The rats were kept in stainless wire rat cages for one week to acclimatize before the commencement of the experiment. Five rats were randomly assigned to each cage that represents different experimental design.

Treatments: The rats were divided into five groups; A, B, C, D and E. Group A in cage A, received 0 ml of the extract and serve as control group. Group B in cage B received 1ml of the extract dissolved in 100ml of distilled water, group C in cage C received 2ml dissolved in 100ml of distilled water, group D in cage D received 3 ml dissolved in 100 ml of distilled water while group E in cage E received 4ml dissolved in 100ml of distilled water. The solution was given to the rats in drinkers which serve as their daily water. All rats were fed (Guinea Feed Growers Mesh) and water *ad libitum.* The experiment was replicated three times.

Analysis: The blood samples of the experimental animals were collected before the commencement of the experiment, then after one week, administration of the extract started. The blood samples were collected weekly for eight weeks. The blood samples were collected using capillary tubes and centrifuged with haematocrit centrifuge at 10,000 rpm for 5 minutes. The data were obtained using haematocrit reader.

The data collected were subjected to descriptive statistics at 95% confidence limit and the output presented in percentages (Steel and Torrie, 1990).

RESULTS

From the analysis of the data, it was observed that there was significant difference ($P < 0.05$) between the rats in different cages which received different dosage of the leaf extract and the control. There was progressive increase in the PCV levels as the week progressed (Figure 1). Rats in cage E had the highest PCV, followed by rats in cages D, C and B sequentially, while rats in cage A had the lowest PCV (Figure 2). This showed that *Prosopis africana* boosted the PCV level in rats.

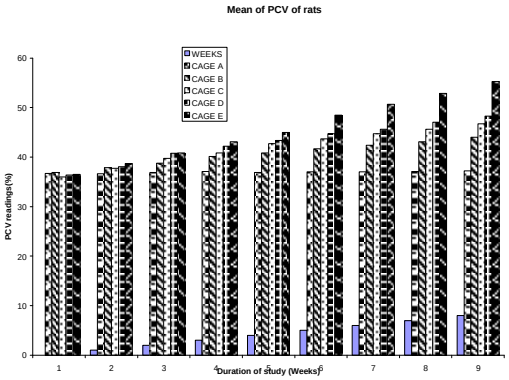

Figure 1: Weekly increase in packed cell volume of *Rattus norvegicus* treated with *Prosopis africana* ethanolic leaf extracts

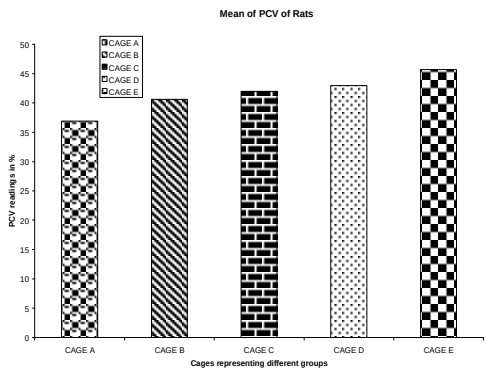

Figure 2: Dose dependent increase in packed cell volume of *Rattus norvegicus* treated with *Prosopis africana* ethanolic leaf extracts

DISCUSSION

It was observed that the extract of *Prosopis africana* has positive influence in the PCV of rats. Edeoga *et al.* (2005) reported that the *Prosopis africana* contains a myriad of complex chemical compounds which health wise is beneficial to humans and animals. *Prosopis africana* extracts in this study boosted PCV in animals. At the end of the experiment, it was discovered that rats in cage E had the highest level of PCV while the control group had the lowest level of PCV. It has been reported that this plant is used traditional in the treatment of various types of ailments (Adewumi *et al*. 2001; Tagboto and Townson, 2001; Aderbaner *et al*., 2008). The result obtained in this study indicated that this plant can help fight anaemia in animals because it increases the level of PCV.

