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A METHOD TO DETERMINE ADHESION OF SUPPOSITORY MASS ON EXCISED INTESTINAL TISSUE

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ABSTRACT

A method to determine adhesion of suppository mass to intestinal tissue was developed using excised pig intestine. The method which employs the principle of drainage unto and subsequent detachment from the mucosa, of an adherent suppository mass is simple, inexpensive and accurate. Fully optimised, it can be used to assess differences between suppository formulations, as shown by the preliminary results obtained with commercial Anusol formulations.

Keywords: Adhesion, Suppository mass, Excised intestinal tissue, Perfusion, Detachment

INTRODUCTION

The potential use of the rectum for the systemic delivery of drugs is a relatively recent idea, even though administration of drugs in the rectum, using the suppository dosage form, is an old practice (Lieberman and Anschel, 1979; Helliwel, 1993; Adikwu and Okafor, 2006).

The advantage of rectal delivery must be the reduced extent of hepatic first pass elimination of drug, especially when the drug is administered in the lower region of the rectum (Onyechi and Martin, 1995).

The limited results obtained with oral bioadhesive systems for enteral delivery drive the search for formulations for rectal delivery. Such systems should prolong the gastrointestinal (GIT) transit time of the drug delivery system and subsequently improve the bioavailability of drug loaded into it (Kellaway *et al*., 1984; Pritchard *et al.*, 1996; Helliwel. 1993; Smart *et al*., 1994; Leung and Robinson, 1990; Lehr *et al.,* 1990).

To date drainage and perfusion techniques are used in bioadhesion studies. These techniques provide some approximation to *in vivo* conditions (Kellaway et al, 1984; Pritchard *et al*, 1996; Helliwel, 1993; Smart *et al*, 1994; Onyechi and Martin, 1995; Leung and Robinson, 1990; Lehr *et al.,* 1990).

The aim of this study was to design intestinal drainage and detachment techniques as well as model drainage and detachment of commercial Anusol products. Such techniques developed would then be applied to the development of sustained release (SR) bioadhesive formulations for Anusol products.

MATERIALS AND METHODS

Animal Model: Intestinal tissue was obtained from freshly slaughtered pits. After cleaning and bathing in normal saline, the tissue was cut into 10 – 12 cm pieces and stored in self-sealed freezer bags at -16^{0}C until required.

Formulations: Suppository samples formulated at Parke-Davies were used in the drainage studies. For detachment experiments, 0.4 % w/w Fluorescein (BDH Chemicals Limited, Poole, England) was incorporated into the suppositories as marker. Suppositories containing Fluorescein were prepared by melting the supplied suppositories with minimum amount of heat and incorporating the required weight of material. The suppositories after cooling were stored at room temperature.

Experimental Design

***In-Vitro* Drainage Tests:** A metallic stand (Figure 1) was constructed which allowed intestinal tissue to be mounted and maintained vertically in the stand. Once the tissue was mounted, the stand was suspended vertically in a high humidity environment (Figure 2), and allowed to equilibrate in a hot air oven with temperature set at 39 ^{0}C. The suppository under test was weighed and inserted at the upper end of the mounted, excised pig intestinal tissue and allowed to drain. A sample vial (of known weight) was placed beneath the tissue assembly to collect the molten and detached suppository mass. After complete drainage, the sample vial and contents were stored in a desiccator overnight and weighed. Storage in a desiccator enabled removal of moisture adhering to the vial and contents. A fresh piece of intestinal tissue was used for each run (n=4).

***In-Vitro* Detachment Studies:** A water-jacketed glass support was constructed which allowed the mounted tissue to be maintained horizontally (Figure 3). Once the tissue containing the test suppository was mounted, the support was clamped vertically in an environment of high relative humidity and introduced into a hot air oven with temperature set at

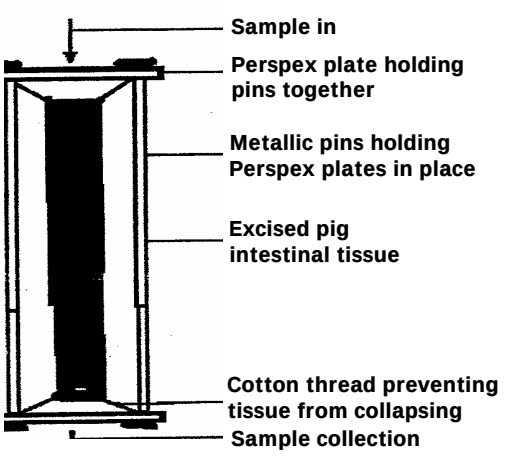

Figure 1: Device for suppository drainage/ adhesion experiments

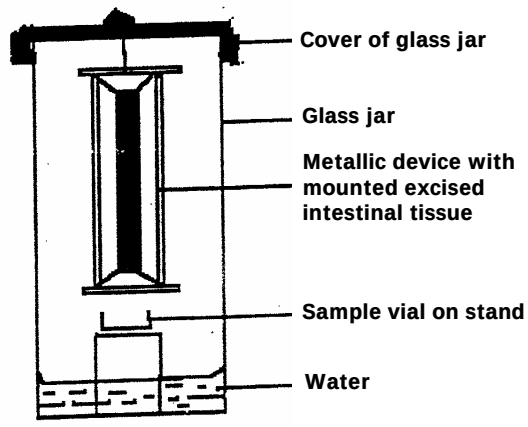

Figure 2: High humidity chamber for drainage experiments

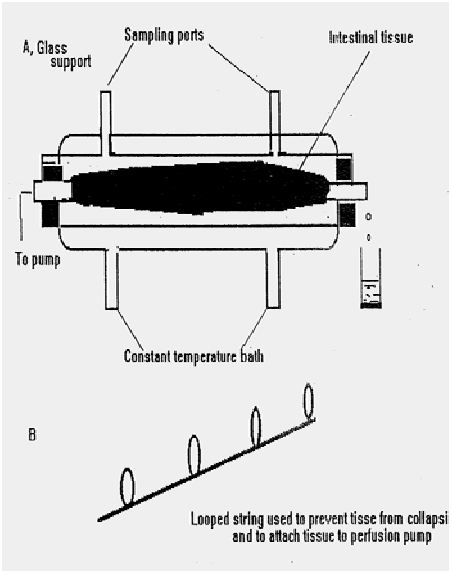

Figure 3: Water-jacketed support for *in-vitro* detachment experiments

39 ^{0}C. The suppository was allowed to drain. Any molten suppository mass which drained down the lumen of the intestinal tissue and broke away was collected and weighed. When drainage ceased, the intestinal tissue was mounted horizontally in the glass support and challenged with a flowing medium, at a fixed rate of 4 ml/minute. Distilled water maintained at 39 ^{0}C was perfused through the intestine with the aid of peristaltic pump. Perfusate samples were collected over a period of 3 hours. Sampling jars were replaced at 30 minutes intervals. A fresh tissue was used for each run (n = 3). The contents of the vial were assayed for fluorescein by spectrophotofluorimetry.

Analytical Method for the Determination of Fluorescein: Analytical techniques employed for the quantitative determination for drugs in delivery systems play a significant role in the evaluation and interpretation of data in adhesion studies. It is essential to employ well-characterised and validated analytical methods to yield reliable results which can be satisfactorily interpreted. The appropriateness of a technique is influenced the ultimate objective of the study. The size of sample involved in the detachment studies and the need for specificity, speed and economy influenced the choice of fluorimetry in the work.

The determinations were performed with a Perkin Elmer Spectrophotofluorimeter Model MDF-2A (Perkin Elmer, Norwalk, Connecticut, USA). Standard solutions of fluorescein in 0.001 M NaOH were prepared ranging from 0.05 to 0.2 ppm. The solutions were assayed at 513 nm; the wavelength of maximum emission for fluorescein and in 0.001 M NaOH is 495 nm. The calibration curve of emission intensity against concentration fluorescein constructed at this wavelength is shown in Figure 4.

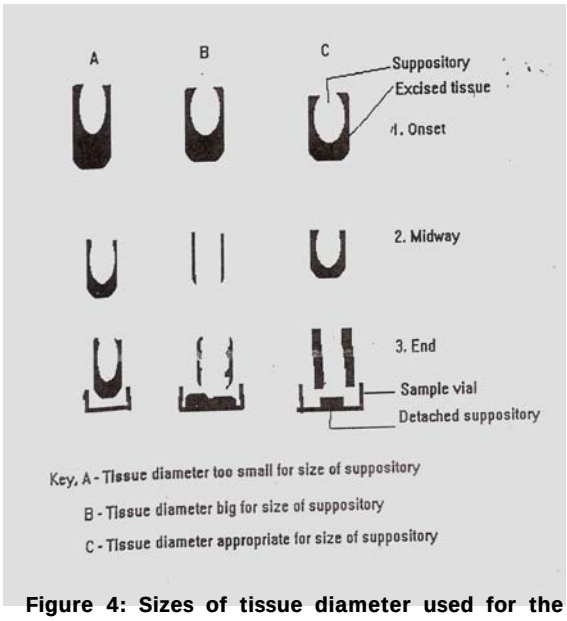

Figure 4: Sizes of tissue diameter used for the suppository

Determination of the Fluorescein Content of Anusol Suppositories: The Anusol suppositories prepared for the drainage experiments were assayed for fluorescein content. A limitation imposed by the number of suppositories prepared led to the use of only a single suppository for the test.

Aliquots of this single suppository were used. An accurately weighed amount of the suppository (5 mg) was transferred to a 500 ml volumetric flask. 25 ml of 0.001 M NaOH was added and warmed to 80 ^{0}C for 2 minutes in an ultrasonic water bath. The solution was cooled and made up to volume with 0.001 m NaOH. The resultant solution was filtered and assayed for fluorescein content. The determination was repeated 5 times.

Validation of Assay Technique: The recovery of fluorescein from the suppository mass was evaluated in order to validate the method of assay. An accurately weighed sample of the suppository mass containing fluorescein was transferred into a 200 ml volumetric flask. 25 ml of 0.001 M NaOH was added and warmed to 80 ^{0}C for 2 minutes in an ultrasonic water bath to dissolve the suppository and content. 0.001 M NaOH solutions were used to make up to volume. After filtration the solution was assayed for fluorescein content. The mass of suppository used for the validation of the assay technique was varied between 2 and 20 mg. The experiments were repeated three times for each sample size.

Determination of Fluorescein Content of Solution from Detachment Experiments: Perfusate samples were collected at 30 minute intervals from the tissue used for the detachment studies. Each sample was transferred quantitatively into a 500 ml volumetric flask and placed in an ultrasonic water bath and warmed up to 80 ^{0}C for 2 minutes. The solution was cooled and made up to volume with 0.001 M NaOH. After filtration, the samples were assayed for their fluorescein content using the analytical procedure described above. Samples were diluted before assay where necessary using 0.001M NaOH solution. The results of the detachment experiments have been expressed as percent fluorescein detached over the entire 3 hours test period. Total fluorescein detached was determined from the fluorescein content of the perfusate for the 3 hours time. The fluorescein remaining in the excised intestinal tissue at the end of the experiment was not considered in calculating percent fluorescein detached.

RESULTS AND DISCUSSION

The calibration curve of Figure 5 and the assay results of Table 1 showed that the suppositories into which fluorescein were incorporated can be assayed satisfactorily by spectrophotofluorimetry. The recovery of fluorescein using varying sample sizes (Figure 6) indicated that there exists a linear relationship between sample size and fluorescein content.

Drainage Experiments: Considerable effort was spent modifying the metallic stand for the drainage experiments. The original design involved inserting used intestinal tissue around three metallic pins fitted to a wooden base. The pins were used to hold the tissue apart to permit drainage.

Table 1: Fluorescein content of Anusol suppositories

Weight of Suppository Mass taken (mg)	Fluorescein Content (mg)	Unit Fluorescein Content (mg)
4.70	0.01928	0.00410
6.20	0.02518	0.00406
5.90	0.02302	0.00390
8.80	0.03722	0.00423
7.20	0.03027	0.00420

Mean = 0.00420, CV% = 3.17

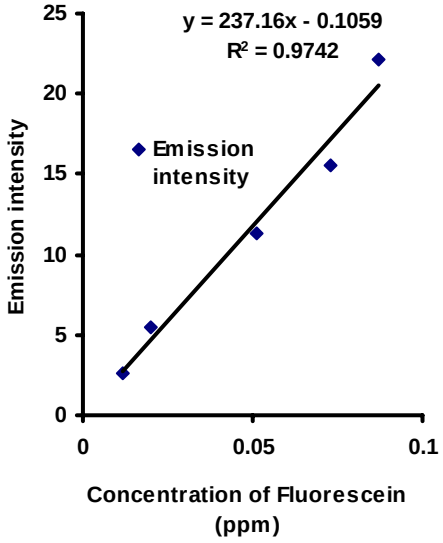

Figure 5: Calibration curve for the determination of fluorescein in 0.001 M NaOH

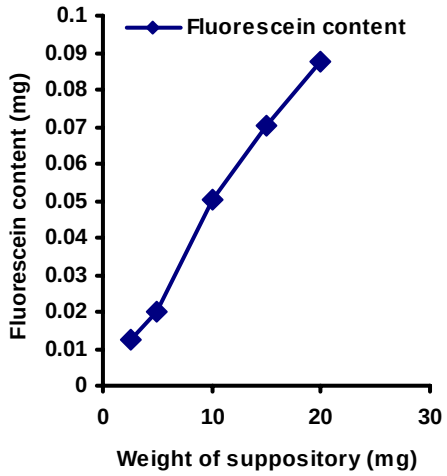

Figure 6: Validation of assay technique for the assay of fluorescein contained in Anusol suppositories

The metallic pins proved not to be stable and the tissue when mounted was difficult to hold in place especially when the suppository was inserted to initiate the experimental procedure. Also, contact between suppository and tissue wall was minimised by the presence of the pins within the gut tissue.

Table 2: Validation of fluorescein assay technique for Anusol suppositories

Weight of Sample	Fluorescein Content (mg)	Unit Fluorescein Content (mg)
2.8	0.01380	0.00492
2.6	0.01126	0.00492
2.4	0.01343	0.00559
5.4	0.02351	0.00435
5.7	0.01733	0.00304
5.4	0.02104	0.00390
11.7	0.04252	0.00363
10.0	0.04103	0.00410
11.7	0.06252	0.00534
15.0	0.06713	0.00448
15.6	0.07207	0.00534
15.5	0.07328	0.00473
21.8	0.08857	0.00406
22.2	0.08633	0.00389
21.3	0.08539	0.00401

Mean = 0.00433, CV% = 15.5

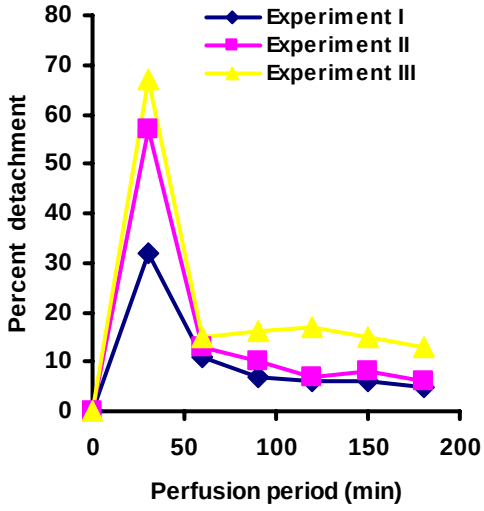

Figure 7: Percentage detachment of Anusol suppositories from gut tissue in in-vitro adhesion studies

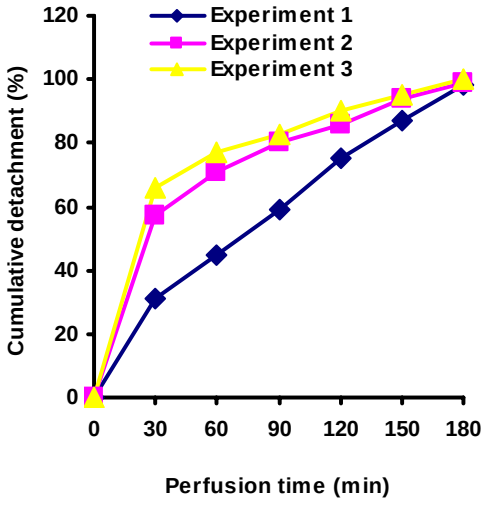

Figure 8: Cumulative percent detachment of Anusol suppositories (batch Number 3 x 126) manufactured on January 19, 1994

There was appreciable delay in the onset of drainage as a result of the positioning of the pins.

In the new improved design, cotton thread was used to prevent the tissue from collapsing. The thread was tied to metallic pins which in no way interfered with the mounting of the tissue. The contact between suppository and gut wall was therefore maximised. The other advantage of using the cotton thread to hold the tissue apart is that it allows a realistic tone on the gut wall to be established.

Results obtained with Anusol GSL suppositories (Batch No. 3 x 126) are shown in Table 2. The time taken for the suppository mass to drain is given results in this manner is that the suppository mass was found to emerge as a bolus from the lower side of the vertically mounted tissue. The time lags between the emergence of the bolus from the tissue tip and its detachment, for the samples of suppositories examined, varied between 30 and 120 sec. It was therefore decided to record the time between the start of the experiment and that for the hanging bolus of molten suppository to detach, as the drainage time.

A considerable length of time was spent identifying factors which affect the drainage experiments. The factors can be separated into tissue, suppository, test device and test environment related factors.

The internal diameter of tissue relative to the size of suppository being tested was the single most important factor. Rat and rabbit intestinal tissue were used initially to establish the technique. In the situation where the size of suppository was too large relative to that of the tissue, there was considerably delayed onset of drainage which eventually ceased despite the presence of molten suppository mass. Where the size of suppository was too small, the coating to the intestinal wall was patchy and drainage time too fast. With an optimum size of tissue, the suppository coated the intestinal wall uniformly and copiously. A diagrammatic representation of what was observed is shown in Figures 7 and 8. It is reasonable to suggest that there is an optimum tissue to suppository size which will yield acceptable and reproducible coating of the excised intestinal wall lumen.

Samples of Anusol FSL suppositories were used for repeat drainage experiments. The experiment was aborted after 5 hour of test at 39 ^{0}C. The excised intestinal tissue with the suppository was left on the shelf to dry out and cut open vertically. The suppository appeared molten, had distended the excised tissue but could not flow or drain out. The central portion of the suppository had started coating the tissue but was not fluid enough at the test temperature, to drain unaided.

The influence of the test device is related to its length. The length of tissue and therefore the time taken for any enclosed, molten suppository mass to drain out will depend on the length of the device. The device used in this study accommodates 9 cm of excised intestinal tissue.

Table 3: Drainage experiments with Anusol GSL suppositories (Batch No 3x126)

Sample Number	1	2	3	4
Wt of sample (g)	2.67410	2.65349	2.65865	2.71971
Weight of sample drained out (g)	1.23161	0.98631	1.12381	1.35421
% Suppository retained on tissue	53.94	62.83	57.73	50.21
Mean % retained	56.18 (9.60)*			
Time taken for drainage (min)	50	50	45	69
Mean drainage time (min)	53.50 (19.81)*			

**CV%*

Table 4: Detachment of Anusol suppositories (Batch Number 3 x 126)

Sampling time (min)	I		II		III		Mean	
	Detachment							
	mg	%	mg	%	mg	%	mg	%
30	0.21	57.02	0.25	66.39	0.09	31.19	51.53	(35.38)*
60	0.05	13.95	0.05	12.20	0.04	15.38	13.84	(11.50)
90	0.04	9.96	0.02	6.20	0.04	14.54	10.1	(39.33
120	0.02	6.37	0.02	5.60	0.04	14.54	8.83	(56.04)
150	0.03	7.95	0.02	5.60	0.04	13.75	9.10	(46.10)
180	0.02	4.75	0.02	4.00	0.03	11.72	6.82	(62.42)

Table 5: Cumulative Percent Detachment of Anusol Suppositories (Batch Number 3 x126)

Sampling time (min)	I	II	III	Mean Cumulative Detachment
30	57.0	66.39	31.19	51.35 (35.38)*
60	70.97	78.59	46.57	65.38 (25.58)
90	80.39	84.78	60.71	75.48 (17.13)
120	87.30	90.39	75.25	84.31 (9.49)
150	95.25	95.99	89.00	93.41 (4.11)
180	100	100	100	100

** CV%*

It was observed that when the drainage experiment was performed in an oven, there was drying out of intestinal tissue as the experiment progressed. There was the need to perform the experiment in a high humidity environment. A glass jar containing some water (or normal saline) was used to maintain a high humidity environment (see Figure 2). This step was necessary because in the body, tissues and organs are bathed in physiological fluids.

Other factors which were identified which may have effects on the reproducibility of test results were: (i) use of fresh or thawed, previously-frozen, intestinal tissue samples (ii) treatment of intestinal tissue before drainage or detachment experiments and (iii) use of the small or large intestines. Fresh samples were found to be coated more efficiently than stored samples. Draining suppository masses tend to egress with some intestinal tissue attached to them when the mounted intestinal tissue had been previously stored at -20 ^{0}C. The influence of the portion of intestine used is thought to be related to the internal diameter of the excised tissue compared to that of the suppository. Small intestinal tissue portions have a smaller internal diameter and therefore promote better coating and adhesion of the melted suppository to the tissue mucosa, as discussed above.

Perfusion / Detachment Experiments: The suppository mass was allowed to adhere to the pig intestine prior to the perfusion apartments. A looped string made of stainless steel was used to support the intestinal (Figure 3) and prevent it from collapsing while allowing the inserted suppository sample to drain (Figure 2). The molten suppository mass drained out of period of time, the time for this drainage being dependent on the diameter of the supporting wire (Table 3). It was necessary to optimise the size of this metallic support, to ensure full coating of the lumen of the excised intestinal tissue. The perfusion experiments were started only after complete drainage of suppository mass and coating of intestinal tissue had occurred.