Conclusion: *Prosopis africana* has positive influence on the PCV of rats. We therefore suggest that this plant can be used in the treatment of anaemia. The extract can also serve as blood tonic for animals. Interest in further research may include adapting the findings to humans.

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EFFECT OF DIETARY VITAMIN A SUPPLEMENT ON SERUM PROTEIN OF RATS INFECTED WITH *Trypanosoma brucei*

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ABSTRACT

The role of dietary vitamin A supplement on the serum protein of trypanosome-infected rats was studied. The rats were inoculated with trypanosomes intraperitoneally and samples were collected on fourth, eight, twelfth and sixteenth days post infection. The experiment was carried out at the Department of Biochemistry, Nnamdi Azikiwe University Awka. Sixty parasite free-albino rats were used, which were divided into four groups. Group A (control) was left uninfected with trypanosomes, group B and C were infected with trypanosomes and treated with 50mls and 100mls of vitamin A per kg of feed respectively and group D was infected and left untreated with vitamin A. Analyses of the sera using Bradford method and cellulose acetate electrophoresis showed that vitamin A influenced the state of hypoproteinaemia in the trypanosome-infected rats. This was manifested by a positive increase in the level of total serum protein concentration, albumin and beta-globulin. Vitamin A also delayed the proliferation of the parasites associated with trypanosomiasis.

Key words: Vitamin A, Dietary supplement, *Trypanosoma brucei*, Serum protein, *Rattus novegicus*

INTRODUCTION

For several decades, trypanosomiasis has continued to contribute adversely to the economic well being of sub-Sahara Africa. Trypanosomiasis is one of the most important livestock diseases in sub-Saharan Africa (Morrison *et al.,* 1981). This scourge remains a pressing challenge especially to African scientists in formulating possible action plan that would eradicate trypanosomiasis from the African continent. The articulation of such plan would include both preventive measures and treatment modalities.

Trypanosome is known to attack red blood cells and vascular endothelium. It concentrates more in the peripheral circulation (Jackson, 1979). These trypanosomes exhibit remarkable antigenic variation of their surface glycoprotein with hundreds of antigenic type. One antigenic type will coat the surface of the parasites for approximately 10 days followed by other types in sequences in the new progeny. This variation is due to sequential movement of the glycoprotein genes to a preferential location of the chromosome were only that specific gene is transcribed into messenger RNA. These antigenic variations allow the organism to continually invade the host immune response. The first wave of parasitemia is accompanied by depressed packed cell volume, neutropenia and thrombocytopenia (Krampitz, 1970).

Trypanosomiasis in rats is associated with a decreased serum protein as infection progressed. Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and also influences the rate of recovery (Katungka-Rwakishaya, 1996).

It was also discovered that supplementary feeding significantly reduces the severity of trypanosomiasis (Agyemang *et al.,* 1990; Little *et al.,* 1990). Vitamin A is essential for the development of bursa of fabricius, thymus and immunity in chickens (Raza *et al.*, 1997). Vitamin A supplements strongly increase serum protein and glycoprotein synthesis which maintain synthesis of surface coating mucopolysaccharides. Hence increase mucosal immune system, together with the epithelial rebuilding character of vitamin A raise macrophage activity and increase humoral and cell mediated response of vitamin A sufficient chicken; strongly suggest a much high resistance to infection of higher animals (Sijtsma *et al*., 1991; Johannsen *et al*., 1998).

Over the years, vitamin A has been used to tackle all kinds of infections and it is very popular both among the low, middle and higher socio-economic class (Benynen *et al*. 1989; Bang *et al*., 1995). This study was design to evaluate to what extent this vitamin A can influence the state of hypoproteinaemia in trypanosome-infected rats.

MATERIALS AND METHODS

Twenty 90-day old male albino rats (*Rattus norvegicus*) weighing approximately 145g, were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house. The cages were labeled A to D corresponding to four groups and each group had five rats. Diet 1 was given to rats in Cage A which contained 1kg of chick mash without vitamin A. Diet 2 was given to rats in Cage B which contained 1kg of chick mash mixed with 50mls of vitamin A. Diet 3 was used to feed rats in Cage C which contained 1kg of chick mash mixed with 100mls of vitamin A and Diet 4 was used to feed rats in Cage D which contained 1kg of chick mash without any vitamin A. Rats in Cage A were not infected while rats in Cages B, C and D were infected with *Trypanosoma brucei*. One rat was first inoculated with trypanosome of NITR type from Veterinary Medicine Faculty, University of Nigeria, Nsukka. It was isolated from other animals and after 14 days of inoculation, the blood of that rat was used to inoculate others. Each experimental rat that was inoculated was given 0.1ml of infected blood in normal saline, which contained about eight thousand trypanosomes, using a matching chart (Herbert and Lumbsden, 1976) to determine the level of parasitemia. Rats in Cages A and D served as control groups. Each experimental set up was replicated three times. The rats had unlimited supply of clean water.

Five (5) ml of the blood of the rats were collected in each experimental day which was four days intervals for sixteen days of the experimental period to determine the total serum protein. The collected blood was allowed to clot for about 30 minutes at room temperature. Then each sample was centrifuged at 3,000 rpm for 10 minutes and the serum was removed. The sera were used immediately for serum protein determination using Bradford method. The absorbance of the solutions was read at 520nm-wavelenghts using spectrophotometer.

Statistical Analysis: The data on change in total serum protein level were statistically analysis using analysis of variance (ANOVA) at 95 percent probability level. Results obtained were reported as mean concentrations.

RESULTS

The administration of vitamin A positively influenced the serum protein of trypanosome-infected rats. There was increased total serum protein, raised albumin and beta-globulin in the infected and treated rats. The lowest level of mean total serum protein of 49.27 ± 5.32g/l, albumin of 17.85 ± 3.35g/l and beta-globulin of 3.46 ± 0.82g/l were observed in infected and untreated rats (Cage D). Furthermore, 50.79 ± 5.05g/l of total serum protein, 20.77 ± 3.53g/l of albumin and 5.15 ± 0.48g/I of beta-globulin were observed in infected rats treated with 50ml of vitamin A per kg of chick mash (Cage B). Then infected rats which were treated with 100ml of vitamin A per kg of chick mash had 52.2 ± 4.24g/l of total serum protein, 22.99 ± 2.97g/l of albumin and 5.36 ± 0.64g/l of beta-globulin (Cage C).

Table 1: Total serum protein, albumin and beta-globulin of rats infected with *Trypanosoma brucei* fed dietary vitamin A supplement

Length of post-infection	Group A (uninfected and untreated)	Group B (infected and treated with 50ml vitamin A/kg of feed)	Group C (infected and treated with 100ml vitamin A/kg of feed)	Group D (infected and untreated)
		Total serum protein (g/l)		
4	60.63	59.80	59.82	59.53
8	59.84	55.88	56.24	54.04
12	61.65	50.83	52.46	48.79
16	61.86	36.67	40.31	34.71
Total	243.98	203.18	208.83	197.07
Mean	60.99 ± 0.47	50.79 ± 5.05	52.21 ± 4.24	49.27 ± 5.32
		Albumin (g/l)		
4	32.68	30.02	30.21	27.40
8	30.93	20.43	24.62	16.51
12	32.69	19.75	21.06	15.73
14	32.64	12.87	16.09	11.74
Total	128.94	83.07	91.98	71.38
Mean	32.24 ± 0.44	20.77 ± 3.53	22.99 ± 2.97	17.85 ± 3.35
		Beta-globulin (g/l)		
4	7.88	6.45	6.92	5.84
8	8.66	5.18	5.77	3.19
12	8.16	4.21	4.86	2.68
14	8.35	4.76	3.91	2.12
Total	33.05	20.60	21.46	13.83
Mean	8.26 ± 0.16	5.15 ± 0.48	5.36 ± 0.64	3.46 ± 0.82

The uninfected and untreated rats (Group A) had the highest mean levels of total serum protein (60.99 ± 0.47 g/l), albumin (32.24 ± 0.44g/l) and beta-globulin (8.26 ± 0.16g/l) (Table 1).