The choice of a 4ml/minute flow rate for the perfusion experiments was arbitrary. It was planned to vary the perfusion rate and the 4ml/minute flow rate was a convenient starting point. The extent of adhesion to gut tissue was determined by calculating percent detachment during the period of perfusion. This was done over a 3 h period. In Table 4, the percent detachments for the suppositories prepared. The data are represented graphically in Figure 7. The cumulative percent detachment for the same samples over a 3 hour period is shown in Table 5. This is depicted graphically in Figure 8. The data in the tables and figures showed that there was considerable adhesion to the intestinal mucosa. Within the first 30 minutes of the experiment, loose and detached suppository mass which formed during the draining of excess suppository mass, seen to egress rapidly in the perfusate. This occurred within the first 5 minutes. Thereafter, there was gradual release of suppository mass into the perfusate. The preliminary result suggests that with further optimisation of the technique, reproducible results can be obtained.

Conclusion: The practical basis for the use of excised pig intestinal tissue for adhesion experiments has been established.

A device which can be used to mimic *in vitro* what happens to the suppository *in vivo*, was developed. Preliminary data obtained with the device showed that the results were reproducible and accurate. The factors that may influence results obtained with this device were identified and relate to the type of formulation being investigated, type of test tissue, test device and the test environment. Perfusion models to confirm the results of the detachment experiments were established. The various factors that may influence results obtained with this model are currently being optimised. It is expected that the test system developed will be used in satisfactory assessment of formulation variables of the commercial Anusol products.

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PHYCOLOGY AND FISHERIES DEVELOPMENT – A REVIEW

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ABSTRACT

Algae are the chief primary producers in the aquatic environment. Thus, they supply proteins, carbohydrates and mineral salts to the primary consumers and consequently sustain many fishes of commercial interest. Despite algae being a good source of food for some fishes, some cyanobacteria are of low nutritional value and with dinoflagellates may secrete toxins that kill fish. Algae population varies according to seasons and this affects algae - dependent organisms. Optimal algal population is favoured by eutrophication but when there is rapid eutrophication, algal blooms may result. This may lead to the death of zooplankton, game fish and even man. The realization of fish as a major source of protein in Nigeria spurred various workers to investigate the natural food for such fishes as clupeids, Synodontis spp., Chrysichthys spp., Schilbe spp., Tilapia, Alestes, Lates sp. Hydrocynus sp., Siluranodon sp., Eutropus, Bagrus docmac, B. bayad, Heterobranchus sp. Cymnarchus sp., Clarias spp, Hemicynodontus sp. and Brachysynodontis sp. Important fish species cultured in Nigeria include Tilapia nilotica, Tilapia melanopleura, Tilapia galilea, Cyprinus carpio (Common carp), Heterotis niloticus, Lates niloticus (Niger perch), Chrysichtys nigrodigitatus (Catfish) C. gariepinus (Catfish) and others. Aquaculture has not advanced as much in Nigeria as in developed countries where algae could be cultured as feed for fish, shrimps, prawns and other crustaceans in commercial quantities. The economic importance of algae in fisheries cannot be overstressed. Their absence could cause disruption of equilibrium, while excess of them could result to mortality of aquatic fauna.

Keywords: Algae, Primary producers, Algal blooms, Algal toxins, Fish food, Fisheries

INTRODUCTION

The term Phycology is the study of Algae. Algae are photosynthetic thallophytes that show little tissue differentiation. Plant life is the base of all food webs in nature, whether aquatic or terrestrial ecosystem, hence the ultimate source of animal food (Lee, 1992). Algae form an important component of the natural economy of inland waters and oceans in that they function as photosynthetic systems (Talling, 1975; Cooper, 1975; Harris, 1986). In the food chain/web in the aquatic environment, algae sustain the primary consumers.

Algae are usually abundant in eutrophic water bodies and are found as phytoplankton floating in the water; as attached algae (aufwuchs/periphyton) and also as submerged vegetation on some substratum of mud or stone. They thus form an important component of the food of benthic feeding animals (Talling, 1975). Other than seaweeds and certain aquatic plants, they are the principal source of organic materials as primary producers (Boney, 1975). They thus deserve the often-applied term “pasturage of sea” (Round, 1977).

Apart from serving as food for primary consumers, they help aerate the aquatic habitat. On the other hand they may constitute a nuisance when in bloom, resulting in far reaching ecological consequences (Codd *et al.*, 2005; EPA, 2006).

MATERIALS AND METHODS

A comprehensive literature search was made from the Internet and serial materials of Nnamdi Azikiwe E-library, University of Nigeria, Nsukka. Various journal articles, proceedings of learned societies of Botany and Phycology, and Food and Agricultural Organization documents and textbooks were consulted vis-à-vis the ecological implications and role of phycology to fisheries development. The data collected were presented in table and figure.

RESULTS AND DISCUSSION

Food Relationships Involving Algae: In a simple food chain, algae as primary producers form the base of the chain and are fed on by primary consumers – zooplankton, crustaceans, Protozoa, rotifers) and benthic vertebrates (oligochaetes, molluscs etc) (Table 1). Lévèque (1979) reported that in lake Chad that 25 % of sunlight is used for photosynthetic yield. Of this, zooplanlankton take up 6 % of the gross

Table 1: Some algal groups and primary consumers

Algal groups	Primary consumers
Flagellated green algae	Protozoa, rotifers, fly larva, ostracods.
Non-motile green algae	Mini crustaceans, frogs, fly larva.
Diatoms	Mini crustaceans, frogs, protozoa, fly larva, oligochaetes and molluscs.
Chara and *Nitella* (Charophyceae)	Water boatman

production. The algae consumed include diatoms such as *Navicula*, *Amphora*, *Fragillaria*. *Cymbella*, *Cocconeis*, which are taken by chironomids (Mackey, 1978); Conchostraca (Royan, 1976); oligochaetes and molluscs (Moore, 1979). Other algae include Chlorococcales like *Ankistrodesmus falcatus* var *mirabilis* and *Scenedesmus obliquus* (Obrdlik *et al*., 1979); *Anabaena flos aquae* and a host of others consumed by cladocerans (Gras *et al*., 1971; Doma, 1979). Other zooplankton that feed on algae include rotifers nymphs of *Povilla adjusta* which in Kainji lake feed on *Microcystis*, *Anabaena* and *Tetraedron* species, and Ephemeroptera (Obrdlik *et al*., 1979; Bidwel, 1979).

The role of phytoplankton in the food web is thus of supplying proteins, carbohydrates and mineral salts to the zooplankton which are termed primary consumers (Boney, 1975; Odum, 1979; Lagler *et al*., 1977). The zooplankton are in turn consumed by fish of commercial interest like *Clarias*, *Alestes*, *Schilbe, Chrysichthys* species in lakes Kainji, Tiga, and Anambra river basin (Beadle (1974; Otobo and Imevbore, 1979; Sturm, 1980; Ezenwaji, 2004). Other zooplankton feeding fishes found include *Brachysynodontis batensoda*, *Hemisynodontis membranaceous* (Gras *et al*., 1979), *Bagrus*, *Aplochelichthys*, and *Stolothrissa* (Beadle, 1974) *Barbus barbus* (Obrdrlik *et al*., 1979; Balirwa, 1979).

There could be extensions of the food chain to form longer chains and complex webs with more trophic levels, with the primary producers being eaten by primary consumers, which in turn are eaten by secondary consumers, which are in turn consumed by fishes (Figure 1). The secondary consumers include nymphs of *Dolania Americana* which feed on chironomid larvae (Tsui and Hubbard, 1979); *Daphnia* species which feed on protozoan and rotifer species (Obrdlik *et al*., 1979); *Asplanchna*, *Synchaeta*, *Choaborus punctipennis* (Edmondson, 1974); *Alestes baremoze*, small fish like *Tilapia*, and other young fry.(Boney, 1976; Moore, 1979; Leveque, 1979). A food web involving algae is presented in Figure 1 (Otobo and Imevbore, 1979)

Increased phytoplankton makes for a larger microfaunal foraging population. Lakes with numerous flora maintain correspondingly dense population of animals (Prescott, 1962). In other words, the rate of primary production together with allotrophic supplies will set an upper limit on the rate of primary production, but a given primary production can be associated with rather different rates of secondary production depending on the effects of such factors as morphometry and seasonal changes in radiation and temperature (Edmondson, 1974; Beadle, 1974). For instance in the temperate region during summer, with longer days and shorter nights, net primary productivity in a lake can be high with consequent rapid increase in biomass of all organisms. Conversely, in winter, characterized by dull days, and long nights, the net primary production is often insufficient to maintain the rest of the population of organisms whose biomass decrease accordingly (Beadle, 1974; Moore, 1979).

In the tropics since there is sufficient light throughout the seasons, more even rate of primary production is to be expected, but this is not so due to fluctuations in nutrients available to the plants that are correlated with movements of water (Beadle, 1974). There is usually dilution of nutrients available in the rainy season and vice versa during the dry season (Holden and Green, 1960; Nweze, 2006).

The effect of these factors is through variation in abundance of the different organisms. For animals, the daily ration and assimilation are proportional to the population density of the food organism. Without continued production of food, there will be decrease. There is thus a relationship between production of food and consumers production (Edmondson, 1974). An investigation by Hrebácêk (1969) on fish stock which were in their third year of life in a pond of about 500 specimens of Carp per hectare disclosed that there was an increase from the original size of the fish of 0.5 kg at the beginning of the year to about an average of 1.5 kg at the end of the dry season. The increase he attributed to their feeding on zooplankton, which were very numerous during the season.

Primary and secondary consumers are fed on by carnivorous fishes like *Cymnarchus niloticus*, *Lates niloticus*, *Heterobranchus bidorsalis*, *Hydrocyanus lineatus*, *H. forskali*, *Clarias gariepinus*, *C. macromystax, Bagrus docmac*, *Bagrus bayad* and *Schilbe mystus*, which reach about 10 kg in weight (Imevbore, 1969; Obrdlik *et al*., 1979; Olatunde, 1979; Ezenwaji, 2004).

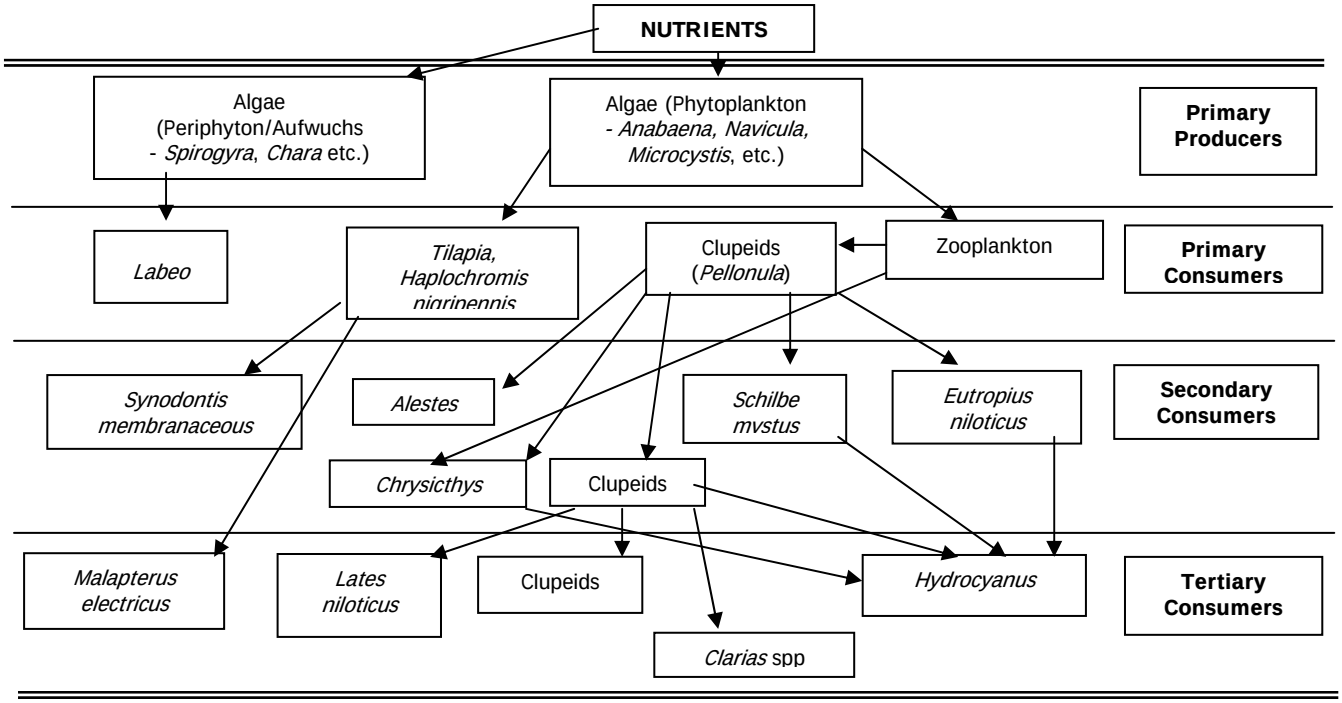

Figure 1: Food web showing the relationships between primary producers, primary consumers, secondary consumers and tertiary consumers

Algae as Direct Food for Fry and some Adult Fishes: Herbivorous fishes such as *Tilapia* and *Labeo* (Beadle, 1974; Round 1977; Otobo and Imevbore, 1979), *Haplochromis gariepinus* (Beadle, 1974), *Siluranodon auratus* (Olatunde, 1979), *Barbus* species, *Synodontis resuionatus* and *S. batensoda* (Willoughby, 1979a) that feed directly on plants have a more efficient production in the sense that there are no intermediaries to dissipate the energy on the way (Figure 1). Fishes that feed on phytoplankton like *Tilapia*, *Menhaden* and *Drosoma* have the phytoplankton separated from water by gill rakers, which are a filtering device on the gill arches (Adiase, 1969; Lagler *et al*., 1977). Many young fishes (fry) with tiny mouths and often still with yolk in their yolk sac feed on zooplankton (Lagler *et al*., 1977). It has been observed that fishes that are carnivorous (i.e. zooplankton feeders), under famine conditions feed on algae. Reynolds (1969) working on the biology of Clupeids in Volta Lake confirmed this. He found that *Pellomula* fed on phytoplankton when the potassium available was very low. Also, during high water tide some species of *Synodontis* that are known to be zooplankton feeders browse intensively on phytoplankton most of which are blue green algae (Willoughby, 1979a). Ezenwaji (2004) observed *Spirogyra* sp, *Zygnema* sp. and *Scenedesmus* sp. (green algae) *Microcystis* sp (blue green alga) and *Navicula* sp. (diatom) amongst other food items in the gut of *Clarias macromystax* in Anambra river basin.

Despite the fact that algae are good sources of food for fishes, some of them are of low nutritional value. Some especially the blue green algae have a polysaccharide layer of cellulose surrounding them that makes them indigestible by gastric enzymes of most fishes (Beadle, 1974). Filamentous algae especially *Spirogyra* species have been found to be the mid summer diet of *Pachychiton pictum*, *Leuciscus* and *Rutilis rubilio* despite its generally poor nutritive quality (Kitcheli *et al*., 1978). Balirwa (1979) observed that in Lake Victoria, East Africa, filamentous algae pass through the gut of some *Barbus* species without being digested.

On the other hand studies using cultures of algae labelled with radioactive carbon ^{14}C have shown that *Tilapia nilotica* and *Haplochromis nigripinnis* from Lake George do in fact digest and assimilate up to 70% of the carbon contained in blue-green algae - *Microcystis* and *Anabaena*, the figure depending on the time of day during which the fish fed (Beadle, 1974). Also, a lot of *Microcystis* has been found to be consumed by *Synodontis membranaceus* in Lake Kainji (Willoughby, 1979b). Experiments have shown that acid conditions in the gut of about 1.4 was responsible for making the polysaccharide coat permeable to gastric enzymes (Beadle, 1974).

Algae as Natural Fish Feed: Algae are the most available primary producers in the aquatic environment all through the seasons. For fishes that take them, an increase in the algal flora will mean an increase in the food available to the fish.

With high concentration of essential ions like phosphorus, nitrogen and right pH, between 7.2 and 9.5, free carbon dioxide, dissolved carbon dioxide and

optimum temperature between 25 and 30^{O}C there would be an optimum algal population. Such a body of water with these optimum conditions is said to be eutrophic. Such waters are usually shallow and have a high degree of transparency.

Increasing the algal flora of a fish pond is by addition of super phosphate fertilizers in the right concentration, which increases nutrients available to the algae. This is used mostly in experimental fish ponds (Banerjea and Ghosh, 1971) and in artificial lakes and ponds in homes across the country where *Tilapia* spp. and *Clarias gariepinus* are cultivated. This will increase the plankton flora of the fish pond and both herbivorous and carnivorous fishes can thrive. Moreover, the algae aerate the pond. As noted by Anon (1977), in Nigerian pisciculture, pond fertilizers used include organic fertilizers and inorganic fertilizers. Organic fertilizers such as cow dung, poultry manure, pig dung, horse dung, green manure or compost etc. are applied at the rate of 2,000 kg/hectare/year. Inorganic manures used are NPK, ammonium sulphate, potassium chloride, super phosphate etc, at the rate of 200 to 400 kg per hectare per year. Impoundment of rivers may increase algal crops growing on submerged vegetation, which result in increase in fish crop of the lake thus formed. Typical examples are lake Kainji and Asa Dam both in Kwara State (Anon, 1977).

Also, algae are cultured and used in aquaculture - in fish ponds and in production of crustaceans such as prawns, crayfish, shrimp, oysters and crab for commercial purposes. Algae are cultured for food for larvae of these crustaceans. Algae such as diatoms, *Chlorella*, *Chaetoceros*, *Tetrasimilia* etc, have been successfully cultured in Philippines, for larval rearing of *Panaeus monodon* that is of great economic importance (Platon, 1978). Platon used wooden tanks of about 60 cm depth and 1-ton capacity for algal culture. He noted that shallow tanks had the advantage of allowing light penetration to the bottom of tanks. Ojeda-Ramirez *et al*. (2008) observed greater growth and survival in late nursery cultivation of Cortez oyster (*Crassosterea corteziensis*) using the alga *Pavlova* spp. (Prymnesiophyceae). *Lyngbya aestuarii* (Mert), *Anabaena* species (symbiotic with *Azolla*, a fern) have been used extensively in fish farming (Phang, 1992; Van Howe and Lejeune, 2002). In ornamental fish culture, Pan and Chien (2009), in the culture of red devil, *Cichlasoma citrinellum* (Gunther) used the alga *Haematococcus pluvialis* as a dietary carotenoid to increase colour intensity, growth and viability.

Harmful Effect of Algae on Fisheries: Despite their importance as natural feed, when in excess during algal bloom, algae are deleterious. Algal bloom is caused by rapid primary production resulting from pollution from sewage and other organic wastes and excessive use of chemical fertilizers on neighbouring land combined with efficient drainage system, which transports them into lakes, rivers and streams (Svobodova *et al*., 1993; EPA, 2006).

The bloom on the surface of the water reduces the secchi disk depth (which is the depth to which light can penetrate). There is consequently a reduction in primary production below the bloom, no matter how favorable other conditions are (Cooper, 1975; Nweze, 2003). An investigation by Lam (1979) working with *Oscillatoria* noted that no matter the level of nutrient added, there is an optimum concentration above which there would be no increase in productivity.

During blooms, the algae on the surface usually die due to intense sunlight. There is auto shading and increased bacterial decay of algae leading to depletion of oxygen below the point required for fish (2 – 3 parts per million) and other animals. The bottom organisms die, e.g. chironimid larvae (Round, 1977; Lee, 1992). The algae themselves die also and sediment at the bottom. More oxygen is used up for their decay and the density of the water is thereby increased. This prevents mixing or recycling of nutrients for a long period or even permanently (Beadle, 1974). There is thus enormous loss of game fish (Prescott, 1962; Round, 1977). Biswas (1981) noted that there was a decrease in fish catch in Zambia as a result of urbanization in Samfya. Urbanization resulted in pollution of the water, which consequently resulted in excessive growth of cyanophycean algae.

The harmful effect of blue green algae in lakes that could be termed productive due to rich algal flora has been proved (Hrebacek, 1969). It was found that there was a low figure of about 2 – 5½ % for the effective transfer of primary production to net fish production when the primary production was due to *Aphanizomenon flos-aquae*, a blue green alga.

Incidences of harmful algal blooms (HABs) have been reported in both marine and freshwater habitats, sometimes called red tides and CyanoHABs respectively (Codd *et al*., 2005). Cyanobacteria – blue green algae (*Microcystis aeruginosa*, *Aphanizomenon flos aquae*, *Cylindrospermopsis raciborskii and Anabaena* spp.), are notorious for bloom forming in the freshwater habitat. Blue green algae have been found to produce poisonous substances like hydroxylamine from the decay of proteins. They release toxins such as microcystin, cyanoginosin, nodularin, cylindrospermopsin (Hepatotoxins); saxitoxins (cause paralytic shellfish poisoning, PSP),

anatoxin and aphanatoxin (neurotoxins) and lipopolysaccharides (endotoxin) into water (Fritz, *et al*., 1992; Burch and Humpage, 2005). The toxins produced are allelopathic to other phytoplankton (Suikkanem *et al*., 2004; 2006); genotoxic (Shen, *et al*., 2002); suppressive to zooplankton grazing leading to reduced growth, reproductive rate and changes in dominance (Ghadouani *et al*., 2003); hepatoxic on fish (Anderson *et al*., 1993; Burch and Humpage, 2005); accumulate in invertebrates (Lehtiniemi *et al*., 2002) and fish (Megalhaes *et al*., 2001; Jos *et al*., 2005); induce oxidative stress in fish (Jos *et al*., 2005), and reduce fecundity in snails (Gerrad and Poullain, 2005).