DISCUSSION

Several scientific researches have been done on trying to identify and standardize active food supplement that would be active in treatment of trypanosomiasis. This trypanosomiasis has contributed adversely to the economic and social well being of Sub-Sahara Africans. A lot of scientific investigations have proved that retinol and retinoic acid nutrient produced significant enhancement on the immune system of rats (Nauss *et al*., 1985).

The observed effect of vitamin A supplement from this study on the serum protein of trypanosome-infected rats is attributed to its effect on the haemopoietic system. The effect of vitamin A on the infected and treated rats when compared with the infected and untreated rats showed that vitamin A had positive influence on the defense capacity of infected and treated animals. Therefore, this study has provided evidence that vitamin A has a potential for influencing the state of hypoproteinaemia in the trypanosome-infected rats. Even if vitamin A cannot destroy the trypanosomes, it can ameliorate the stress of trypanosomiasis and as enhanced the hosts' immune system to fight the invaded pathogens.

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ARTHROPOD FAUNA OF THE UNIVERSITY OF NIGERIA, NSUKKA, SEWAGE POND

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ABSTRACT

A survey of arthropod fauna of the University of Nigeria Nsukka sewage pond was carried out within May and June 2011. The aim was to determine the various arthropod species and its abundance in the sewage pond. The analysis was carried out by two methods, physico-chemical analysis and arthropod faunal studies. The physico-chemical parameters of the pond which included dissolved oxygen, temperature, water hardness, carbon dioxide, pH, depth and alkalinity were recorded. The arthropod faunal study was gotten through sample collections of shoreline zone with the use of insect net and scoop net, the mid benthic zone with the use of Eckman grab. The arthropods found were of the class insecta and class arachnida. The sewage pond had a high accumulation of organic waste with mean dissolved oxygen calculated to be 6.48. As a result of this high content of organic waste, most aerobic organisms cannot survive in the pond, and this resulted to low abundance of arthropods.

Keywords: Arthropod fauna, Sewage pond, Physico-chemical parameters, Biological characteristics

INTRODUCTION

Arthropoda is the most extensive phylum in the animal kingdom. They are everywhere and about 85% of all known animals in the world are part of this group. There are far more species of arthropods than there are species in all the other phyla combined. This can be seen in the work of Eyo and Ekwonye (1995) in a survey of the macro invertebrate fauna of the Fadama pools of Anambra, where they documented over 86% of macro invertebrate fauna belonging to the phylum arthropoda. Arthropods can be seen as an enormous assemblage of species that dwell in both marine, fresh water, air and terrestrial habitats and share common characteristics of having a hard exoskeleton (which acts as a template for body form which facilitates levers for locomotion, allows development of hard structures such as jaws and reduces water loss) and several pairs of jointed legs that may be used for a variety of purposes including swimming, walking, leaping and digging etc. (Barnes, 1968). These arthropods were originally thought to be related to annelids which have paired segmental appendages. The phylum arthropoda is divided into five classes: (i) arachnida e.g. scorpions, spiders, (ii) crustacea e.g. shrimps, crabs, (iii) diplopoda e.g. millipede, (iv) chilopoda e.g. centipede and (v) insecta e.g. grasshoppers, dragonfly (Smith, 1973). Karen and Flake (1995) reported that generally sewage ponds had higher nitrogen and phosphorus levels than industrial and radioactive ponds and higher number of insects

(notonectidae, dytiscidae, chironomidae, daphnidae, eucopepoda, ostracoda and odonata) than industrial and radioactive ponds. Rouhollah *et al.* (2007) reported that out of 1032 collected samples from four sewage maturation ponds, the most prevalent insect groups were: Diptera (52%), Hemiptera (24%), Ciclopodidae (12%), Hydroacarina (9.5%), Coleopteran (0.77%), Aranida (0.67%), Hymenoptera (0.58%) and Odonata (0.48%). Also the families of Chironomidae and Culicidae from Dipteran order, Notonectidae from Hemiptera order were also dominate.

The specific objectives of this study were to determine the various arthropod species inhabiting the University of Nigeria, Nsukka, sewage pond, their percentage composition, abundance and the physico-chemical parameters influencing them.

MATERIALS AND METHODS

Description of the Study Area: The sewage pond of the University of Nigeria Nsukka is located beside the junior staff quarters and is about 181.5 meters yards long and 158.1 meters wide. It is immediately surrounded by farmland. There are two operational open ponds in the sewage unit, each about 125 meters long, 45.70 meters wide and 36 cm feet deep. The pond is connected to four soaked away pits that measures about 30 – 35 cm deep while the rest of the enclosure is planted with grasses.

Physico Chemical Analysis

Temperature: The temperature of the pond water was measured using clinical mercury in glass thermometer. This was done by dipping the thermometer in the pond water and allowing it to stay for about five minutes to stabilize before reading.

Alkalinity: This was determined by adding 2 drops of methyl orange indicator in 100ml of pond water in a conical flask. 0.02m standard H_2SO_4 was filled in a burette and used to titrate against the solution in the conical flask, the end-point was attained when the colour of the solution changed from orange to pink. Alkalinity calculated using the formula: Alkalinity = A x M x 50 x 1000 (mg/L) / V, where A = the titre value, M = molarity of the acid and V = volume of sample (100 ml).

Carbon iv oxide: Free carbon iv oxide was determined by adding 10 drops of phenolphthalein indicator to 100ml of pond water and then shaken thoroughly. The solution was titrated with standard sodium carbonate (Na_2CO_3) until the end point was reached (when the solution turns pink). The pink colour lasted for about 20 seconds before the colour disappeared again. Free carbon iv oxide was calculated thus; Free CO_2 = A x N x 22,000 (mg/l) / V, where: A = titre value, N = molarity of Na_2CO_3 and V = volume of sample (100 ml).

Water hardness: This was determined by adding 2ml of buffer solution to 100ml of water in a conical flask. 8 drops of Erichrome Black T indicator was added and the solution shaken thoroughly. The solution was titrated with standard Ethylenediaminetetracitic acid (EDTA) and the end point was reached when the solution turned blue. The water hardness was calculated thus: Hardness = TV x M x 1000 mg/L / V, where TV = Titre value, M = molarity of EDTA used and V = volume of sample (100 ml).

Dissolved oxygen: This was determined using the Winkler's method. The water sample was collected under water using a 500 ml volumetric flask. The water sample was fixed at the site using 2 ml of manganese sulphate and 2 ml of alkaline potassium iodide, a precipitate was formed which was dissolved by adding 2ml of concentrated tetraoxosulphate vi acid (H_2SO_4) solution turned to very light orange colour. In the laboratory 100ml of the fixed water was titrated using sodium thiosulphate ($Na_2S_2O_3$), the colour changed from orange to a milky solution. Dissolved oxygen = Titre value x M x 8 x 1000 (mg/L) / V, where M = molarity of $Na_2S_2O_3$, 8 = Atomic no of oxygen and V = volume of sample (100 ml).

Arthropod Faunal Study

Shore/littoral zone: This zone is exposed to air, solar radiation and at times covered by water. The scoop net or pond net was used for collecting organisms from the shallow area of the pond (Inyang *et al.,* 2006).

Mid channel benthic zone: The organisms found in the benthic zone of the pond were sampled using Eckman grab.