Marine algae such as dinoflagellates are known to cause red tides (Lee, 1992). Some dinoflagellates (*Gonyaulax, Prorocentrum, Dinophysis, Gymnodinium, Pyrodinium)* are toxic. Their toxins are concentrated in shellfish that consume them and when man consumes the shellfish there is severe food poisoning known by various names such as paralytic shellfish poisoning (PSP) or saxitoxin poisoning, ciguatera fish poisoning (CFP) diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP) and mussel poisoning (Lee, 1992). Domoic acid, a neuroexcitatory amino acid that causes amnesic shellfish poisoning (ASP) is secreted by *Pseudonitzchia australis*, a diatom and concentrated in bivalves (Fritz *et al*., 1992; Engstrom-Ost, *et al*., 2002). These result in the death of zooplankton, fish and aquatic birds and man.

Conclusion: As there has been dearth of work on the direct use of algae in fisheries in Nigeria, the importance of algae in fisheries should not be overlooked. Several investigations have been carried out the type of food taken by different families of fishes found in the country. Such workers like Otobo and Imevbore (1979), Olatunde (1979), Willoughby (1979ab), Gras *et al*. (1971), Leveque (1979) and Ezenwaji (2004) have found out the natural food for such fishes of commercial interest like *Alestes sp, Synodontis sp., Labeo, Sarotherodon sp., Citharinus sp*., *Lates niloticus*, *Clarias spp* and others. Such fishes of commercial importance are cultivated intensively in large fish ponds and homestead ponds; and semi intensively in floodplain ponds (Ezenwaji, 2004) to produce more protein for the masses.

Aquaculture has not been developed in Nigeria as in some countries where algae could be cultured as feed for fish, shrimps, prawns and other crustaceans in commercial quantities (Platon, 1978). Nevertheless, improvements could be made by impoundment of rivers, which could increase algal crops on submerged vegetation resulting in corresponding increase in fish crops of the lake thus formed. Moreover, utilizing other ideas got from research already carried out in other countries could be applied. For example, algae could be cultivated by the method used by Platon (1978) and used for the breeding of fish such as *Labeo* and *Tilapia* which are common in Nigeria's inland waters in commercial quantity. There is therefore the need for interdisciplinary research to produce natural feed from algae for fisheries in Nigeria.

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COMPARATIVE EFFECT OF CRUDE OIL PRODUCTS ON NITROGEN CONTENT OF *Clarias gariepinus* JUVENILES

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ABSTRACT

The comparative effect of crude oil products was investigated using Clarias gariepinus juveniles. The fish (average weight 100.20 ± 0.08g) was introduced into graded concentrations (2.00, 4.00, 6.00 and 8.00 mll $^{-1}$) of Bonny light crude oil (BLCO), premium motor spirit (PMS) Dual purpose Kerosene (DPK) in toxic phase that lasted for two hours. Nitrogen level was estimated from both serum and blood of C. gariepinus. Result showed that there was increase in nitrogen content of fish in the treated samples compared to control experiment. This may be due to the presence of the crude oil. The biological parameter investigated showed significant differences (p<0.05) when compared to the control. Increase nitrogen content suggested that there was an alteration in the water chemistry predisposing the fish to stress and disease.

Keywords: *Clarias gariepinus*, Nitrogen, Bonny light crude oil, Kerosene, Premium motor spirit

INTRODUCTION

Low survival rate in aquaculture systems of juveniles of the much cherished African catfish has been attributed to nutritional problem (Faturoti *et al.,* 1986). In addition, adults and juveniles of these fish species have been reported to be sensitive to aquatic pollutants such as cadium and crude oil (Oluah, 2001). This author reported that alterations in water chemistry usually predispose the fish to stress and disease and elicit quick physiological responses especially on the haematological parameters.

Contamination of water body by petroleum products has been shown to produce many changes in fish when either chronically or briefly exposed (Kenney *et al.,* 2002). The crude oil and its fractions when spilled have both physical and chemical effect on aquatic organisms. The physical effects are caused by oil, coating the organism or their immediate environment thus causing suffocation, loss of buoyancy and asphyxiation. Oxygen uptake in water is thus reduced due to lower dissolved oxygen concentration which may result to death.

Crude oil it products vary considerably in its toxicity and the sensitivity of fish to this products vary according to species. The water-soluble fractions of crude oil can stunt fish growth (Lopes *et al.,* 2001). Its direct mortality effect is primarily on fish eggs, larvae and early juveniles, with limited effect on the adults. The aim of this study was to determine the effect of various concentrations of crude oil and its fractions on nitrogen content of *Clarias gariepinus*, a highly priced Nigerian food fish.

MATERIALS AND METHODS

The experiment was carried out at Heldin's fishery unit, New Haven, Enugu, Nigeria. One hundred and fifty (150) juveniles of *Clarias gariepinus* (mean weight 100.2 ± 0.08 g) were transported from a private fish hatchery at Ugwuomu, Emene in Enugu State, Nigeria in 90 liter capacity plastic container to the fishery unit of the Department of Applied Biology, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria. Water temperature of 23 ^{0}C was maintained on transit by addition of ice cubes.

The fish juveniles were acclimatized in the plastic basin (90 litre) for 14 days and maintained on 38 % crude protein diet at 3 % body weight daily. Twenty six (26) juvenile of *C. gariepinus* were subjected to different concentrations (2.00, 4.00, 6.00 and 8 mIL^{-1}) of Bonny light crude oil (BLCO), premium motor spirit (PMS) and Dual purpose kerosene (DPK). Each of these there toxicants was introduced in duplicates (R1 and R2) in 24 plastic containers and two plastic containers served as control (without any treatment). The fish juveniles were randomly stocked in completely randomized Block Design (CRBD) in 26 plastic containers. Each container was filled to 10 litres mark with rain water and labeled according to treatment. The fish in each setup was exposed for two hours to the different concentrations of the toxicants.

Blood samples from fish juveniles exposed to different concentrations of the toxicant were collected from razor cut into the musculature behind the opercula region on the dorsal surface. The collected blood samples were allowed to cloth in order to get blood serum.

Nitrogen concentration of the blood and the serum were determined according to the method of king and king (1954). All assays were conducted spectrophotometrically at 549 nm wave length. Data collected were analyzed using the analysis of variance (ANOVA) and FLS-D to indicate statistical significance ($p>0.05$) among treatment means.

RESULTS

The values obtained for nitrogen content of *C. gariepinus* exposed to different toxicants ranged from 23.00 – 24.80 i.u/L (Table 1).

Table 1: Nitrogen contents of *Clarias gariepinus* exposed to different crude oil products

Toxicants	Concentration (ml^{-1})	Nitrogen concentration (i.u/l)		Total (i.u/l)	Mean nitrogen concentration
		RI	R2		
Kerosene (DPK)	2ml	23.50	23.30	46.80	23.40 ± 0.014^{a}
	4ml	23.60	23.50	47.10	23.50 ± 0.007^{b}
	6ml	23.50	23.30	46.80	23.40 ± 0.014^{b}
	Control	24.20	24.20	48.60	24.30 ± 0.014^{a}
Crude oil (BLCO)	2ml	23.60	23.50	47.10	23.50 ± 0.007^{b}
	4ml	23.80	23.60	47.40	23.70 ± 0.014^{b}
	6ml	23.50	23.40	46.90	23.40 ± 0.007^{b}
	Control	24.80	24.70	49.50	24.70 ± 0.00^{7a}
Petrol (PMS)	2ml	23.60	23.40	47.00	23.50 ± 0.014^{b}
	4ml	23.90	23.80	47.70	24.80 ± 0.007^{a}
	6ml	23.50	23.30	46.80	23.40 ± 0.014^{b}
	Control	24.20	24.10	48.50	24.10 ± 0.007^{a}

Table 2: Comparative effect of toxicants on the nitrogen concentration in *Clarias gariepinus* juveniles

Toxicants	Total concentration (mll^{-1})
Petrol	189.30
Kerosene	190.90
Bonny light crude oil	189.80
Control	184.00

Kerosene (190.90 i.u/L), recorded highest level of nitrogen concentration among the tested samples, seconded by Bonny light crude oil (189.80 i.u/L), while the least was recorded in petrol (189.30 i.u/L). Comparable with the control (184.00 i.u/L) (Table 2), the degree of toxicity was highest in kerosene followed by Bonny light crude oil and petrol has least toxic effect. Generally, there was significant differences ($p>0.05$) among nitrogen concentrations of fish exposed to the different products. Decreased nitrogen levels were recorded at low exposures to toxicants.

DISCUSSION

The increase in nitrogen concentrations in the treated samples may be due to the toxic effects of the toxicants. Though not all the concentrations caused increase in nitrogen concentrations, the toxic effect of the crude oil products varied according to components the fractions. There was no specific pattern of variation in the nitrogen concentration, the biological parameter investigated showed significant difference ($p>0.05$) when compared with the control and increase in the nitrogen content may be due to alteration of the protein synthesis in the blood and the immune response mechanism of the fish, this is in the line with the report of Oluah (2001). In some concentrations, there was either rapid metabolism of the products thereby reducing or terminating its toxic effect on the liver. It has been suggested that metabolism of these products will lessen their effects in fish (Oluah, 2001). On the other hand, elevation of nitrogen may be as a result of injury sustained by the fish in the liver due to its inability to metabolize the toxicant immediately.

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CO-PARASITISM AND MORPHOMETRICS OF THREE CLINOSTOMATIDS (DIGENEA: CLINOSTOMATIDAE) IN *Sarotherodon melanotheron* FROM A TROPICAL FRESHWATER LAKE

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ABSTRACT

In ever competitive environment of nature, evolution of most attributes of an organism - anatomy, physiology, and behaviour are determined by the environment through selection. The same is the microhabitats of Sarotherodon melanotheron where three Clinostomatids; Clinostomum tilapiae, Clinostomum complanatum, and Euclinostomum heterostomum, were recovered from Opi Lake (GPS N06.75275*, E007.49104*), were studied from (November 2007– October 2008) using multiple fishing gear techniques; cast nets, hook and line, and seine nets (150 mm – 200 mm), showed significant difference ($p < 0.05$) in all other anatomical parts but the distance between oral and ventral suckers. The prevalence was low; (20.8 %) was recorded in C. complanatum, (6.4 %) in E. heterostomum and (21.1 %) in C. tilapiae. But mean intensity was high which was suggestive of heavy parasite burden; C. complanatum (2.7), C. tilapiae (5.8) and E. heterostomum (5.1). In the rank-abundance curve for parasite communities C. tilapiae was more abundant than the other two species. Differential parasitic implications was due to selection for relatively better adaptiveness to host's microhabitats, more population size, better host location, and larger body size. Consequently, this resulted in a trade-off between larger morphometric parts and population size among the parasites.

Key words: *Clinostomum* sp, co-parasitism, Morphometrics, *E. heterostomum,* Opi Lake, Selection

INTRODUCTION

The present study investigated co-parasitism and morphometrics of Clinostomatids in relation to higher susceptibilities to infection, better adaptiveness and selection in *S. melanotheron.* This has become important as most studies have been based on taxonomy and basic parasite biology. For instance, species of *Clinostomum* have been described from freshwater fish in Nigeria and many parts of the world. Ukoli (1966) described *Clinostomum tilapiae* in the intestine of *Oreochromis niloticus* and *Sarotherodon galilaeus* in the river Niger. Other species of *Clinostomum,* e.g. *Clinostomum complanatum* has been recorded in the fish of river Niger (Ukoli, 1969), *Clinostomum* sp. were found to be common in *Tilapia* sp. While those of *Euclinostomum* sp. were recovered mainly from the osteoglossid *Heterotis niloticus.* However, in the species of *Tilapia*, the metacercariae occurred in the sub mucosa of the mouth cavity, gill chamber, below the operculum and pharyngeal bone, orbit, muscles of the body, around the heart, abdominal cavity, mesentery, viscera and the swim bladder (Awachie, 1965). *Clinostomum tilapiae* n. sp. and *Clinostomum phalacrocoracis* Dubois, 1931 from Ghana (Ukoli, 1966). The adult trematode of *Clinostomum* are attached to the upper and lower jaws of cattle egrets, herons etc. Family *Clinostomatidae* has a widespread distribution. Members of this family have been recorded from the area where mean temperature is about 10^0C (Grabda-Kazubska, 1974).

Infected cases of fish have been reported from Japan and Korea (Chung *et al*., 1995; Hiral *et al*., 1987; Isobe *et al*., 1994). *C. complanatum* and *C. tilapiae* have been recovered from African continent with *Euclinostomum heterostomum* being cosmopolitan. Metacercariae of *Clinostomum complanatum* have been recovered from *Perca fluviatilis* and *Rutilus rutilus* (Grabda-Kazubska, 1974), *Plecoglossus altivelis* (Lo *et al*., 1987), *Misgurnus anguillicaudatus* (Lo *et al*., 1992), *Carassius carassius, C. gibelio langsdrfi, C. cuvieri, Cobitis anguillicaudatus, Cyprinus carpio, Pseudogobio esocinus, Pseudorasbora parva, Rhodeus lanceolatus, R. ocellatus* and *Hypomesus transpacificus* (Aohagi *et al*., 1993), *Carassius* sp (Aohagi and Shibaharam, 1994), *Zacco temminki, Acheilognathus rhombea, Microphysogobio yaluensis, Carassius auratus* (Chung, 1995), *Lateolabrax* japonicus, *Leuciscus hakonensis* (Aohagi *et al.,* 1995), and *Eutycea neotenes* (Mitchell, 1995). Adult stage of the parasite has been recovered from *Nycticorax nycticorax* (Aohagi *et al*., 1992) and *Ardea cinnerea* (Lo *et al*., 1987; Aohagi *et al.,* 1992). Metacercariae may affect growth and survival, or disfigure fish so that they lose their market value as a food or ornamental product (Paperna, 1991). Some metacercariae in fisheries and aquaculture products (fish and shellfish) are a source for infections in humans and domestic animals (Deardoff and Overstreet, 1991).

MATERIALS AND METHODS

Study Area: Opi lake is a tropical freshwater lake located between 6^0 45′ 0″ – 45′ 28″N and 7^0 29′ 28″ – 7^0 29′ 35″ E (GPS N06.75275*, E007.49104*) in the valley of river Uhere, Northeast of Nsukka, Enugu State, Nigeria. The lake is about 300 meters from Uhere river. The soil is porous and subject to sever erosion. The vegetation and climate of the lake area has been described (Hare and Carter, 1984). The lake has no permanent inlet, but during the flood period the lake overflows through a small channel at the southern end. The lake has a gentle sloppy shoreline with thick marginal vegetation (Inyang, 1995). The western side has a wide beach overgrown with saprophytes dominated by *Crytosperma senegalenses* (Schott); Jussiaea *repens Var diffusa* (Forsk) and *Rynchospora* sp. Its surface area and maximum depth (Zmax) fluctuate seasonally and range between 1.3 and 2.0 ha and 2.0 and 3.9 m respectively (Inyang, 1995). The mid lake deposit is mud mixed with coarse organic matter from the marginal vegetation on the other parts of the shoreline. The ichthyofauna of the lake includes *Tilapia zillii, Hemichromis fasciatus, Parachana obscura, Malapterus electricus, Chrysicthys auratus, Epiplatys sexfasciatus, Hetrobranchus longifilis, Clarias angullaris, C. gariepinus, Barbus aboinensis, Nannaethiops unitaeniatus, Mormyrops engystoma* and *M. hasselguisti* (Inyang, 1995).

Sampling: One hundred and seventy seven species of *S. melanotheron* (Olaosebikan and Raji, 1998) were caught using multiple fishing gear techniques (cast nets, hook and line and seine nets (150 mm – 200 mm)) monthly (November 2007 – October 2008). Harvested fishes were transported in ice to Parasitology and Public Health Research laboratory, Department of Zoology, University of Nigeria, Nsukka for analysis.

Prevalence: Freshly caught fish were examined for parasites using procedure in Arthur and Albert (1994). Prevalence was calculated as the number of host infected divided by number of host examined expressed in percentage. Treatment, fixation and preservation of parasites were according to Ash and Orihel (1987).

Morphometrics: Eye piece and stage micrometers were used to measure the diameters of oral sucker (OS), ventral sucker (VS), and pharynx to the nearest 0.1 micrometers. Other measurements taken were body length (BL), and the distance between oral and ventral suckers (DOVS) (nm).

Data Analysis: Data generated were analyzed using the infection statistics of Bush *et al.* (1997), Rank-abundance, species diversity and quantitative index of Shannon-Wienner index (Molles, 2002). Differences in various morphometric characters were established using analysis of variance with Duncan's post hoc. All statistical analysis were done using SPSS version 15 statistical package.

RESULTS

The prevalence of the clinostomatids was low; *C. complanatum* (9.4 %), *E. heterostomum* (10.4 %), and *Clinostomum tilapiae* (4.8 %) (Table 1). High mean intensities suggestive of heavy parasite burden were recorded thus; *C. complanatum* (4.2)*, C. tilapiae* (2.0) and *E. heterostomum* (4.5). In all sampled fish species, fish size range 10 – 12 cm was most infected whereas fish size above 16 cm had the lowest level of infection. *C. complanatum* infection in fish size range of 10 – 12 cm gave the highest prevalence (11.3 %) and the lowest prevalence (1.13 %) occurred in fish size above 16 cm. In the infection of *E. heterostomum* also, fish size range 10 – 12 cm had the highest prevalence (2.25 %) and the least was recorded by size range above 16 0.56 %. Similarly, in the infection of *C. tilapiae* fish size range of 10 – 12 cm had the highest prevalence of 10.16 % while the size range above 16 cm had lowest value of 1.13 % (Table 2). Out of 177 *S. melanotheron* examined, 19 were infected by C*. tilapiae,* 38 infected by *C. complanatum* and 41 *S. melanotheron* by *E. heterostomum*. 158 *C. complanatum,* 85 *E. heterostomum* and 87 *C. tilapiae* were recovered from the infected hosts. *E. heterostomum* (pi 0.48) was more abundant than the other two species (pi 0.26) (Figure 1). Duncan's one way analysis of variance for comparison of means of morphometric characters indicated that except for the oral sucker, *C. complanatum* and *E. heterostomum* differed in all other variables significantly from *C. tilapiae* ($P < 0.05$) (Table 3). The three major microhabitats viz: buccal cavity, skin and eye were inhabited by clinostomatid parasites. The buccal cavity had the highest infection when compared to the other microhabitats (eye and skin). The presence of these clinostomatids led to inflammation and haemorrhages developed during penetration and early migration. Damage of important organs (eye and skin) was recorded. Eye damage due to corneal infection resulted in total blindness and other degrees of eye infection; exophtalamus, necrotic cells and ulceration of the lining membranes. Roughening of the skin by bumps/ yellow grubs caused by encysting metacercariae was observed.

DISCUSSION

The prevalence of infection of the parasites in relation to size showed that the larger the fish the lower the infestation, possibly due to development of immunity against it. It was found that fish above 16 cm had lowest parasitic infection while those in the range of 10 – 12 cm had the highest level of infection. This agrees with the work of (Malek and Mobedi, 2001) usually one expects larger fish to have more parasites as they have been exposed to infection for longer time. The prevalence of parasites significantly decrease with increase in length and no significant difference were observed in abundance. Decrease in the prevalence of infection in the larger fish could be as a result of increase in the mortality of infected fish, increase in the built up humoral and non specific

Table 1: Clinostomatids composition, overall prevalence and prevalence in relation to microhabitats in *Sarotherodon melanotheron* from Opi Lake

Parasite species	Host fish and No of infected hosts	Sex of hosts	No of infected host	Microhabitats in host fish	Total No of Parasites	*Prevalence (%)	¶Mean intensity	♯Abundance
Clinostomum complanatum	*S. melanotheron* (n = 177) infected hosts = 37	Male	8	B. cavity	56	4.52	1.51	0.32
		,,	10	Skin	16	5.65	0.43	0.09
		,,	10	Eye	14	5.65	0.38	0.08
		Female	3	B. cavity	7	1.69	0.19	0.04
		,,	3	Skin	4	1.69	0.11	0.02
		,,	3	Eye	3	1.69	0.08	0.02
						(20.89)		
Euclinostomum heterostomum	*S. melanotheron* (n = 177) infected hosts = 11	Male	1	B. cavity	4	0.56	0.36	0.02
		,,	1	Skin	2	0.56	0.18	0.01
		,,	1	Eye	2	0.56	0.18	0.01
		Female	2	B. cavity	13	1.23	1.18	0.07
		,,	4	Skin	9	2.26	0.82	0.05
		,,	2	Eye	2	1.23	0.18	0.01
						(6.4)		
Clinostomum tilapiae	*S. melanotheron* (n = 177) infected hosts = 37	Male	10	B. cavity	70	5.65	1.89	0.40
		,,	14	Skin	56	7.91	1.51	0.32
		,,	6	Eye	15	3.39	0.41	0.08
		Female	2	B. cavity	41	1.23	1.11	0.23
		,,	3	Skin	27	1.69	0.73	0.15
		,,	2	Eye	8	1.23	0.22	0.05
						(21.1)		

Key: *B. cavity = Buccal cavity *Prevalence: number of host infected divided by the number examined expressed as a percentage. ¶Mean intensity: Mean number of parasites per infected host.♯ Abundance: Mean number of parasites per host examined. Number in parenthesis = total prevalence.*

Table 2: Overall variations in the abundance and prevalence of Clinostomatids in different length groups

Length groups (cm)	N	Buccal cavity	skin	Eye	Prevalence %	Mean intensity	Abundance	% Prevalence of Buccal cavity	% Prevalence of skin	% prevalence of eye
C. complanatum										
< 10	4	9	3	2	2.30	3.50	0.08 ± 0.98	14.29	15.00	11.76
10 – 12	20	34	8	8	11.30	2.50	0.38 ± 0.44	53.97	40.00	47.05
12 – 14	8	12	6	4	4.51	2.75	0.12 ± 0.69	19.05	30.00	23.53
14 – 16	3	6	2	2	1.69	3.33	0.06 ± 1.13	9.52	10.00	11.76
Above 16	2	2	1	1	1.12	2.00	0.02 ± 1.39	3.17	5.00	5.88
E. heterostomum										
< 10	2	2	2	1	1.12	2.50	0.03 ± 1.27	11.76	18.20	25.00
10 – 12	4	9	7	2	2.25	4.50	0.08 ± 0.90	52.94	63.60	50.00
12 – 14	2	3	2	1	1.12	3.00	0.03 ± 1.27	17.65	18.20	25.00
14 – 16	2	2	-	-	1.12	1.00	0.01 ± 1.24	11.76	-	-
Above 16	1	1	-	-	0.56	1.00	0.00 ± 1.80	5.88	-	-
C. tilapiae										
< 10	6	18	10	3	3.38	5.16	0.18 ± 0.85	16.22	12.05	13.04
10 -12	18	51	39	12	10.16	5.67	0.58 ± 0.49	45.95	46.99	52.22
12 – 14	8	24	20	5	4.52	6.13	0.27 ± 0.74	21.62	24.10	21.74
14 – 16	3	11	9	2	1.69	7.33	0.12 ± 1.21	9.91	10.84	8.69
Above 16	2	7	5	1	1.13	6.50	0.07 ± 1.48	6.31	6.02	4.35

0.00 is for values lower than 0.01

immunity against the parasites. Since temperature is not limiting in the tropics it is expected that antibody production and other immunological reactions are more active in tropical fish than temperate ones. As the small size fish survives the infection it grows to occupy new niches and acquire better microhabitat against parasitic infestation.

Clinostomatid metacercariae had predilection for the mesenteries of blood capillaries in the buccal cavity; this explains their high prevalence and abundance in the buccal cavity. The buccal cavity blood capillaries provide nutrients which the parasites feed on either through passive, active or facilitated diffusion.

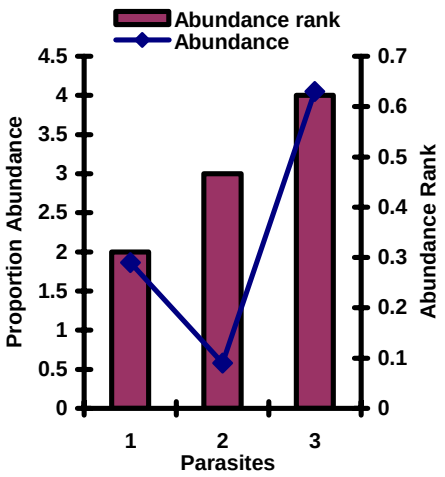

Figure 1: Communities of parasites in *Sarotherodon melanotheron*

Table 3: Morphometric parts of Clinostomatids from *Sarotherodon melanotheron*

Morphometric characters	*Clinostomum complanatum*	*Euclinostomum heterostomum*	*Clinostomum tilapiae*
OS (mm)	2.35 ± 1.10^{c}	1.43 ± 0.50^{a}	1.81 ± 1.02^{b}
VS (mm)	4.50 ± 1.14^{b}	3.22 ± 0.94^{a}	3.37 ± 1.93^{a}
D.O/V (mm)	8.17 ± 2.50^{a}	7.60 ± 2.07^{a}	7.45 ± 2.54^{a}
BL (mm)	55.96 ± 12.04^{b}	49.79 ± 8.52^{a}	53.22 ± 11.89^{ab}
Pha (mm)	1.17 ± 0.36^{c}	0.89 ± 0.33^{a}	1.02 ± 0.38^{b}

Key: *Letters a, b, and c show significance difference at 95 % confidence interval (P < 0.05 %), the figures with similar letters indicate no significance difference, while those with different letters show significance difference.*

Relative larger morphometric body length of *C. tilapiae* would be more influx of nutrients than the other two species. Malek and Mobedi (2001), the higher prevalence and abundance of parasites in the area under the mouth to behind the operculum are probably due to the presence of blood, which *Clinostomum complanatum* feed on. It disagrees with the finding that the main habitat of *Clinostomum complanatum* in the *Carassius* sp was the muscles around the gills (Aohagi and Shibahara, 1994).

Two forms of occurrence of metacercariae in fish (encysted and excysted) possibly indicate some fish developed resistance to the metacercarial stage of *Clinostomum*. The later form; excysted metacercariae in the various localized sites could be more harmful to human health when they are consumed in semi cooked form. This is because attachment to the mucus membrane of pharynx of the definitive hosts (Egret, Herons etc) causes laryngopharyngitis. There were more excysted forms of *E. heterostomum* than the other two parasite species. Out of 32 *E. heterostomum* only 10 were not excysted, 37 *C. tilapiae* and 44 of *C. complanatum* were encysted respectively. These excysted forms caused serious damages to the infected fish. These effects include blindness, myositis, muscle bumps (yellow grubs) etc. This will affect the palatability and marketability of the infected fish as well as the acceptance of fish as the primary source of animal protein.

Adaptation is a heritable trait that either spread because of natural selection or has been maintained by selection to the present or currently spreading relative to alternative traits because of natural selection. In all such cases, the trait in question has conferred and continues to confer or is just beginning to confer higher genetic or reproductive success on dominant species of the parasites with favorable alternative traits in the various fish species. In evolutionary biology, 'fitness' is a measure of an individual's reproductive or genetic success, so that 'fitness benefit' refers to the positive effect of a trait on the number of surviving offspring produced by an individual or the number of genes it contributes to the next generation whereas 'fitness cost' refers to the damaging effects of the trait on these measures of individual genetic success. Much the same type of thing has been documented for two strains of laboratory mice that are genetically identical in every respect, except for a single gene that encodes an enzyme called α-calcium-calmodulin kinase. Because of this one genetic and enzymatic difference, members of the two strains differ in the construction of their hippocampus, a region of the vertebrate brain involved in spatial learning. These hippocampus differences between the two kinds of mice underlie differences in their performance on spatial memory tests (Silva *et al.*, 1992). Suggestive pressures on selection for the fittest. Natural selection would favour *C. tilapiae* in *S. melanotheron* during the cause of time and possible domination in other fish hosts in this freshwater lake.

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KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) OF SCHOOL TEACHERS ON MALARIA, HELMINTHIASIS AND ASSOCIATED RISK FACTORS IN PRIMARY SCHOOLS IN ONITSHA, ANAMBRA STATE, SOUTH-EASTERN NIGERIA

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ABSTRACT

Structured questionnaires were administered to 160 teachers from different Nursery and Primary Schools in GRA Onitsha, Nigeria to assess their knowledge, attitude and practices (KAP) on malaria, helminthiasis and associated risk factors in the schools' premises. Educational attainments of the teachers were Masters Degree (0.8 %), Bachelors Degree (39.2 %), Diploma Certificate (50.8 %), and Secondary School Certificate (9.2 %). There were more female (99.2 %) than males (0.8 %). A high percentage of the teachers (75.4 %) attributed malaria to eating too much oily food, hereditary (0.7 %), intense sunlight (2.1 %), drinking of dirty water (0.7 %), butter (3.5 %), and fried foods (1.4 %). On malaria prevention, about 64.2 % of teachers heard about insecticide treated net (ITN) but have never used it. Other preventive measures mentioned were use of clean environment (31.9 %), mosquito nets (20.2 %), and antimalarial drugs (12.3 %). KAP on helminthiasis indicated that some of teachers attributed worm infection to eating sugary foods (19.2 %), drinking dirty water (9.3 %), natural occurrence (1.3 %), eating with dirty hands (13.9 %), unwashed fruits and vegetables (10.6 %), unripe fruits (3.3 %), and over ripped fruits (2.0 %). About 48.33 % had seen worms in pupil's stool., while 37.5 % had de-wormed pupils; 53.3 % of them using Ketrax®. Teachers' perceived methods of preventing worm infection were avoidance of sugary foods (27.9 %), washing hands before eating (10.46 %), washing fruits and vegetables before consumption (26.2 %), and drinking clean water (10.46 %), while 22.1-30 % did not know how to prevent nor treat helminthiasis. Risk factors for parasitic infections observed in most of the schools included indiscriminate defecation, unhygienic lavatories, blocked drainages, container breeding habitats and open dumping of wastes. Health education for teachers in nursery and primary schools on transmission, prevention and treatment of malaria and helminthiasis is highly advocated.

Keywords: Malaria, Helminthiasis, Parasitic infections, Risk factors, Primary schools children, KAP,

INTRODUCTION

Nearly 13 million children die each year in developing countries and majority of the deaths are attributable to parasitic diseases (Tomkins and Watson, 1989). Malaria, caused by protozoan parasite of Genus *Plasmodium*, debilitates and kills more people than any other single infectious disease (Sherman, 1998) and continues to be a major public health burden in Nigeria, which has the largest population at risk of stable malaria in the world (de Savigny *et al.,* 2004). Each year, one fifth of the world's population is at risk of malaria, with over 300 million coming down with the illness, resulting to more than one million deaths, with a child somewhere in the globe dying every 30 seconds (UNICEF, 2004). In Nigeria, malaria is holoendemic with intense all year round transmission (Bruce-chwatt, 1983) more especially in the wet season, and with fifty percent of the population experiencing at least one episode of malaria each year (Coker *et al.*, 2001). Studies in several parts of Nigeria have demonstrated deficiencies in knowledge, attitude and practices (KAP) of malaria (Agomo *et al.*, 1999; Fawole and Onadeko, 2001). According to Service (1993), community perception of malaria had suffered major set backs mainly due to misconceptions and cultural barriers within the communities. In West Africa, the community awareness on the causes of malaria is generally very poor. For instance, the sun and groundnut consumption were believed to cause malaria in the Benin-Republic (Kombila, 1994) while excessive drinking, heat, fatigue, flies and unsafe water were some perceived causes in Ghana (Okyere,

1994). In some parts of southern Nigeria, excessive heat, over work, excessive sex, sunlight, certain foods and drinks, noise, heredity and witch-craft and other superstitions were thought to be responsible for malaria (Brieger *et al.*, 1997; Nebe *et al.*, 2002; Ozumba and Ozumba, 2004). Current global estimates indicate that Soil transmitted helminthiasis is among the most common of all parasitic infections (Stephenson, 1987). Intestinal helminthiasis is particularly common in school children and these children carry the heaviest burden of morbidity due to infection (Ozumba and Ikpeze, 2007) and may contribute significantly to transmission as reported by Bundy *et al.* (1990). Increasing evidence suggest that intestinal helminthiasis affects health, nutritional status and growth, cognitive performance and school attendance of school children (Stephenson, 1987; Pawlowski *et al.,* 1989; Stephenson *et al.,* 1989; Connolly and Kvalsvig, 1992; Eneanya and Anikwue, 2006; Ozumba and Ikpeze, 2007). There is a dearth of information on KAP of nursery and primary school teachers on malaria, helminthiasis and associated risk factors in Onitsha. The need to improve on KAP of nursery and primary school teachers on malaria, helminthiasis and associated risk factors which will help in the control of parasitic infection among pupils stimulated the present study in nursery and primary school at Onitsha.

MATERIALS AND METHODS

The Study Location: The study was conducted between January and June, 2009, using Nursery and Primary Schools in GRA Onitsha (Latitude 6˚ 08' N and Longitude 6˚ 48' E), southeastern Nigeria.

Data Collection: Copies of a well-structured questionnaire were administered to 160 nursery and primary schools teachers who were requested to respond to questions regarding their KAP on malaria, helminthiasis among their pupils and the associated risk factors in their schools. Only 120 teachers responded and returned the completed questionnaire. Data on the questionnaires were collated and further corroborated with oral interviews and on-the-spot photographs of risk factors for parasitic infections observed in various schools inspected during the study period.

RESULTS AND DISCUSSION

Gender and educational attainment of the respondents are shown in Table 1. There was only one male teacher, which indicated that primary school teaching is being dominated by females.

Table 1: Gender and educational attainment of teachers in nursery and primary schools in Onitsha, Anambra State, Nigeria

Teachers	Number	Percentage
Gender		
Male	1	0.8
Female	119	99.2
Total	**120**	**100**
Educational attainment		
Masters degree (M.Ed)	1	0.8
Bachelor's degree (B.Sc, B.Ed, B.Tech)	47	39.2
Diploma certificate (NCE, HND)	61	50.8
Secondary school education (TCII, WASC)	11	9.2
Total	**120**	**100**

This observation may not be surprising. Onitsha is a commercial city where the get-rich-quick syndrome had gone into the heads of many males, hence teaching is not considered a lucrative vocation for men in the town (Ikpeze and Eneasator, 2008; Ikpeze, 2008). About 50.8 % of the teachers possess NCE and HND certificates, while 39.2 % have bachelor's degrees in Teaching and Technical subjects. Less than 10 % have Teachers Grade Two Certificate and the West African School Certificate, but only one person has a Masters degree in Education.

Despite their educational qualifications, it appeared that majority of the teachers have poor KAP on parasitic infections. KAP of teachers on causes and signs of malaria (Table 2) shows that about 75.4 % of the teachers perceived that eating of too much palm oil in foods was a major cause of malaria. It was surprising to note that only 7.1 % associated malaria with dirty environment, while 1.4 % mentioned stagnant water. Only 1 teacher (0.7 %) thought that mosquito bite could cause malaria. Nevertheless, most of the respondents possessed good knowledge of the signs of malaria. Responses, perhaps from personal experiences, ranged from fever and high temperature (26 %), head ache (22.8 %), body weakness (16.9 %), loss of appetite (10.9 %), cold (6.2 %) and vomiting (5.3 %).

About 31.9 % agreed that clean environment could prevent malaria. Other responses were installation of fly-proof window and door nets (20.2 %), taking anti-malaria drugs (12.3 %) and sleeping under insecticide treated bed nets (10.4 %). It is also observed that about 45.3 % of the respondents practice self-medication when they think they have malaria. This practice may be responsible for development of drug resistance, which is a problem in the control of malaria in Nigeria and other

Table 2: KAP for causes and signs of malaria amongst teachers sampled from nursery and primary schools in Onitsha, Anambra State, Nigeria

Knowledge, attitude and practice (KAP)	Number	Percentage
Causes of Malaria?		
Hereditary	1	0.7
Mosquito bite	1	0.7
Drinking dirty water	1	0.7
Eating too much of palm oil in food	107	75.4
Butter	5	3.5
Dirty environment	10	7.1
Fever	4	2.8
Poor feeding	6	4.2
Stagnant water	2	1.4
Fried food	2	1.4
Hot sun	3	2.1
Total	**142**	**100**
Signs of malaria?		
Body weakness	57	16.9
Hotness of the body	7	2.1
Head ache	77	22.8
Stomach ache	5	1.5
Fever (high temperature)	88	26
Yellowish eye	1	0.3
Joint pain	7	2.1
Yellowish urine	2	0.6
Loss of appetite	37	10.9
Vomiting	18	5.3
Body pain	6	1.8
Cold	21	6.2
Bitterness in the mouth	1	0.3
Weakness of the eyes	6	1.8
Oil on the face after sleep	1	0.3
Shivering	3	0.8
Reddish eye	1	0.3
Total	**338**	**100**

endemic areas of the world where self-medication is in vogue. However, about 25 % would opt to see a medical doctor, while 10.1 % would rely on herbs. Some of the teachers who use herbs declared their loss of confidence in proprietary malaria drugs, some of which are feared to be expired, faked or adulterated. The Onitsha bridge head drug market is reputed to be the largest in Black Africa, and the drug traders there are suspected to be engaged in fraudulent activities. Thus one becomes wary of any drug, especially malaria drugs, sold in the town. It is most alarming that a few persons (0.7 %) could erroneously think that immunization could protect people from malaria, others (10.1 %) had no idea whatsoever on the treatment of malaria. This poor perception may account for high morbidity and mortality seen in cases of non-complicated falciparum malaria in most communities in this country.

Table 3: KAP for prevention and treatment of malaria amongst teachers sampled from nursery and primary schools in Onitsha, Anambra State, Nigeria

Knowledge, attitude and practice (KAP)	Number	Percentage
Prevention of malaria?		
Fly-proof net on windows and doors	33	20.2
Clean environment	52	31.9
Early laboratory diagnosis	5	3.1
Spraying of insecticides	1	0.6
Taking anti-malaria drugs	20	12.3
Sleeping under insecticide treated net	17	10.4
Avoiding oily food	5	3.1
Drinking clean water	30	18.4
Total	**163**	**100**
Treatment of malaria?		
Taking malaria drugs (self-medication)	67	45.3
Avoid fried food	1	0.7
Using herbs	15	10.1
Seeing a medical doctor	37	25.0
Seeing a chemist	5	3.4
Immunization	1	0.7
Taking lots of fruits	2	1.3
Reduce eating of oily food	5	3.4
No idea	15	10.1
Total	**148**	**100**

Table 4 is the result of KAP for worm infection amongst teachers in nursery and primary schools sampled at Onitsha during the study period.

About 19.2 % of the teachers attributed worm infections to eating sugary things, 1.3 % to nature, where 27.2 % did not know any cause of worm infection. Other causes mentioned included drinking of dirty water (9.3 %), eating with dirty hands (13.9 %), unwashed fruits and vegetables (10.6 %), un-ripped fruit (3.3 %), over ripped fruit (2.0 %), dirty and spoilt food (2.6 %), worm infected food (2.6 %), and improperly cooked food and vegetables (8.0 %). Signs of worm perceived by the respondents included stomach pains and troubles (32.3 %), vomiting (27.2 %), and loss of appetite (4.6 %), while 12.9 % have no idea. On the reason for development of pot-belly amongst the pupils, 51.6 % that have noticed some pupils with pot belly had no idea of the cause, but others attributed pot-belly to worm infection (25.4 %), nature (2.4 %), sickle cell (3.9 %), malnutrition (4.8 %), over eating (1.6 %), stomach pain (1.6 %), other illnesses (3.9 %), cold food (0.8 %), anaemia (1.6 %), Kwashiorkor (0.8 %), and constipation (1.6 %). About 22.1 % of the teachers did not know how to prevent worm infection, 27.9 % suggested avoiding sugary foods.

Table 4: KAP for worm infection amongst teachers in nursery and primary schools sampled at Onitsha, Anambra State, Nigeria

Knowledge, attitude and practice (KAP)	*Number*	*Percentage*
Causes of worm infection?		
Eating of sugary foods	29	19.2
Drinking of dirty water	14	9.3
Eating of dirty and spoilt food	4	2.6
Eating of worm-infected food	4	2.6
Eating with dirty hands	21	13.9
Eating of unwashed fruits and vegetables	16	10.6
Eating of un-ripped fruits	5	3.3
Eating of over-ripped fruit	3	2.0
Natural (worm infections come and go)	2	1.3
Eating of improperly cooked food and vegetables	12	8.0
No idea	41	27.2
Total	**151**	**100**
Signs of worm infection?		
Stomach pain and trouble	70	32.3
Spitting of saliva	5	2.3
Vomiting	59	27.2
Loss of appetite	10	4.6
Anaemia	2	0.9
Noisy stomach	7	3.2
Blood in urine	1	0.5
Frequent watery stool	6	2.8
Blood in stool	2	0.9
Swollen tummy	10	4.6
Loss of weight	4	1.8
Headache	2	0.9
Fever	6	2.8
Weakness	4	1.8
Cold	1	0.5
No idea	28	12.9
Total	**217**	**100**
Ever seen worms in pupils' stool	58	48.33
Never seen worms in pupils' stool	55	45.83
No idea	7	5.83
Total	**120**	**99.99**
Do you de-worm your pupils?		
Yes	45	37.5
No	75	62.5
Total	**120**	**100**
With what do you de-worm your pupils?		
Ketrax	24	53.3
Combantrin®	21	46.7
Total	**45**	**100**
Prevention of worm infection?		
Washing hands before eating	18	10.46
Avoid eating of sugary things	48	27.9
Washing fruits and vegetable before consumption	45	26.2
Drinking clean water	18	10.46
Going to hospital for check-up	1	0.58
Cooking food well	3	1.7
Health Education	1	0.58
No idea	38	22.1
Total	**172**	**99.98**

Others methods of prevention pointed out by the teachers included washing of fruits and vegetables before consumption (26.2 %), drinking clean water (10.46 %), washing hands before eating (10.46 %), health education (0.58 %), cooking food well (1.7 %) and going to hospital for check-ups (0.58 %). About 37.5 % had previously dewormed their pupils with either Ketrax® or Combatrin®.

Figures 1 and 2 shows the photographs of some of the risk factors for parasitic infections observed in nursery and primary school premises and among the pupils under study at Onitsha. Figures 1A and B show the poor drainage system commonly seen in the schools. The PVC pipe discharges it waste into the shallow drainage gutter, the content of which has become stagnant and provide good breeding place for disease vectors, like mosquitoes. Pupils studying the illustrations on the adjacent wall are at risk of being bitten by these vectors. Pupils are equally exposed to the dirty surrounding provided by the stagnant drainage gutters. Indiscriminate voiding of faeces is common amongst the pupils (Figures 1C and D), thus encouraging the rapid breeding of flies that carry pathogenic bacteria and protozoa. Figure 1E shows that the used tyres delineating the play ground constitute very good habitats for container breeding *Aedes* and *Culex* mosquito species that transmit filariasis and other mosquito borne diseases. The play area (Figure 1 F) is already overgrown with weeds, which again, are excellent breeding grounds for arthropod pests. Pupils playing bare-footed (Figure 1G) are exposed to possible Geohelminth infections because faecal matter is thrown away on the area after washing the basins used by the pupils in defecating (Figure 1 C and D). Health risk posed by the refuse heap (Figure 1H) needs not be overemphasized. Apart from being unsightly, it is also a good breeding place for snakes scorpions, and rats which are proven transmitters of plague and other bacterial and Rickettsial infections in man.

Figure 2 also shows other risk factors noticed in course of the study.

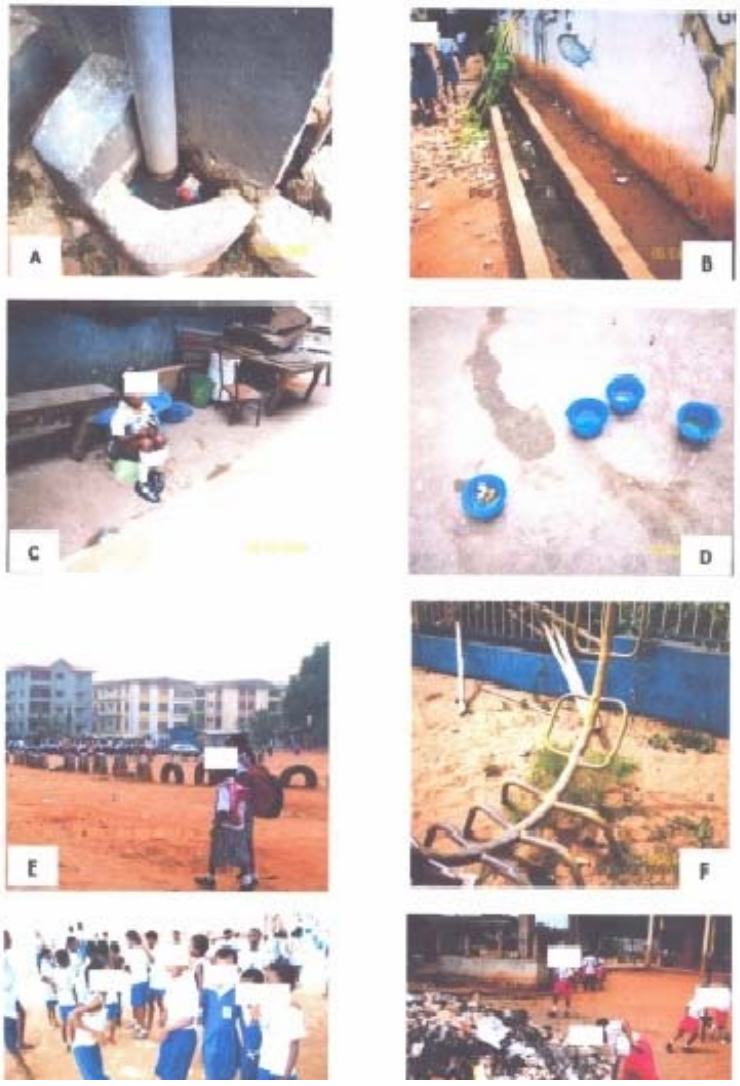

Figure 1: Risk factors associated with parasitic infection as observed in the study population (A to H are explained in the text)

Figures 2 A, B, C, D and E show that indiscriminate defecation and urination is very common in nursery and primary schools. Figure 2 A and B show pupils defecating in the faecal receptacles without supervision. The faecal receptacles were left uncovered afterwards, attracting flies. The floor of the open area used as toilet room is filled with excreta and urine. Myiasis causing flies visiting these sites might also perch on the pupils to lay eggs leading to myiasis. Pupils were observed to be putting on shoes while on bed during siesta. This action can carry the eggs of *Ascaris lumbricoides* to bed; and these eggs have been reported to be inhaled (Paniker, 1997). The water closets (WC) in most of the schools studied were without covers and non-functional., while excreta were left to accumulate and not flushed, thereby posing health risk to pupils of the schools. Figures 2C and D are typical examples where flies had already infested the toilet buckets and the seats. Pupils left to ease themselves without supervision contribute a lot in contaminating the toilet areas (Figures E and F). A child passing excreta in this toilet can easily pick up excreta and other things left on the seats by the fly, which include cysts of *Entaemoeba histolytica* that cause Amoebiasis, *Ascaris lumbricoides* eggs that cause Ascariasis, and possibly segments of tapeworms that may give rise to bladder-worms in the muscles of the pupils if ingested.

Most times pupils wash hands collectively (Figure 2D) due to lack of domestic water, which is a serious problem in the schools since the State Water Corporation, has been out of operation for more than nine years. This basin containing water is exposed to houseflies, which are mechanical vectors of diseases. They can carry pathogen like *Vibrio cholera*, which causes cholera, cysts of *Entamoeba histolytica,* which causes Amoebiasis several enteric parasitic diseases (Greenberg, 1971). At the end of school, pupils, perhaps with dirty hands, would eat snacks (Figure 2H) on their way home and may in this way contract parasitic infections, like Giardiasis caused by *Giardia lamblia*.

Conclusion: This work has exposed the level of KAP of teachers of nursery and primary school pupils on malaria, helminthiasis, and risks factors associated with parasitic infections in nursery and primary schools in Onitsha, some aspects of which are not very encouraging. Similar conditions may exist in other parts of the country, so appropriate health education on prevalent parasitic infections in the country is advocated for all categories of teachers in the primary and secondary levels of education in the country.

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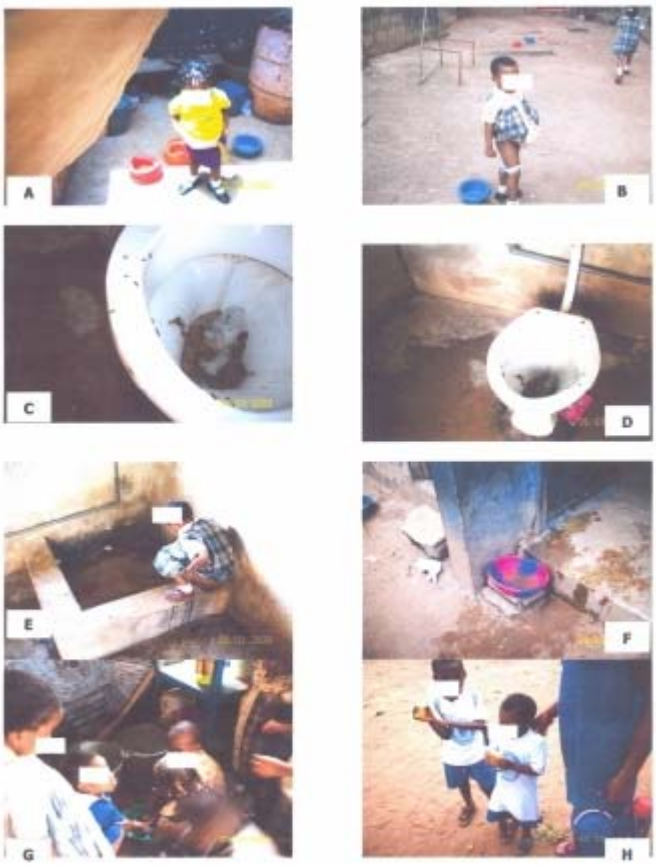

Figure 2: Other risk factors associated with parasitic infection as observed in the study population (A to H are explained in the text)

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ASPECTS OF THE BIOLOGY OF *Heterotis niloticus* CUVIER 1829 (OSTEOGLOSSIFORMES: OSTEOGLOSSIDAE) IN THE ANAMBRA FLOOD RIVER SYSTEM, NIGERIA

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ABSTRACT

Studies on some aspects of the biology of Heterotis niloticus in Anambra river were carried out for 22 months. Analysis of the stomach content of 546 species of H. niloticus in Anambra river established the preponderance of plantivorous crustacean, copepods and cladocera throughout the four maturation stages examined. Seasonal variations occurred in the dietary components of the fish. The length-weights relationship and the relative condition revealed that females had better condition. The mean length and weight were 94.5 ± 60 cm (29 – 167 cm) and 120 ± 21.8g (10 – 250g) respectively. A sex ratio of 1:0.8 (M: F) at Otuocha was more pronounced than at Ogurugu and Nsugbe. Digestive enzyme assays in the different gut regions (oesaphagus, stomach, pyloriccaeca, ileum and rectum) of H. niloticus showed an array of glycosidase (amylase, sucrase, malatase, lactase, cellulase); protease, (pepsin, trypsin, chymotrypsin) and lipases. The pattern of spread and relative activity of the enzymes is consistent with its planktophagous diet. Haematocrit values, haemoglobin concentrations, erythrocyte and leucocytes counts, mean cells haemoglobin concentrations, plasma protein, glucose, albumin and inorganic ion (sodium, chloride, calcium, potassium, magnesium, phosphorus) for H. niloticus were established. Correlations were found between some blood parameters and standard length.

Keywords: *Heterotis niloticus*, Length-weight relationship, Stomach content, Digestive enzymes, Haematology

INTRODUCTION

The freshwaters of the forested region of Nigeria supports large population of the West African Osteoglossid fishes. *Heterotis niloticus* is an important element in the artisanal fishery of the Anambra river basin. The fish is a regular feature in the Anambra river basin and is highly cherished because of its socio-cultural benefits, particularly among the Igbo people of Nigeria. Considerable biological studies have been undertaken and documented on some economically important tropical fish families in Anambra river basin, for instance, Clariidae (Eyo and Mgbenka, 1992; Mgbenka and Eyo, 1992; Ezenwaji and Inyang, 1998), Distochontidae (Nwani, 1998), Clupeidae (Ezenwaji and Offia, 2003) among many others. Studies on aspect of the biology of *Heterotis* are vast (Balogun, 1980; Lawal, 1991; Ugwumba, 1992; Akegbejo-Samsons, 1995; Fagbenro *et al.,* 2000), nevertheless non from the Anambra river basin. To be able to manage a resource of such commercial importance, knowledge of its biology is imperative. The study of dietary habits of fish, based on stomach content analysis, is widely used in fish ecology as an important means of investigating trophic relationships in aquatic communities. The ability of an organism to digest a given material is dependent on the presence of appropriate enzymes. No information is available on the quantitative and qualitative of assays of digestive enzymes in the gut *of H. niloticus* of Anambra river, in contrast to other African freshwater fishes whose digestive enzymes assays have been established (Uys and Hecht, 1987; Olatunde *et al*., 1988; Fagbenro, 1990; Fagbenro *et al.,* 1993; Fagbenro *et al.,* 2000). The use of haematological characteristics in diagnosing the health status of fish under captive rearing is well established. The knowledge of the haematological profile of a fish also indicates its dietary sufficiency and physiological stress. The haematological profile of a few African fish species have been reported (Olatunde *et al*., 1988; Fagbenro, 1990; Fagbenro *et al.,* 1993; Fagbenro *et al.,* 2000). Despite the foregoing, it does appear that no investigation has been conducted on the aspect of biology of *H. niloticus* of Anambra river basin. Thus, this study, which forms part of a larger and on-going investigation, on the fish and fisheries of the river basin, addresses aspects of the biology of the species viz; qualitative and quantitative food composition, occurrence, distribution and relative activities of glycosidases, proteases and lipases in the different gut regions, haematological and serological profiles, age, growth and reproduction.

MATERIALS AND METHODS

Study Area: The Anambra river is about 207.4k m in length and 14014km^2 in area (Awachie, 1976). It rises from the Ankpa hills (ca. 305 – 610m above sea level), flows in southerly direction through a narrow trough that gradually broadens as it courses down. It crosses the Kogi/Anambra States boundary a bit north of Ogurugu, then meanders through Ogurugu

to Otuocha and Nsugbe. From there it flows down to its confluence with the Niger at Onitsha. The basin lies between latitude $6^0 10^1$ and $7^0\ 20^1$, longitude $6^0\ 35^1$ and $7^0 40^1$ east of Niger river into which the Anambra river empties.

There are two main seasons, the dry season October/November – March and the rainy season (April – September / October) approximately corresponding to the dry and flood phases of the hydrological regime (Ezenwaji, 1986).

The vegetation is derived Guinea Savannah. Also the riparian vegetation, ecology and productivity of the river basin have been extensively studied (Awachie and Ezenwaji, 1981). The abundance and distribution of macroinvertebrates in the flood river system has been documented (Eyo and Ekwonye, 1995).

Sampling: Monthly *H. niloticus* were sampled at Ogurugu, Otuocha and Nsugbe stations, along Anambra river for twenty two months with 200 baited hook and line (no.17), baskets traps, seine gill nets of 3 cm stretched mesh in deep waters. The 200 baited hook and line, seine gill nets of 3 cm mesh size set overnight (1800 – 0700 h) in each sampling location were taken as unit efforts, and used to determine the distribution and abundance of the osteoglossid. Total length (TL) to nearest 0.1 cm and body weight to the nearest 0.1 g of each fish species were measured and the sex determined.

Age and Growth: The length-frequency data were grouped into 2-cm TL intervals and the length-based Powell-Wetherall method (Sparre and Venema, 1992) was employed in estimating the asymptotic length ($L\infty$) of the von Bertalanffy growth function from the linear relationship, $L = a + bl$, where L = mean length of the fully recruited fish computed from L upward, a and b being regression constants. The growth coefficient (K) was derived from Ursin (1994) thus: $K = 0.27 \exp(0.038T)$, where T = 27.5C (Ezenwaji, 1982). The age at length zero (to) was estimated from the empirical relationship of Pauly (1979) thus: $\log(-to) = -0.3922 - 0.2752 \log L\infty - 1.038 \log K$. Mean lengths at age were then estimated from the resulting von Bertalanffy growth function. The length growth performance index (ǿ) was calculated according to Pauly and Munro (1984) thus: $ǿ = \text{Log } iK + 2\log L\infty$. The relationship of body weight (W) to TL was determined using the power curve ($W = aTL^b$). Relative condition factor (Kn) was estimated as ($Kn = W/aTL^b$ (Le Cren, 1951).

The length-weight relationships (LWR) were determined for the same species collected at different periods. These different estimates were considered separate 'population'. Fulton condition factor, k, was calculated as: $K = W/L^3 \times 100$. Where W = fish weight (g) and L = Fish length (cm).

Food and Feeding Habits: The stomach of each *H. niloticus* was dissected out and its degree of fullness estimated on 0 – 20 points scale; where 0, 2.5, 5, 10, 15 and 20 points were allotted to empty, trace, quarter-full, half-full, three quarter-full and fully distended stomachs respectively.

Stomach contents were sorted into categories and identified. The contents were further analyzed using relative frequency (RF) and point's method (Hynes, 1950; Hyslop, 1980). In the RF, the frequency of a particular food item in all stomachs was expressed as a percentage of the frequencies of all food items.

For the point's scheme, each stomach was allotted 20 points regardless of the fish size and these were shared amongst the various contents, taking account of their relative proportion by volume. The points gained by each food item in all stomachs examined were computed and expressed as a percentage of the total points of all food items. The point's scheme gave an indication of bulk contribution of each food category to the diet composition. %RF and %PP were then used to determine the index of food significance as follows: IFS = (% RF x % PP) / (Σ % RF x % PP) x 100 (Hyslop, 1980). Food items with IFS $\geq$ 3 % were regarded as primary, $\geq$ 0.1 to < 3 % as secondary, whereas food with < 0.1 % were regarded as incidental. The IFS data were used to compute diet breadth based on Shannon-Wiener function (H) as follows: $H_{(IFS)} = \Sigma (n_i/N) \text{Log}_e (n_i/N)$ (Shannon-Wiener, 1963). Where Ni = IFS of each food item, N = total IFS of all food items. Food richness was defined as the number of food items in the diet with IFS $\geq$ 0.1%.

Digestive Enzyme Assays: Twenty-three adult *H. niloticus* specimens (TL 29-167 cm) were kept unfed for 72 hours inside out door concrete cisterns in order to bring them to a similar physiological state as well as to ensure the emptiness of their entire gut. They were anaesthetized with 0.5 % MS 222 and dissected to remove the entire gut. The gut was later separated into anatomically distinct regions. The different gut regions were pooled and homogenized. The homogenates were then cold centrifuged (4 ^{0}C) at 1200 rpm for 5 minutes. The supernatants were used as crude extracts without further purification. Benedict's qualitative reagents were used for the qualitative assays of glycosidase (Olatunde *et al.*, 1988), while quantitative assays were conducted using the dinitrosalicyclate (DNS) methods (Plummer, 1978). Qualitative and quantitative assays of proteases followed the methods of Balogun and Fisher (1970).

Haematological and Serological Profiles: Twenty three live specimens (TL 32 – 51 cm) capture from the river basin were kept undisturbed in large glass aquaria (120 litre capacity) supplied with filtered and aerated tap water for two weeks of acclimation to laboratory conditions (pH 7.8, dissolved oxygen concentration >6 mg/l, water temperature 26 - 28.5 ^{0}C), prior to blood sampling. During the acclimatization period the fishes were fed 5 % of their body weight with 40.40 % crude protein fish diet (Eyo and Ezechi, 2004) in divided rations, twice daily.

Table 1: Abundance and percentage of occurrence (%FO) of *Heterotis niloticus* in the Anambra flood river system, Nigeria

Location	Forest plain habitat		Grass plain habitat		Marshy habitat		Location
	No	%FO	No	%FO	No	%FO	total
Ogurugu	16(0.8)	71	11(0.5)	68	19(12)	94	46(2.5)
Otuocha	12(0.5)	93	14(0.7)	80	16(0.8)	77	46(2.0)
Nsugbe	18(0.9)	86	19(1.0)	79	12(0.7)	82	49(2.6)
Habitat total	46[a]	32.6	44[a]	31.2	47[a]	33.3	141

Key: *%F0 = percentage frequency of occurrence, a = the figures in the same column or row with the same superscript are not significantly different p=0.05, values in parentheses = weight in kg*

Table 2: The relationship between body weight and total length ($W = a TL^b$) of the *Heterotis niloticus* from the Anambra flood river system, Nigeria

Length range		Sex	N	A	B	r^2	P
Min (cm)	Max (cm)						
59	**142**	**Female**	54	$3.9x10^{-3}$	2.978	0.970	<0.001
39	**150**	**Males**	48	$1.2x10^{-2}$	3.109	0.869	<0.001
98	**292**	**Both sexes**	102	$9.3x10^{-3}$	2.79	0.872	<0.001

Table 3: Sex ratio of *Heterotis niloticus* in the Anambra river basin

Month	Ogurugu			Otuocha			Nsugbe			Anambra river		
	M	F	Sex ratio	M	F	Sex ratio	M	F	Sex ratio	M	F	Sex ratio
January	19	11	1:0.6	21	13	1:0.6	-	-	-	10	26	1:0.7
February	7	2	1:0.3	13	6	1:0.5	-	-	-	15	5	1:0.3
March	-	-	-	5	7	1:1.4	-	-	-	5	7	1:1.4
April	12	13	1:0.6	13	7	1:0.5	-	-	-	34	26	1:0.8
May	23	21	1:0.9	16	19	1:1.2	8	11	1:14	47	51	1:1.1
June	29	11	1:0.4	15	18	1:1.2	20	14	1:0.7	64	43	1:0.7
July	35	26	1:0.7	26	13	1:0.5	-	-	-	11	39	1:0.6
August	24	11	1:0.5	22	20	1:0.9	-	-	-	46	31	1:0.7
September	-	-	-	20	27	1:1.4	8	13	1:1.6	20	40	1:2.0
October	-	-	-	19	29	1:1.5	-	-	-	19	29	1:1.5
November	-	-	-	9	6	1:0.7	-	-	-	9	6	1:0.7
December	32	17	1:0.5	30	4	1:0.1	-	-	-	62	21	1:0.3
Total	181	112	1:0.6	209	169	1:0.8	36	38	1:1.1	342	324	1:0.7

All fish were considered healthy on the basis of their appearance and absence of obvious signs of disease. No sexual selection was made. The fish were caught individually using hand net and anaesthetized with 0.5 % MS 222. Blood was collected from the caudal vein of each fish using heparinized disposable syringes and hypodermic needles. Haematocrit (PCV) was measured after centrifugation at 15000 rpm (MSE Microcentrifuge). Haemaglobin (HB) content, leucocyte count (WBC), erythrocyte count (RBC), total plasma protein, plasma glucose, plasma albumin and plasma ion (Na, CL, Ca, Mg, K, P) concentrations were determined using the methods of Svobodova *et al.* (1991). Blood grouping was performed based on agglutination tests, while the genotype was determined by haemoglobin electrophoresis (Delany and Garratty, 1969).

Data Analysis: Data for corresponding months were pooled together for analysis. Abundance data were analyzed by two-way analysis of variance (ANOVA).Food composition and sex ratio were analyzed by students-test and X^2 test respectively (Bailey, 1994).

Differences were considered significant at 5% level of probability. The methods described by Ogunbiyi and Okon (1976) were used to determine lipase activity both qualitatively and quantitatively. Controls were run simultaneously for all assays. Regression analysis was carried out between the various haematological parameters and standard length. The coefficient of regression (r) was then analyzed for statistical significance by student's t-test (p=0.05).

RESULTS

Distribution and Abundance: The number of *H. niloticus* in the various stations showed no significant difference (P = 0.05). The osteoglossid fish appeared to be evenly distributed, and frequently occurred in all the stations studied. These osteoglossids occur throughout the year but with the peak from June – August (Table 1).

Size Range: The length of *H. niloticus* ranged from 29.00 – 167.00 cm Total Length (mean 94.5 $\pm$ 6.00 cm TL), while the weight ranged from 10.00 – 250.00 g (mean 120 g $\pm$ 21.8 g). Sexes were not different in lengths and weights (p>0.05) (Table 2).

Table 4: Relative importance and occurrence of food items in stomachs of *Heterotis niloticus* fry, fingerling, juveniles and adult in the Anambra river

Diet and fish variables	Stage 1 post fry 10.3 cm	Stage 11 fingerling 17.1 cm	Stage 111 juveniles 25.0 cm	Stage IV adults 31.8 cm
Copepods	38.5	40.0	50.0	50.0
Cladocera	38.0	45.6	49.0	52.0
Ostracoda	30.0	28.5	36.5	38.1
Diatoms	20.1	28.0	38.5	39.6
Protozoan	15.5	-	20.6	27.8
Insect parts	26.1	-	10.5	9.7
Bivalves	20.1	8.6	10.5	13.0
Annelids	21.6	7.1	9.0	8.9
Plant remains	-	10.5	9.7	10.0
Plant detritus	-	8.6	10.4	10.8
Sand	-	7.1	15.5	11.9
Unicellular algae	-	8.0	11.7	12.0
Total no of Fish Examined	60	60	60	71
Number with food	35	41	46	42

Ta**ble 5: Trophic spectrum of the diet for all sizes of *Heterotis niloticus***

Diet	% PP	% RF	IFS
Copepods	18.45	9.70	28.15
Cladocera	18.01	12.69	30.70
Ostracoda	14.06	5.22	19.28
Diatoms	16.61	16.67	33.28
Protozoan	9.40	14.68	24.08
Insect parts	8.90	5.70	14.60
Bivalves	6.01	8.00	14.01
Annelids	2.00	6.01	8.01
Plant remains	7.91	14.92	22.83
Plant detritus	2.46	3.48	5.94
Sand	2.64	2.74	5.38
Unicellular algae	10.46	14.18	24.64

Age and Growth: From the length-based Powell-Wetherall method, L = 36.4 cm Tl (or 340mm TL). Estimated K and t_o were 8 yr^1 and -17 yr^1, respectively.

Sex: The monthly sex ratio ranged from 1:0.1 for Otuocha in December to 1:1.6 for Nsugbe in the month of September, the overall sex ratios of *H. niloticus* in Anambra river ranged from 1:0.3 to 1:1.5 with a modal sex ratio of 1:0.7 in favour of the males (Table 3). The Otuocha and Ogurugu stations showed more pronounced sex ratio similarity than the Nsugbe station.

Food and Feeding Habits: One hundred and sixty four (164) (65.34 %) of the 251 stomachs examined contained 12 different food items (Table 4). Out of the food items isolated with increasing maturity, foods of animal origin were most important in the juvenile's diet than those of plant origin (Table 4). A shift from carnivorous to omnivorous dietary pattern occurred with age (Table 4).

The trophic spectrum (Table 5) of the diet for all sizes of *H. niloticus* indicated that the crustacean (Cladocerans, Copepods and Ostracoda) were more dominant in juvenile's and adult diets. The insect parts were of primary importance as food items fry, juveniles and adult stages respectively. Other food items, plant detritus, plant remains, sand and unicellular algae were of secondary importance. The plantivorous crustacean particularly the copepods and cladocera form the main food throughout the four stages of fish examined. The food items were mostly available during the dry season (Table 6).

Qualitative food composition was higher in the dry than in the wet season (Table 7). Copepods, ostracods, chironomid larva, plant remains and sand were significantly more in the dry than in the wet season. The converse was true for the cladocera, insect parts, bivalves, protozoan and unicellular algae. The occurrence of other food items was not different between the seasons. Food richness and diet breath were dependent on season.

Digestive Enzyme: Enzymes detected in the different regions of *H. niloticus* gut, their distribution and activity varied along the gut length. Significant quantities of glycosidase were detected in the oesophagus, stomach, pyloric caeca, and duodenum. Cellulase activity was recorded only in the pyloric caeca (Table 8). The protein hydrolyzing enzymes found in the stomach are pepsin-like while those in the pyloric caeca are alkaline proteases, possibly trypsin and /or chymotrypsin. Lipase activity occurred along the entire gut length with peaks in the pyloric caeca and duodenum (Table 8). Generally, no enzyme activity occurred in the rectum.

Haematological and Serological Profiles: The mean values for the blood parameters are presented in Table 9. The linear regression analysis of the blood parameters as functions of total length gave the following relationships: PCV (%) = 9.56 + 0.83 TL (cm), r=0.187, p = 0.05; RBC ($10^{12/l}$) = 0.18 + 0.05 TL (cm), r = 0.007, p = 0.05; Plasma Na (nM) = 7.64 + 0.19 TL (cm), r = 0.285, p = 0.05 and MCHC (g/dl) =1.769 x 10^{-3} + 8.24 x 10^{-6} TL(CM); r = -0.143, P = 0.05. The results of the serological studies (Table 10)

Tables 6: The monthly IFS of *Heterotis niloticus* in Anambra river

Diet and Fish variables	N	D	J	F	M	A	M	J	J	A	S	O
Copepods	2.16	0.40	0.20	0.01	-	11	0.30	-	2.01	0.01	-	0.29
Cladocera	2.10	0.40	-	0.69	0.61	-	-	0.1	0.3	-	-	11.4
Ostracoda	0.34	0.13	-	0.31	0.09	-	-	0.5	-	-	-	0.32
Diatoms	0.6	0.19	0.1	1.79	-	8.70	0.1	0.3	-	35.22	0.31	1.10
Protozoa	0.31	0.17	0.21	-	-	-	-	-	0.61		0.78	0.86
Insect parts	0.86	0.10	-	-	-	-	-	-	-	0.01	0.05	0.18
Bivalves	1.10	0.31	0.25	0.53	0.31	-	-	-	-	1.11	-	0.86
Annelids	0.27	0.20	-	-	-	0.01	-	0.4	-	3.75	-	-
Plant remains	0.40	0.37	0.09	0.01	0.91	2.0	-	0.6	-	-	-	2.02
Plant detritus	1.12	0.61	0.55	0.61	0.2	-	-	-	-	0.03	-	1.62
Sand	0.36	0.54	0.40	-	-	-	-	-	0.3	10.06	30.0	-
Unicellular algae	0.17	0.08	0.33	-	-	-	-	0.8	-	-	-	0.18
Food richness	12	12	8	7	5	4	2	6	4	7	4	10
Diet breath	1.72	1.20	1.50	1.63	1.47	1.80	1.0	0.79	1.51	1.37	1.35	1.79

Table 7: Seasonal variation in IFS of *Heterotis niloticus* in Anambra river

Diet and Fish variables	Dry	Wet	P
Copepods	3.01	-	<0.05
Cladocera	1.71	-	
Ostracoda	3.62	0.01	
Diatoms	2.91	0.10	<0.05
Protozoa	0.71	-	
Insect parts	0.88	0.03	
Bivalves	0.07	-	
Annelids	0.09	-	
Plant remains	17.81	0.08	<0.05
Plant detritus	3.61	-	
Sand	24.08	0.16	
Unicellular algae	0.09	-	
Food richness	13	5	
Diet breadth	1.86	1.03	

showed a similar anti-gene reaction to that observed in human blood. The predominant blood group of *H. niloticus* is O+ (90%), while the genotypes are AA (51%) and AS (49%).

DISCUSSION

The abundance of *H. niloticus* in Anambra river may be influenced, to variable extent, by number of factors, including food availability, short life span, high natural mortality and environment. Abundant foods may also permit rapid growth and high recruitment. Early sexual maturity and all year round breeding in fishes are generally survival strategies and adaptations aimed at perpetuating the species in response to high fishing and/or natural mortality. The survivors prey on the rich variety of food available in the river; they grow very fast and become recruited into the fishery. It seems probable that it is in this way that large numbers of *H. niloticus* are maintained in the Anambra river. While a fairly good knowledge of the breeding biology of *H. niloticus* is beginning to emerge in Nigerian lentic and lotic habitats, we still need as Marshall (1993) noted for *Limnothrissa miodon*, to ascertain the environmental factors determining reproductive success, the effect of fishing on the sexually mature individuals and the relationship between stock and recruitment. Furthermore, knowledge of other demographic characteristics such as (growth and mortality) of *H. niloticus* is also important in order to gain an overall understanding of factors determining its abundance in the Anambra River.

Fish growth is determined by the combined effects of food quality and quantity. Analysis of food composition in stomach of *H. niloticus* from the Anambra river basin showed a predominant microphagous diet plus insect larvae. Coupled with the possession of numerous densely-packed gill rakers (developed into fine sieves), this suggests a filter-feeding habit. Brief accounts of the dietary habit of *H. niloticus* in Epe Lagoon (Balogun, 1980), Badagry creek (Lawal, 1991), Eleyele Reservoir, Oba Dam (Ugwumba, 1992) and coastal wetlands of southwest Nigeria (Akegbejo-Samsons, 1995) confirmed *H. niloticus* as a micro ore. The inclusion of sand grains was possibly an accidental ingestion along with insect larvae, annelids, prawns and bivalves, while the high occurrence and prominence of detritus (Tables 4, 5, 6) suggest frequent bottom feeding on benthic invertebrates, which dominated the diet in the river habitat. It was evident that *H. niloticus* was strictly a planktonic microphage in the lentic habitats, while it adopted a mud-eating microphagous habit in the river. Ecologically, these habits seem to be common and characteristic of osteoglossid fish species (Akintunde, 1977). This adaptiveness to the natural diet is responsible for the success of *H. niloticus* in the various habitats. *H. niloticus* exhibit, wide plasticity in their feeding, primarily consuming a combination of two or more of crustaceans, insects, plankton and plant detritus, depending on availability and abundance of these foods the main food throughout the three stages of fish that were examined. Changes observed in food composition and feeding habits of fish in relation to the size or age of fish are biological phenomenon, which are common in many tropical fish species (Hellwell, 1972). Bhatt (1972) observed similar trend

Table 8: Summary of qualitative and quantitative assays of digestive enzymes in the gut of *Heterotis niloticus*

Digestive enzymes	Oesophagus	Stomach	Pyloric caeca	Duodenum	Ileum	Rectum	SE
GYCOSIDASES[1]							
A-amylase	7.42	8.04	192.5	165.9	87.3	ND	2.69
Sucrase	ND	11.6	16.0	32.6	29.0	ND	0.58
Maltase	ND	42.7	49.1	40.6	48.0	ND	1.08
Lactase	ND	26.2	34.1	37.0	31.2	ND	0.64
Cellulose	ND	ND	21.8	ND	ND	ND	-
PROTEASES[2]	ND	125.8	240.8	262.0	102.7	ND	3.29
LIPASE	ND	40.5	287.4	243.0	110.4	ND	3.67

ND=not detected; [1] mg glucose/min/mg protein at 37 ^{0}C; [2] change in optical density at 595nm/hr/mg of L-tyrosine/hr at 37^{0}C; 3 =mill equivalents of fatty acids /mg protein/hr @ 37^{0}C; SE = standard error

Table 9: Haematological characteristics of *Heterotis niloticus* in Anambra river

Haematological parameter	Mean (±SD)	Range
Total length (TL) (cm)	30.22 (0.92)	30.4-39.5
Body weight (Wt) (g)	419.40 (31.17)	300.8-610.1
Erythrocyte (RBC) (10 $^{12/1}$)	1.50 (0.02)	0.58-2.17
Leucocytes count (WBC)(10 9/1)	57.2(4.9)	53.9-58.7
Haematocrit (PCV) (%)	28.12 (2.98)	13-39
Hemoglobin concentration (Hb) (g/dl)	4.06 (0.43)	2.2-6.2
Erythrocyte sedimentation rate (%)	31.16(7.84)	11-80
MCV (mean corpuscular volume) (11)	187.16 (12.79)	128-304
MCH (mean corpuscular haemoglobin) pg	19.27 (1.28)	28-36
MCHC (mean corpuscular haemoglobin concentration) (g/dl)	0.14 (0.01)	0.11-18
Plasma protein (g/l)	53.6 (5.4)	47-78
Plasma glucose (mg/dl)	57.38(4.17)	46-79.0
Plasma albumin (mg/g)	3.87 (.75)	0.3-6.9
Na (mM)	112 (3.6)	87-127
Cl (mM)	76 (1.07)	6.4-145
K (mM)	18.45 (1.02)	15.3-27.6
Mg (mM)	7.23(0.46)	5.4-9.2
P (mM)	521.23(49.10)	196.2-682.1
Ca (mM)	0.87(0.12)	0.54-1.12

Table 10: Percentage occurrence of serological characteristics in *Heterotis niloticus*

Blood group	
A	-
B	-
AB	-
O+	91
O-	9
Genotype	
AA	-
AS	51
SS	49
Agglutinations test	
+ve	-
-ve	100

in India catfish, *Mystus seeghala* (Sykes) and *Mystus vittatus* (Block) respectively. Fagade and Olaniyan (1973) noted similar occurrence in the most African shad, *Ethmalosa fimbriata* (Bow dish). However, the dominance of plantivorous crustacean was established in the study irrespective of the additional food items that emerged later.

Except for the occurrence of amylase in the oesophagus, Akintunde (1985) observed a similar general pattern of digestive enzyme distribution in *Sarotherodon galilaeus* Linnaeus 1758. Worthy of note is the occurrence of enzyme secretion in the oesaphagus of *H. niloticus* (Table 8) which is rare, having been reported in only a few fish species (Kawai and Ikeda, 1971). The variety of glycosidase indicates the ability of *H. niloticus* to digest a wide range of carbohydrate food components. Cellulase activity was recorded only in the pyloric caeca and its origin is attributed to gut micro flora ingested along with the detritus which featured prominently in the diet (Table 8). Detritus-inhabiting microflora (which produces microbial cellulase) imparts the ability to digest cellulose to their host animals (Chow and Halver, 1980).

The relatively high activity levels of proteases, particularly in the pyloric caeca and duodenum (Table 8), were not surprising in view of the large proportion of protein components (mainly zooplankton) in the diet (Table 8). Pepsin would hardly be expected to occur in the two distal gut regions since it is active only in strongly acid media

found in the stomach. Lipase distribution and activity along the entire gut (Table 8) was also reported in *Clarias isheriensis* Sydenham, 1980 (Fagbenro, 1990). From the foregoing, it is evident that *H. niloticus* is enzymatically well equipped to digest the carbohydrate, protein and lipid components of its diet.

Generally, the ranges of the blood parameters determined for *H. niloticus* (Table 9) are similar to those reported for Africa fresh water catfish species, except for those of erythrocyte count (RBC) and haematocrit (PCV) which are higher in *H. niloticus.* The mean haematocrit value of *H. niloticus* is comparable to those of African clariid catfishes, *C. isheriensis* (Kori-Siakpere, 1985). *C. gariepinus* Burchell, 1822 and *Heterobranchus longifilis* Valenciennes, 1840 (Erondu *et al.,* 1993). The mean RBC value for *H. niloticus* was lower than those reported for *Heterobranchus bidorsalis* (Erondu *et al.,* 1993; Fagbenro *et al.*, 1993) but comparable or slightly higher than the values reported for other catfish species *C. isherensis*, *C. gariepinus* and *Chrysichthys nigrodigitatus* (Kori-Siakere, 1985; Erondu *et al.,* 1993). Except for *H. bidorsalis,* the mean leucocytes count (WBC) value of *H. niloticus* is higher than those reported for freshwater fishes by Erondu *et al.* (1993).

The high values of erythrocyte count and haemoglobin concentration (Table 9) reflect a high oxygen carrying capacity of the blood which is consistent with the correlation of haemaglobin concentration and fish activity. As suggested by Lenfant and Johansen (1972), haemaglobin concentration is higher in the fishes capable of aerial respiration. *H. niloticus* can tolerate very low values of dissolved oxygen because it is able to undertake aerial respiration via the air-bladder (D'Aubenton 1955).Thus the high Hb values in *H. niloticus* are indicative of its air-breathing character and high activity. The results of the serological studies (Table 10) showed similar anti-gene reaction to that observed in human blood. The predominant blood group of *H. niloticus* is O+ (90%). While the genotypes are AA (51%) and AS (49%). These may suggest the use of *H. niloticus* as animal model for medical sciences research involving humans.

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HISTOLOGICAL STUDIES OF THE VOMERONASAL ORGAN OF AFRICAN GIANT RAT (*Cricetomys gambianus,* WATERHOUSE)

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ABSTRACT

The vomeronasal organ (VNO), a chemoreceptive organ was studied in African giant rat (Cricetomys gambianus, Waterhouse), a macrosmatic animal, by means of gross dissection and light microscopy. The VNO was located in the rostral part of the base of the nasal septum. It was tubular in shape, about 10.6 mm in length and opened in the rostral region of the nasal cavity, with a blind caudal end that terminated in glandular branches. Its lumen in the middle segment was lined by sensory epithelium on the medial wall and non-sensory epithelium on the lateral wall. The sensory epithelium showed sensory, supporting and basal cells, whereas the non-sensory wall contained psendostratified columnar cells with ciliated epithelium. Vomeronasal glands were present in the lateral wall and on the dorso-lateral region between the sensory and non-sensory epithelia. Intraepithelial blood vessels were observed on the medial sensory epithelium. Nerve bundles were also apparent in the medial sensory wall. The vomeronasal capsule incompletely housed the organ and showed ossified areas. The histological observations suggest that the VNO is an important organ in sexual behaviours as in other rodents and will throw more light on future studies of the vomeronasal organ.

Keywords: African giant rat, Histology, Chemoreception, Vomeronasal organ

INTRODUCTION

It is well established that the mammalian vomeronasal organ (VNO) is involved in the control of sexual behaviour (Estes, 1972; Wysocki, 1979; Meredith and Fernandez-Fewell, 1994). The VNO is a chemoreceptor organ; the receptor cell project their axons to the accessory olfactory bulb and other higher centers of the brain and is involved in detection of con-specific chemical signals (pheromone). The organ has a tubular structure, with a lumen surrounded by two types of epithelium: the vomeronasal epithelium and non-sensory epithelium. Mammalian VNOs has been extensively studied anatomically (Adams and Wiekamp, 1984; Johnson *et al.,* 1985; Salazar *et al.,* 1994; 1995; 1998). The vomeronasal organs of many rodents have also been studied, in rats (Garrosa *et al.,* 1986; Garrosa and Coca, 1991; Zuri *et al.,* 1998; Weiler *et al.,* 1999), in mouse (Addison and Rademake, 1927; Barber and Raisman, 1978), rabbit (Wohrmann-Repenning, 1984; Taniguchi and Mochizuki, 1983), and guinea pig (Sangari *et al*., 2002).

The African giant rat (*Cricetomys gambianus*, Waterhouse), with an average adult mass of 1.4 kg is one of the largest cricetids, occurring in Africa, predominantly confined to moist savannah regions. They are nocturnally active restricting their activity to areas with reasonable vegetational cover (Knight, 1984). It provides supplementary protein diet for rural dwellers. There has been a continuous effort to domesticate it in some parts of Nigeria.

Some aspects of the biology of the African Giant Rat (AGR) have been studied (Ewer, 1967; Ajayi, 1977; Kokkin, 1981; Knight, 1984; Knight and Knight-Eloff, 1987; Ogwuegbu *et al.,* 1983; Oke, 1985; Oke and Aire, 1989; 1997; Olayemi and Adeshina, 2002; Kelani and Durotoye, 2002; Onyeanusi *et al*., 2007). There are no published reports on the vomeronasal organ of the African giant rat. The aim of the present study was to examine the histological structure of the vomeronasal organ in order to shed light on the AGR, which has good potential for use as animal protein and research model.

MATERIALS AND METHODS

Eight adults (5 males and 3 females) over 8 months old from kill-trapping in the fields around the University of Nigeria, Nsukka, were used in this study. Following decapitation, the heads were washed with normal saline and their vomeronasal organ was dissected out with nasal septum and hard palate for

gross observations under dissecting microscope. Some blocks of tissue were sawn with small handsaw after trimming off the palatine and vomer bones. These blocks of tissue containing the VNO was fixed in 10% neutral buffered formalin decalcified using formic acid-sodium citrate solution for 2 – 4 days according to Bhatnagar and Kallen (1974) and Smith *et al*. (1997). The tissues were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were sectioned in transverse plane at 7μm thickness. Every tenth section was mounted on glass slides and stained with haematoxylin and eosin. The middle sections of the organ were observed with a Hund Wetzlar 600H light microscope with Moticam 1000 digital camera attachment and images captured into a computer. Ocular and stage micrometer gauge were used to measure the thickness of the sensory and nonsensory epithelium.

RESULTS

Location and Morphology: The vomeronasal organ was a paired bilateral structure located at the base of the nasal septum, with direct relationship with the vomer bone, palatine process of maxillary bone and the incisive bones. Each organ appeared as a hollow cylinder. The average palatal length was 10.6 mm. It communicated with the nasal cavity through the incisive duct rostrally. It was blind caudally. The VNO was enclosed by an incomplete vomeronasal capsule (VNC), which was a matured hyaline cartilage with some ossified areas. The VNC is incomplete in its dorsolateral region. The lumen of the organ was an elongated bean-shaped opening (Figure 1).

The size and internal contour of the vomeronasal duct varied along its longitudinal axis. Rostrally, the lumen of the tube was bounded by a medial and lateral cartiginous wall. Caudally, as the organ increased in size, the lateral wall of the tube was convex and the medial wall concave. The medial wall lay on the nasal septum, while the lateral wall was covered by the nasal mucosa.

Histological Observations: Three segments with different histological features were observed between the rostral and caudal ends of the vomeronasal organ. The rostral segment had the openings of the organ into the nasal cavity. This segment was covered by stratified squamous epithelium and that of the nasal cavity by pseudostriatified columnar epithelium. The middle segment presented different epithelia in each of its walls (Figure 2, 3 and 4). A pseudostratified epithelium about 31.6 μm thick covered the lateral wall.

The vomeronasal epithelium found on the medial wall was about 140.2 μm thick. The sensory vomeronasal epithelium was absent in the caudal extremity of the middle segment and thereafter the pseudostratified epithelium changed to simple columnar cells. The vomeronasal epithelium in the middle segment was made up of a superficial layer formed by extensions of underlying cells, a layer of elongated supporting (sustentacular) with oval nuclei and 8 – 10 layers of bipolar cells with rounded nuclei (Figure 3). The basal cells were interposed amongst lower bipolar cells without forming a distinct layer. Apical 'brush border' were present on the vomeronasal sensory epithelium of the medial wall (Figure 5). A profuse vascularisation of the VNO at the lateral wall was seen and these represented venous sinuses with a prominent large vein amongst smaller arteries, capillaries and venules. The vomeronasal cartilage (vomeronasal capsule) showed ossified points and did not completely enclose the organ but left a dorso-lateral opening for passage of some glands and nerves of the organ. The caudal segment of the VNO terminated in some glandular branches with simple columnar epithelium around its lumen.

Connective tissue spread from the adventitial layer of the venous sinus reached and merged with that of the vomeronasal capsule (Figure 7). Two groups of glands were observed amongst the loose connective tissue, the upper glandular groups were found at the dorsal transition of the epithelia, whereas the other occupied a lateral position on the non-sensory epithelium. Some glands were also visible through openings in the vomeronasal capsule and seem to have reached the lower regions of the nasal septum (Figures 8 and 9). Few nerve plexuses were observed on the lamina propria. Venous sinuses and glands occupied much of the lateral wall of the organ, which was incompletely housed by the ossified capsule. Intraepithelial capillaries were observed amongst the cells of the sensory medial wall (Figures 10, 11 and 12).

DISCUSSION

Histological descriptions of the vomeronasal organ in most mammals frequently show a uniform organization along the longitudinal axis of the tube: The vomeronasal sensory epithelium in the medial wall and the non-sensory (respiratory epithelium) in the lateral wall. Our present histological observations in the medial and lateral walls of the VNO in African giant rat (AGR) is supported by similar findings in very young rat (Kratzing, 1971a); older rat (Vacarezza *et al*., 1981), rabbits (Taniguchi and

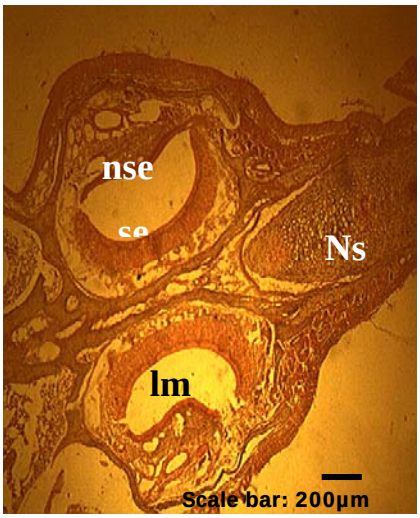

Figure 1: Paired vno sensory (se) and nonsensory (nse) epithelium, lumen of vno (lm), nasal septum (ns)

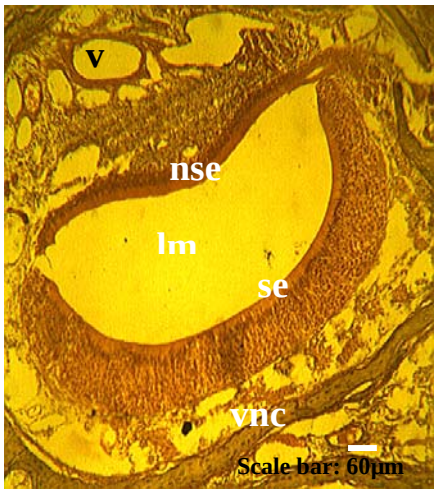

Figure 2: Vomeronasal organ with large vein (v), vomeronasal capsule (vnc)

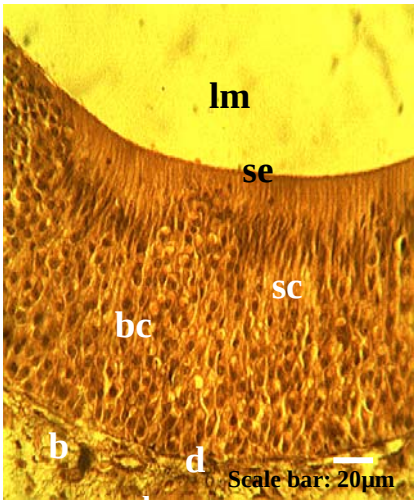

Figure 3: Sensory epithelium: sc - supporting cells, bc - bipolar neurons, b - basal cells, d -dendrites

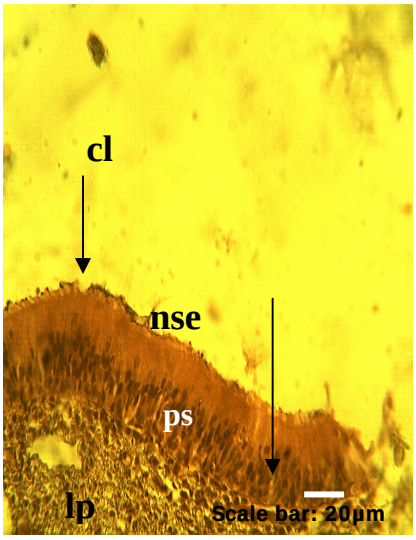

Figure 4: Nonsensory epithelium (nse), ps - columnar cells, lp - laminar propria, basal cells – arrows, cilia - cl

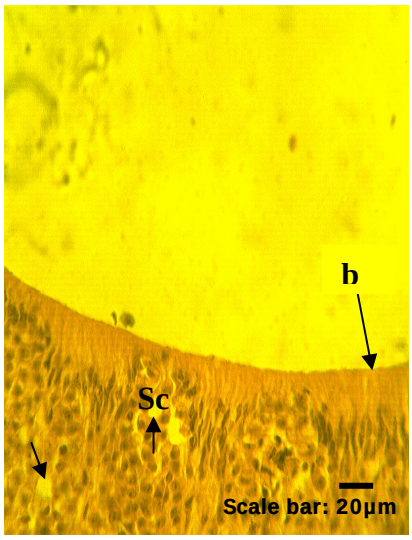

Figure 5: Sensory epithelium: microvillary surfaces (b), supporting cells - sc, intraepithelial capillaries (arrows)

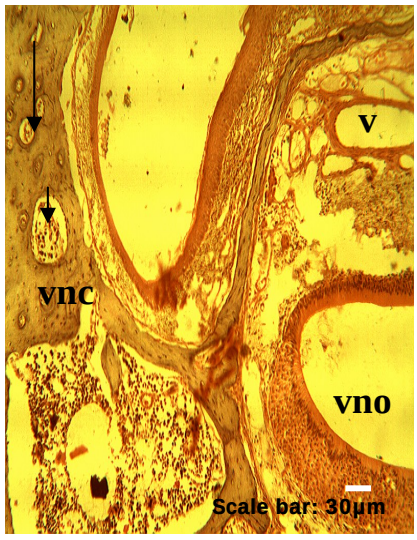

Figure 6: Relationship of vno and capsule (vnc). Note ossified areas (arrows)

Mochizuki, 1983), sheep (Kratzing, 1971b) and golden hamster (Taniguchi and Mochizuki, 1982).

The cells of the vomeronasal sensory epithelium of the African giant rat show some similarities in number and in the arrangement of the apical process and microvillary 'brush borders', as observed in rats (Garrosa *et al*. 1986) several other rodents. This may have important functional implication in reception of stimuli. Many capillaries, several arterioles and large veins on the lateral non-sensory epithelium make the vomeronasal organ a highly vascularised structure. Such an irrigated structure as the VNO with similarities to the neighbouring nasal mucosa suggest that the VNO in AGR consists of an erectile tissue as in most mammals so far studied.

Intraepithelial blood vessels seen in the vomeronasal organ of AGR could be of phylogenetic consideration. Such blood vessels have been described in lower vertebrates, Guinea pig (Sangari *et al.,* 2002), mouse (Cushieri and Bannister, 1975), but have not been observed in VNO of higher mammals where the vomeronasal epithelia are gradually reduced (Jordan, 1972). It can be suggested that the presence of intraepithelial blood vessels is to provide nourishment for the thick epithelium and permit exchange of metabolites (Cushier, 1975). The relationship of these blood vessels with vomeronasal endocrine regulation remains to be verified.

Vomeronasal glands were also observed to be located within the VNO (lateral wall) and some outside the vomeronasal capsule close to the respiratory mucosa. The communication of these extracapsular glands with lumen of the organ is feasible through the gap existing in the capsule, which are also used by the nerves.

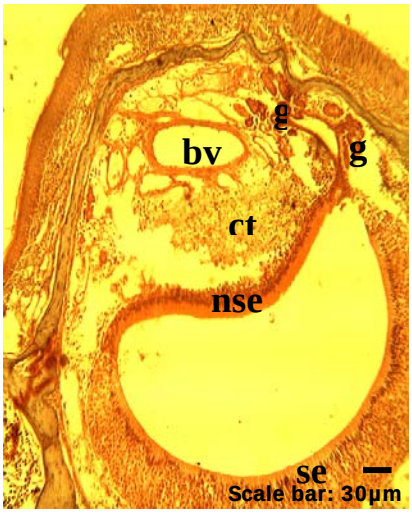

Figure 7: Components of lateral wall: glands (g), blood sinuses (bv), abundant connective tissue (ct)

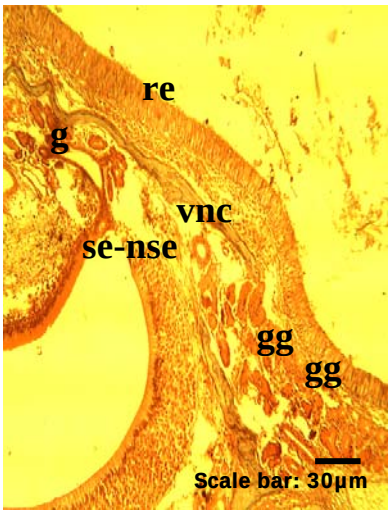

Figure 8: Vomeronasal glands (g) in intracapsular (g) and extracapsular positions (gg), respiratory epithelium (re), epithelia boundary between sensory and nonsensensory (se-nse)

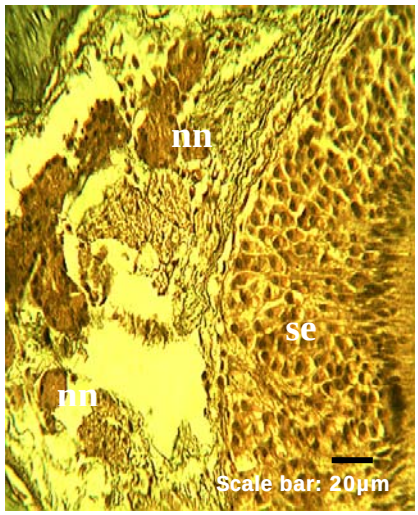

Figure 9: Vomeronasal nerves (nn), vomeronasal sensory epithelium (se)

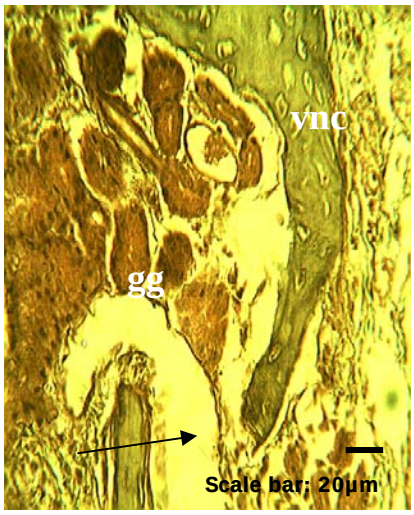

Figure 10: Vomeronasal glands, vomeronasal capsule (vnc) with a gap (arrow)

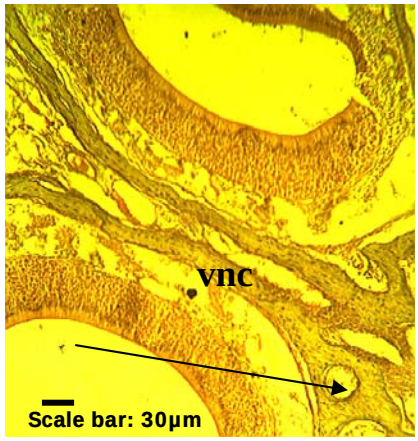

Figure 11: vomeronasal cartilage with ossification (arrow)

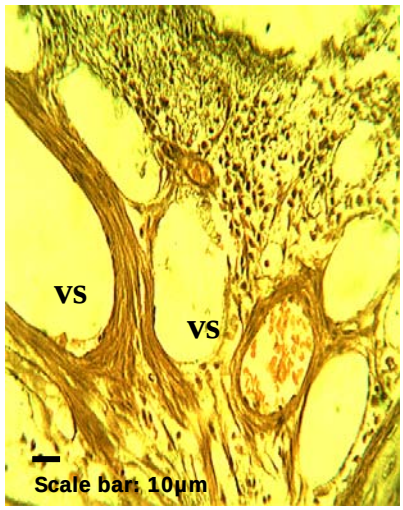

Figure 12: Venous blood sinuses (vs)

The ossification of the vomeronasal cartilage (capsule) in some areas is found in most rodents, but not in higher mammals (ungulates and carnivores) (Salazar *et al.,* 1997). The vomeronasal capsule serves the protective function of the organ and plays a major role in the pump-mechanism associated with reception of stimuli during vomerolfaction.

In conclusion, we hope that this study revealing the histological features of vomeronasal organ in African giant rat will form basis for future studies on the organ.

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EFFECT OF INTRARUMINAL INFUSSION OF SATURATED AND UNSATURATED FATTY ACIDS ON ORGANIC MATTER DEGRADABILITY, TOTAL VOLATILE FATTY ACID AND METHANE PRODUCTIONS IN WEST AFRICAN DWARF SHEEP

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ABSTRACT

This study describes the effect of intraruminal infusion of different proportions of palmitic (saturated fatty acid) and linolenic (unsaturated fatty acid) on rumen degradability of organic matter fraction of Pennisetium purpureum, total volatile fatty acid and total methane productions in West African Dwarf sheep. Five combination proportions of palmitic and linolenic acids viz: 70 % palmitic acid + 30 % linolenic acid, 30 % palmitic acid + 70 % linolenic acid, 50 % palmitic acid + 50 % linolenic acid, 100 % palmitic acid + 0 % linolenic and 0 % palmitic acid + 100 % linolenic acid designated treatments A-E respectively served as the experimental treatments. These treatments were intraruminally infused into five (5) adult WAD sheep of average body weight of 13.49 ± 1.63 kg and the trial performed in a 5 x 5 latin square experimental design. A sixth group of four sheep, that did not receive any fatty acid infusion, served as the control group. The in-sacco technique for degradability studies was adopted in the determination of organic matter disappearance from the rumen at time intervals of 4, 8, 12, 24 and 48 hours in both the experimental and control groups. Appropriate mathematical model for estimation of total volatile fatty acid (VFA) and total methane production were used for determination of VFA and methane productions. The result of the study showed that organic matter degradability was significantly (p < 0.01) highest in treatment A (70 % palmitic acid + 30 % linolenic acid) at 24 hours (84.63 ± 8.6 %) and 48 hours (88.42 ± 4.8 %) compared to other treatments and the control. Higher proportion of linolenic acid (treatments B and E) significantly (p<0.01) reduced potential OM degradability at 48 hours with values at 41.08 ± 5.5 % and 23.92 ± 2.4 % respectively. Total VFA production was significantly (p <0.01) increased in treatment A at 24 hours (3.59 ± 0.07 m mol/l) and 48 hours (3.62 ±0.04 m mol/l) compared to other treatments and the control. At same time post incubation, total methane production was significantly (P<0.01) decreased in treatments B (0.39 ± 0.01 mol/hr) and E (0.34 ± 0.006 mol/hr) compared to treatments A (0.52 ± 0.01 mol/hr) which recorded a significant (P<0.01) increase. The study revealed that high proportion of unsaturated fatty acid suppressed rumen fermentation with resultant decrease in organic matter degradability, total VFA and methane productions. The reverse was however the case with high proportions of saturated fatty acids.

Keywords: Fatty acids, Degradability, Volatile fatty acid, Methane, WAD sheep

INTRODUCTION

Fat and fatty acid metabolism and digestion in ruminants particularly the dairy cows are of considerable interest. This renewed interest is based on several reasons, first, the use of dietary fat supplements by nutritionists to increase the energy density of diets to meet requirements of the high producing dairy and beef cows; second, it is now recognized that fatty acids, both of dietary and rumen origin, can have specific and potent effects on ruminant metabolism and human health (Doreau *et al*., 1997) and third, we now recognize that specific fatty acids produced in the rumen are potent regulators of rumen function and milk fat synthesis in the mammary gland (Bauman and Grinari, 2003). These therefore pose a great challenge in the understanding of the optimal and satisfactory dietary level of fats *vis-à-vis* saturated and unsaturated fatty acid proportions, within the rumen and probably in

the postruminal segments of the gastrointestinal tract, that would not be detrimental to rumen functions and other physiologic indices of fat digestion and utilization particularly in the West African Dwarf sheep.

Energy value of fat supplements varies (Shingfield *et al*., 2003). The variability in net energy for lactation (NEL) among fat supplements has been described as a function of the long chain fatty acid content and digestibility (Borsting and Weisberg, 1989). Digestibility of these fatty acids can be influenced by dry matter intake and amount of fat consumed as well as the characteristics of the supplemental fat (degree of saturation) (Elliot *et al.,* 1999). Degree of fatty acid unsaturation is probably the most important characteristic that influence rumen fermentation and intestinal digestibility of fats (Grummer *et al.,* 1990; Nestle *et al*., 1994). Iodine value (IV) is an indicator of the degree of unsaturation. The higher the IV, the greater the degree of unsaturation. Digestibility may decrease if the IV is below 45 (Firkins and Eastridge, 1994). Maximal digestibility of fats with an IV greater than 40 was 89 % compared with 74 % for fats with IV less than 40 (Jenkins, 1994). Thus saturated fatty acids are less digestible (within the intestine) than unsaturated fatty acids and the difference is greater when predominantly saturated fats are supplemented (Borsting *et al*., 1992). These indicated that unsaturated fatty acids may have synergistic effect on the digestibility of saturated fatty acids.

There are also possibilities that some synergistic interaction between saturated and unsaturated fatty acids occur within the rumen and may exert influence on rumen functions such as microbial population and activity, rumen fermentation (VFA, methane, carbon dioxide and other intermediates) rate of passage and fatty acids pool (Harfort and Hazlewood, 1988). If so then, the extensive metabolism of lipids in the rumen (hydrolysis and biohydrogenation of polyunsaturated fatty acids) would be affected by the proportions of the saturated to unsaturated fatty acids in the rumen at any given time and thus may have major impact on the profile of fatty acids available to the ruminant animal in milk and tissues.

Unsaturated fatty acids are toxic to many rumen bacteria (Baumgard *et al*., 2000). This is however checked by rumen biohydrogenation of the polyunsaturated fatty acids (PUFA). Therefore, the understanding of this toxic level of unsaturated fatty acids is very pivotal to the understanding of the influence of degree of saturation of fatty acids in ruminant nutrition. This therefore refers to the quantitative intake, digestion and metabolism of fatty acids in ruminants with respect to dietary saturated and unsaturated fats. This quantitative model could be useful in studying the physiologic effect of fatty acids in different segments of the gastrointestinal tract of ruminants. Against this background, this study was aimed at investigating the effect of different combination proportions of saturated and unsaturated fatty acid on rumen functions with emphasis on organic matter degradability, total volatile fatty acid and total methane productions in the West African Dwarf sheep.

MATERIALS AND METHODS

Nine West African Dwarf sheep were purchased from Ibagwa market in Igbo-Eze Local Government Area of Enugu State. They were weighed, dusted with an ectoparasite powder (pif-paf) and introduced into the Veterinary Farm, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were further dewormed using levamisol and ivermectin. They were also given prophylactic treatment for trypanosomosis. They were adapted on free range grazing for 21 days. After the adaptation period their body weights were recorded before implantion of the rumen fistula. Following a 24 hour fasting, rumenotomy was performed as described by Remi-Adewumi *et al*. (2006), and rumen fistulation as described by Santra and Karim (2002) and Aka and Kamalu (2005).

Pennisetium purpureum which was oven dried and milled, fine granules were used for the *in-sacco* degradability of organic matter (Aregbede *et al*., 2002). The experiment was designed in a 5 X 5 Latin square model of five sheep, five treatments and three replicates of *P. purpereum*. The proportions of fatty acids used are as follows: 70 % palmitic acid + 30 % Linolenic acid (treatment A), 30 % palmitic acid + 70 % Linolenic acid (treatment B), 50 % palmitic acid + 50 % Linolenic acid (treatment C), 100 % palmitic acid + 0 % Linolenic acid (treatment D), 0 % palmitic acid + 100 % Linolenic acid (treatment E). Three WAD sheep without any fatty acid infusion served as the control group. Each of the fatty acid treatments was infused daily for 10 days and the study performed during the last 2 days. A cross over period of 5 days was allowed for the Latin square model. The chemical composition of the forage sample *P. purpereum* was determined by the AOAC (1990).

The disappearance of the OM fraction from the nylon bags at various incubation time was fitted to Orskov and McDonald (1979) equation thus: $P = a + b (1-e^{ct})$, where p = level of potential degradability, a = immediate soluble fraction, b = water insoluble

but rumen fermentable fraction on time (t), c = rate of degradation of b and t = duration of incubation.

Total rumen volatiles fatty acid production was determined as described by Kennedy and Milligan (1978). Volatile fatty acid production and methane production in the rumen were related to the amount of organic matter (OM) apparently degraded in the rumen (g/d) using the equation: V = 0.00809D + 2.903 and M = 0.00277D + 0.273, where D = OM degradability, M = quantity of methane produce, V = volatile fatty acid, M is measured in mol/hr and V is measure in mMol/l of rumen fluid.

In this experiment the OM for rumen degradability of *P. purpereum* was used to estimate the total VFA and methane production at various time intervals of incubation following the intraruminal infusion of the various proportions of the fatty acids

Statistical Analysis: The results were analyzed statistically by the one-way analysis of variance (ANOVA). Treatment means for OM degradability, VFA and methane productions at 48 hours were tested for significant variation at $p < 0.01$ using the least significant difference (LSD) of mean comparison (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 showed the chemical composition of the forage (*P. purpureum)* used for the study. The organic matter content of *P. purpureum* was 80.53 %. The fraction of 80.53 % that was soluble in water (i.e. 'a' fraction) and the rumen degradable fraction (i.e. 'b' fraction) out of the 80.53 % organic matter of the forage sample (*P. purpureum*) are shown in table 2.

Table I: Chemical composition (% of dry matter) of *Pennisetium purpureum*

Chemical Constituent	Percentage proportion of Dry matter
Dry matter	92.03
Crude protein	12.80
Organic matter	80.53
Ash	11.50
Crude fiber	22.13
Ether extract	4.56
Gross energy (kcal/g)	3.47
Nitrogen free extract	43.78
Calcium	0.30
Phosphorus	0.36
Potassium	5.34
Sodium	1.86

The immediate soluble fraction 'a' was the same for all the treatments. That is, 2.16 ± 0.12 % of OM of *P. purpureum* was soluble in water, and since same forage was used for all treatments, the value was constant. The percentage disappearance of organic matter from the rumen varied between 4 to 48 hours of incubation. The rumen degradable OM was least in treatment E (100 % linolenic) with a value of 21.76 ± 2.41 at 48 hours post incubation. This was followed by 39.72 ± 4.04 in treatment B (70 % linolenic + 30 % palmitic) compared to other treatments that had 86.26 ± 4.75 % (treatment A – highest); 67.86 ± 3.961 % (treatment C – 50 % palmitic + 50 % linolenic), 58.17 ± 4.57 % (treatment D – 100 % palmitic + 0 % linolenic) and 68.24 ± 3.65 % (control). The general observation here was that organic matter degradability was reduced as the proportion of unsaturated fatty acid increased.

The potential degradability of *P. purpureum* when 70 % palmitic + 30 % linolenic acid was infused was significantly ($p < 0.01$) higher than all treatment groups and control, with a value of 84.63 ±8.6 % and 88.42 ± 4.8 % at 24 and 48 hours respectively (Table 3). This was followed by a potential degradability of 64.72 ± 5.0 % and 66.22 ± 4.9 % at 24 and 48 hours respectively, when 50 % palmitic + 50 % linolenic acid (treatment C) was infused. There was no significant difference in the potential degradability (PD) with treatment C and the control group, which had a PD of 61.80 ± 4.9 % and 70.40 ± 3.7 % at 24 and 48 hours respectively. The results show that higher levels of unsaturated fatty acids (30 % palmitic + 70 % linolenic and 100 % linolenic acids) significantly reduced PD with values at 41.08 ± 5.5 % for treatment B and 23.92 ± 2.4 % for treatment E. At 100 % saturated fatty acid infusion potential degradability at 60.33 ±4.6 % was significantly reduced compared to treatments A, C and the control. This trend indicated that organic matter degradability was markedly improved at high proportion of saturated fatty acid than at high proportions of unsaturated fatty acids. Equal proportions of saturated and unsaturated fatty acids gave rise to improved rumen degradability especially at prolonged incubation period compared to the control and treatment A.

These observations probably was as a result of these proportions of fatty acids on the: cellulolytic bacterial population in the rumen; accumulation of lactic acid in the rumen; and changes in intramminal pH. The addition of fats and oils to animal diets particularly ruminants has produced conflicting result with regard to rumen degradability of feed fractions and energy utilization (Van Soest, 1963). In this study where potential rumen degradability was significantly reduced in treatments B and E (high level of unsaturated fatty acid), it is probable that the activity of cellulolytic bacteria were inhibited by high

Table 2: Water solubility ('a') and rumen degradable ('b') organic matter content of *Pennisetium purpureum* in WAD sheep intraruminally infused with varied ratios of palmitic and linolenic acids

Fatty Acid proportions	a	4 hours	8 hours	12hours	24 hours	48 hours
A	2.16 ± 0.12	46.95 ± 2.47	60.43 ± 1.62	70.08 ± 8.75	80.47 ± 6.43	86.26 ± 4.75
B	2.16 ± 0.12	8.74 ± 0.84	19.83 ± 2.99	21.48 ± 4.57	30.67 ± 6.29	39.72 ± 4.04
C	2.16 ± 0.12	37.74 ± 0.49	42.80 ± 2.27	54.87 ± 4.22	62.56 ± 5.01	67.86 ± 3.96
D	2.16 ± 0.12	26.78 ± 1.29	30.75 ± 2.84	33.64 ± 3.29	48.17 ± 4.41	58.17 ± 4.57
E	2.16 ± 0.12	4.63 ± 0.49	6.78 ± 2.10	8.86 ± 1.97	16.73 ± 4.48	21.76 ± 2.41
Control	2.16 ± 0.12	43.47 ± 2.82	44.87 ± 3.09	56.61 ± 4.41	59.44 ± 5.09	68.24 ± 3.65

Table 3: Potential degradability (%) (a+b) of OM fraction of *Pennisetium purpureum* in WAD sheep intraruminally infused with varied combination ratios of palmitic and linolenic acid

Fatty Acid Proportions	% potential degradability of P. purpureum OM at time t				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	40.51 ± 20.5	62.59 ± 1.6	73.64 ± 7.3	84.63 ± 8.6^{a}	88.42 ± 4.8^{a}
B	10.96 ± 0.8	22.00 ± 3.0	24.23 ± 3.6	32.83 ± 6.3^{ab}	41.08 ± 5.5^{ab}
C	39.90 ± 0.5	44.96 ± 2.3	57.03 ± 4.2	64.72 ± 5.0^{b}	66.22 ± 4.9^{b}
D	28.94 ± 1.3	32.91 ± 2.8	35.80 ± 3.3	50.33 ± 4.4^{abc}	60.33 ± 4.6^{abc}
E	6.79 ± 0.5	8.94 ± 2.1	11.10 ± 2.1	18.89 ± 4.5^{c}	23.92 ± 2.0^{4c}
Control	45.76 ± 2.8	47.03 ± 3.1	58.77 ± 4.4	61.80 ± 4.9^{b}	70.40 ± 3.7^{b}

a, ab, b, abc, c= means with different superscript are significantly different at p<0.01

proportions of unsaturated fatty acids either by a coating effect or direct toxic effect. Increased dietary unsaturated fatty acids have been associated with reduced fermentative activity (Leng, 1993). Eastridge (2002) had pointed out that unsaturated fatty acids inhibit cellulolytic bacteria and rumen fermentation. It is also a known fact that cellulolytic bacteria have a low metabolic rate and hence population changes are also slow (Leek, 2004). Therefore, rate of regeneration once inhibited is slow. It could be that once the coating effect or toxic effect were exerted on the cellulolytic bacteria their rate of regeneration became reduced and hence their decreased ruminal population and activity.

Another possible mechanism by which the potential degradability was decreased by high proportions of unsaturated fatty acids could be by changes in rumen pH. The pH optimum of celluloytic bacteria is 6.2 to 6.8 (Leek, 2004). Under the experimental condition, it could be that rumen pH reduced to a level that compromised optimal activity of the cellulolytic bacteria at high unsaturated fatty acid levels. At reduced rumen pH, the conversion of lactic acid and metabolic acids to propionate was reduced (Yang *et al.,* 2002). A situation that could lead to further reduction in rumen pH and possibly negative impact on cellulolytic bacteria. Since experiments with high fat diets have noted ketosis and inefficient use of energy (Van Soest, 1963), accumulation of lactic acid in situation of high dietary or supplemental level of unsaturated fatty acid could be responsible for poor OM degradability in the rumen. Increased supplemental fat or when grains are fed in high amount have been demonstrated to increase intraruminal acidity (Huhtanen and Svseinbjornsson, 2006). Changes in intraruminal pH are known to decrease ruminal microbial population of both the cellulose fermenting and non-fermenting bacteria such as *S. ruminantium, P. ruminocola and B. fibrisolvens* (Russell and Wallace, 1988). *S. ruminantium* also occurs in quite high proportions in the rumen, especially when high amounts of cereal grains or fats are fed (Russell and Wallace, 1988). This species does not degrade cellulose or hemicellulose, but utilize the intermediary products such as cellodextrins and xylo-oligosaccharides (Paynter and Elsden, 1970). Vitamin depletion has been shown to affect proliferation of desirable groups of bacteria in the rumen of animals fed poor quality feeds of high fibre and fatty acid content (Van Gylswyk, 1994). The major cellulose-digesting bacteria, as well as others, have absolute requirement for a range of vitamins (Stack and Hungate, 1984). Low concentration of these vitamins in the rumen could have resulted from moderate to high level of unsaturated fatty acid and very high (100 %) level of saturated fatty acid infusion. Low concentration of vitamins in the rumen has been shown to limit fibre degradability (Harfoot and Hazlewood, 1988). Fatty acid content of diets has been shown to affect the rate of capture of vitamins and minerals contained in ruminant diets (Komisarczuk-Bony *et al.,* 1994).

The cellulolytic bacteria incorporate the straight and branch chain volatile fatty acid (Bc-VFA) mainly into n-C13 to n-C7 straight and branch chain

Table 4: Total volatile fatty acid (VFA) in (mol/hr) in WAD sheep fed *Pennisetium purpureum* and intraruminally infused with varied combination ratios of plamitic and linolenic acid

Fatty Acid Proportions	Total VFA (mmol/l) at time t				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	3.30 ± 0.02	3.41 ± 0.01	3.50 ± 0.06	3.59 ± 0.07^{a}	3.62 ± 0.04^{a}
B	2.99 ± 0.006	3.08 ± 0.01	3.10 ± 0.03	3.17 ± 0.05^{b}	3.24 ± 0.03^{b}
C	3.23 ± 0.004	3.2 ± 0.02	3.36 ± 0.03	3.43 ± 0.04^{c}	3.44 ± 0.04^{c}
D	3.14 ± 0.010	3.17± 0.02	3.19 ± 0.03	3.32 ± 0.04^{c}	3.39 ± 0.04^{c}
E	2.96 ± 0.004	2.97 ± 0.02	2.99 ± 0.02	3.06 ± 0.04^{b}	3.10 ± .02^{ab}
Control	3.27 ± 0.022	3.28 ± 0.05	3.38 ± 0.04	3.40 ± 0.04^{c}	3.47 ± 0.03^{c}

a, ab, b, c, d, = means within column (24 and 48 hours) with different superscripts are significantly different at p<0.01.

Table 5: Total methane production in WAD sheep intraruminally infused with varied combination ratios of palmitic and linolenic acid

Fatty Acid proportions	Methane Production (mol/d)				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	0.41 ± 0.007	0.4 ± 0.004	0.48 ± 0.02	0.50 ± 0.01	0.52 ± 0.01^{a}
B	0.30 ± 0.002	0.33 ± 0.008	0.34 ± 0.009	0.36 ± 0.02	0.39 ± 0.01^{b}
C	0.38 ± 0.001	0.40 ± 0.006	0.43 ± 0.01	0.45 ± 0.01	0.46 ± 0.01^{c}
D	0.35 ± 0.004	0.36 ± 0.007	0.37 ± 0.009	0.41 ± 0.01	0.44 ± 0.01^{c}
E	0.29 ± 0.001	0.29 ± 0.02	0.30 ± 0.006	0.34 ± 0.05	0.34 ± 0.006^{d}
Control	0.40 ± 0.008	0.40 ± 0.008	0.44 ± 0.01	0.44 ± 0.01	0.47 ± 0.01^{c}

a, b, c, d= means within column at 48 hours with different superscript are significantly different at p<0.01.

fatty acids and aldehydes as part of the lipid component of bacterial cells (Wegner and Foster, 1963). It has been suggested that the long-branch chain acids and aldehydes lend fluidly to the lipids of the cellulolytic rumen bacteria as is the case for unsaturated long chain straight acids in aerobic organisms (Allison and Byrant, 1963). In the rumen and other anaerobic environment, there is a strong tendency towards saturation of double bounds due to reducing conditions. The special need for fluidity in the lipids of the cellulolytic bacteria could indicate that it is concerned with cellulolysis. Thus increased infusion of unsaturated fatty acid perhaps incorporate high branch chain fatty acids into the microbial fatty acid pool, which could have led to cellulolysis hence a decreased OM degradability.

Finally, increased unsaturated fatty acid infusion or saturated fatty acid infusion beyond 70 % intraruminally could have exerted undesirable effect on OM degradability by obstructing the uptake of minerals by the rumen bacteria. Growth and activity of *F. succinogenes* and *R. flavefaciens* are known to be dependent on availability and uptake of phosphorus, calcium and manganese within the rumen (Komisarezuk-Bony *et al.,* 1994). Concentrations below 15mg/l for P and 5mg/l for Mg reduced growth and cellulose degradability by *R flavefaciens* (Komisarczuk-Bony et *al*; 1994). This could still be another mechanism by which the fatty acid infusion, at the observed level of reduced OM degradability, exerted their effects.

Though these suspected mechanism discussed here were not studied in this research work, the fact that optimal infusion level of saturated fatty acid (70 %) and unsaturated fatty acid (30 %) had positive effect on OM rumen degradability, beyond which OM degradability was reduced, has been established in this study. Efforts should be made to closely study the underlying mechanism by which high proportions of unsaturated fatty acid reduces organic matter degradability in the rumen.

The total volatile fatty acid (VFA) production was highest is treatment A (70 % palmitic + 30 % linolenic) with a value of 3.59 ± 0.07mmol/l and 3.62 ± 0.04mmol/l at 24 and 48 hours respectively (Table 4). These values were significantly ($p < 0.01$) different from all other treatment groups and control. The VFA in control with a value of 3.47 ± 0.03 mmol/l though significantly less than treatment A, was significantly higher than treatment B (30 % palmitic + 70 % linolenic) and treatment E (100 % linolenic acids). Thus at high unsaturated fatty acid proportions VFA production was significantly reduced compared to other treatments and control. There was no significant difference in VFA production between treatment C with a value of 3.44 ± 0.04 mmol/l compared to treatment D, with a value of 3.39 ± 0.04 mmol/l and the control with a value of 3.47 ± 0.03 mmol/l. These trends suggest that higher proportions of unsaturated fatty acids, beyond 50 %, depressed total VFA production while high proportion of saturated fatty acid not in excess of 70 % increased

total VFA production. It was however observed that at 100 % proportion of saturated fatty acid total VFA was depressed. This, in comparison with 70 % proportion of saturated fatty acids, shows that an optimal level of saturated fatty acids (probably 70 %) is required to support increase in total VFA production in the rumen. That is to say that all saturated or all unsaturated fatty acids were inimical to volatile fatty acid production. Therefore, some forms of interactions between these two fatty acids, are probably required for improved volatile fatty acid production in the rumen... These findings indicate a direct and definitive effect of degree of saturation of dietary fatty acids on total rumen VFA. The more saturated the free fatty acid concentration in the rumen are, the more rumen VFA are produced. This trend which was same for OM degradability indicating a direct relationship between organic mater degradability and rate of volatile fatty acid production. This observation agreed with report of Reynolds *et al*. (2003) and Kristensen (2005). These workers established linear relationship between OM degradability and rumen volatile fatty acid production in steers intraruminally infused with volatile fatty acids. In the analysis of Banik *et al.* (2006), ruminally digested starch was on the average 2.64 and 3.11 kg/d for roughage and concentrate diets respectively and the concentration of VFA in the rumen was 0.22 and 0.31 mol/day for the respective diets, thus indicating a direct relationship. Brown *et al.* (2002) observed high VFA concentration with increase starch degradation with higher propionate and lower acetate proportions. Yang *et al*. (2002) have also demonstrated even higher propionate proportions (740 mMol) VFA when the rate of rumen degradation of starch was improved by defaunation. In the light of these, the suggested possibilities for increased or decreased OM degradability probably apply to increased or decreased volatile fatty acid respectively in this study, thus suggesting a direct relationship between OM degradability and rumen VFA production. Furthermore, VFA production in the rumen has been related to the microbial yield in the rumen (Leng, 1993). The most important concept that bears on the feeding strategies used for ruminants is that microbial protein available and total volatile fatty acids produced in the rumen are inversely related (Brown *et al*., 2002). This arise because under the anaerobic condition of the rumen, the feed nutrients provide both the substrate for microbial cell synthesis and also the potential energy as ATP generated through conversion of feed nutrients to VFA. The efficiency of microbial growth in the rumen appears to be highly dependent on the feeding conditions. The factors that affect microbial growth efficiency and therefore the protein relative to VFA available for digestion and absorption are:

a) A deficiency of any microbial factor (e.g. ammonia, sulfur, phosphorus, amino acid etc.) in the feed or induced some time after feeding in rumen liquor because of rapid absorption of nutrient (Leng, 1993).

b) The relative amounts of carbohydrate and protein that are fermented (fermentative degradation). A high protein to carbohydrate ratio in the diet can lead to a relatively low microbial protein to VFA ration in the end products of fermentative digestion where the dietary protein is easily and rapidly fermented in the rumen (Orskov and McDonald, 1979).

The observed differences in the VFA at different proportions of saturated and unsaturated fatty acids probably resulted from the effects of those fatty acid proportions on the microbial growth factors in the rumen as well as relative fermentative degradation of carbohydrate and protein fractions of the feed.

The relative decrease in VFA production in the control group compared to treatment A showed that in the control condition, the proportions of these fatty acids in the rumen does not support maximum VFA production. In general, changes in rumen pH at different fatty acid combination proportions could be the primary reason for the observed effect on OM degradability and VFA production. The pH at which bacteria grow can affect the fermentation pattern (Hobson and Summer, 1972) and this affects different bacteria differently (Mueller-Harvey and Reed, 1992). Low rumen pH not only in part but sometimes totally abolishes cellulose fermentation (Khazoal and Qrskov, 1994) leading to poor VFA production. It also exposes the animals to problems of acidosis, acetonaemia, laminitis and other feed associated problems. Increase in VFA production stimulates the growth of cellulolytic microorganisms (Palmquist and Eastridge (1991). This again may lay credence to the direct relationship between OM degradability and VFA production as observed in this study.

It has therefore been demonstrate from this study that unsaturated fatty acids are more inhibitory to cellulolytic activity than saturated fatty acids especially in relation to OM degradability and VFA production. This agreed with the observations of Eastridge (2002), Palmquist and Eastridge (1991) and Firkins and Eastridge (1994), that positive ruminal function in relation to OM degradability and VFA production was exhibited at a maximum rumen infusion level of 70 % saturated fatty acid and 30 % unsaturated fatty acid of the NRC (1996)

recommended fat intake (i.e. 3-5 % DM intake/day). Beyond or below this combination proportions OM degradability and VFA production are likely to be inhibited.

At 48 hours, total methane production was highest at 0.52 ± 0.01 mol/ hr when 70 % palmitic + 30 % linolenic (treatment A) was infused (Table 5). This was significantly ($p < 0.01$) different from the total methane production in the control that had a value of 0.47 ±0.01 mol/hr. Total methane production was however significantly reduced in treatment B (30 % palmitic + 70 % linolenic acids) and treatment E (100 % linolenic acid) compared with the control. At the same 48hr incubation period, there was no significant difference in the total methane produced between treatment C (50 % palmitic + 50 % linolemic), treatment D (100 % palmitic) and the control. The general observation was that methane production was suppressed at high proportions (100 % and 70 %) of unsaturated fatty acids.

Unsaturated fatty acids have been shown to be toxic to rumen bacteria particularly the methanogenic bacteria (Philipson, 1970; Eastridge, 2002). This has been evidenced in this study. The relative decrease in methane production at high proportion of unsaturated fatty acids suggests reduction in energy loss and vice-versa. This is so because methane is a high energy compound and its increased production and elimination represents the loss of about 8 percent of the total digestible energy of the diet (Leek, 2004). These results show that methane production was suppressed at high proportions of unsaturated fatty acids. Unsaturated fatty acids have been shown to be toxic to rumen bacteria particularly the methanogenic bacteria (Eastridge and Firkins, 2000). In an earlier report, Eastridge and Firkins (2000) has reported