Identification: All the arthropods collected were sorted out, identified (Hyman, 1959; Barnes, 1968; Hynes, 1970; Russell-Hunter, 1979; Egborge, 1993) and preserved in 5% formalin to which some quantity of glycerol was added. Very large specimens were preserved in 95 parts of 70% alcohol plus 5 parts of glycerol (Russell-Hunter, 1979).

Data Analysis: Data collected were analyzed using descriptive statistics, analysis of variance (ANOVA) and Spearman's correlation at P< 0.01.

RESULTS AND DISCUSSION

The results of the physico-chemical parameters of the sewage pond (Table 1) indicated acidic pH, high free carbon dioxide and low alkalinity among others.

Table 1: Physico-chemical parameters of University of Nigeria, Nsukka, sewage pond

Physico-chemical parameters	Values
Free carbon dioxide	1.319 ± 0.264 mg/l
Alkalinity	0.023 ± 0.36 mg/l
Temperature	27.43 ± 3.04 ^{o}C
Dissolved Oxygen	6.47 ± 2.13 mg/l
Depth	5.0 ± 1.34 m
water hardness	0.22 ± 0.09 mg/l
pH	6.7 ± 2.23 pH

Spearman's correlation indicated that temperature had a perfect positive correlation with carbon dioxide, water hardness and alkalinity ($r = 1$, $P < 0.01$). Carbon dioxide, temperature, water hardness and alkalinity had perfect positive correlation ($r = 1$, $P < 0.01$), while depth and pH had perfect positive correlation ($r = 1$, $P < 0.01$).

The arthropods collected in the sewage pond were insects belonging to 4 orders namely Diptera, Hymenoptera, Coleoptera and Odonata and Chilopoda belonging to the order Scorpionida from the class (Tables 2, 3 and Figure 1). Both aquatic and non-aquatic insects were collected. The non-aquatic insects were collected around the pond and they include grasshoppers, houseflies and butterflies. The higher the diversity index, the more diverse were the arthropod. The scorpionids had perfect positive correlation with the dipterans ($r = 1.00$, $P < 0.01$). The scorpionids may have depended on the dipterans for food and survival. The coleopterans had negative correlation with the dipterans and the group scorpionida ($r = -1$, $P > 0.01$). The odonatans had perfect positive correlation with the dipterans and the group scorpionids ($r = 1.00$, $P < 0.01$). The hymenopterans have no correlation with the other four groups.

The relationship between physico-chemical parameters and arthropods indicted that temperature, carbon dioxide, dissolved oxygen and water hardness had negative correlation with abundance of dipterans, scorpionidans and odonatans ($r = -1$, $P > 0.01$) and a positive correlation with abundance of coleopterans ($r = 1$, $P < 0.01$). The depth of the pond, pH and alkalinity had negative correlation with abundance of dipterans and scorpionidans ($r = -1$, $P > 0.01$) but no correlation with the abundance of hymenopterans and coleopterans.

The sewage pond in University of Nigeria Nsukka was nutrient rich. Organic enrichment led to euthrophic conditions and may be the cause of high algal species diversity and low number of invertebrate taxa found. The reduced number of arthropod taxa in sewage may be due to lack of emergent vegetation in pond (Gordon, 1990). High organic waste content has been reported as another possible cause of low faunal species richness in waste water ponds (Pearson and Penridge, 1987).

Table 2: Arthropods species composition and abundance in University of Nigeria sewage pond

S/N	Species	WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	Abundance	% Abundance
1.	**Dragonfly**	7	8	5	7	10	4	3	6	50	8.10
2.	**Damselfly**	5	7	3	4	5	4	5	5	38	6.16
3.	***Culex* spp**	20	32	17	27	14	36	42	16	204	33.06
4.	**Chironomids**	3	4	5	8	6	4	7	2	39	6.32
5.	**Ants**	4	3	5	6	3	2	4	3	30	4.86
6.	***Laccotrephes* spp**	10	14	9	12	10	9	15	12	91	14.75
7.	***Nepa* spp**	8	5	7	8	6	2	4	3	43	6.97
8.	***Cybister* spp**	2	3	1	2	3	2	1	2	16	2.59

WK = week

Table 3: Major arthropod orders associated with University of Nigeria sewage pond

S/N	Order	WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	Total
1.	**Coleoptera**	2	3	1	2	3	2	1	2	16
2.	**Diptera**	23	36	20	35	20	40	49	18	243
3.	**Hemiptera**	2	1	3	2	1	1	2	3	15
4.	**Hymenoptera**	4	3	5	6	3	2	4	3	30
5.	**Lepidoptera**	2	4	5	2	3	3	2	2	23
6.	**Odonata**	12	15	8	11	15	8	8	11	88
7.	**Orthoptera**	12	15	8	11	15	8	8	11	88
8.	**Scorpionida**	18	19	16	20	16	11	19	15	134

WK = week

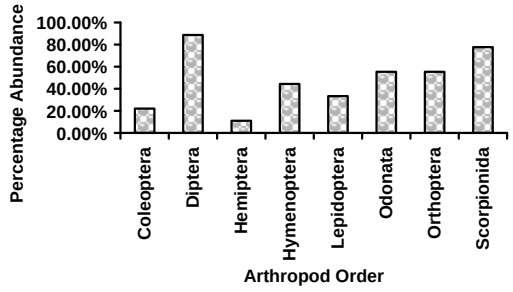

Figure 1: Persentage abundance of arthropods in University of Nigeria, Nsukka, sewage pond

Hilsenhoff (1988) assigned arthropod families from streams in the great lakes region to tolerance values ranging from 0 (lowest tolerance to organic pollution) to 10 (highest tolerance to organic pollution). Eleven of the families for which he presented tolerance values were found in INEC ponds and only two had tolerance values of less than 4. These 11 families and their tolerance values were as follows: Aerhnidae and Tipulidae (3), Baetidae, Elmidae and Leptoceridae (4), Ceratopogonidae (6), Caenidae (7), Chironomidae and Talitridae (8), Coengrionidae (9) and Psychodidae (10). The two families with 3 tolerance rating were represented by only single specimens in INEC wastewater ponds.

Furthermore high invertebrate growth and abundance have been associated with high algal productivity (Hyman, 1959). Taxa found in greater abundance in sewage ponds than in industrial ponds were those that could take advantage of the unique and difficult living conditions. Eutrophic waters typically exhibit lower dissolved oxygen concentrations and greater fluctuations in dissolved oxygen and pH. The population of the non-aquatic insects revealed that members of the dipterans were the most abundant and may be involved in the mechanical transmission of agents of intestinal disease associated with the sewage.

Temperature affected the physical, chemical and biological processes in the sewage pond by altering the concentration of dissolved oxygen, pH and thus rate of photosynthesis. The lives of most of the aquatic organisms were controlled by water temperature as shown in this study. The average temperature of the pond being 27.43 ± 3.04 ^{o}C was most suitable for the growth of arthropods because

temperature influenced water quality and the distribution and abundance of arthropods (Wallace *et al.,* 1984). pH higher than 7 but lower than 8.5 was ideal for biological productivity, while pH lower than 4 was detrimental to aquatic life (Ezekiel *et al.,* 2011). As can be seen in this study 6.7 ± 2.23 pH was most ideal and therefore influenced the low species abundance in this sewage.

When water contains large amounts of organic wastes, the rate of micro-organism activity (effective decomposition) may be high and the water may rapidly become depleted of oxygen (Mancy and Jaffe, 1966). Therefore since the sewage pond had a high accumulation of organic waste with 6.48 mg/l dissolved oxygen aerobic organisms cannot survive thus resulting to low abundance of arthropods.

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ANIMAL RESEARCH INTERNATIONAL

Volume 9 Number 1, April 2012

Global Impact Factor: 0.562

http://globalimpactfactor.com/journals-list/?snap=A

Published by Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria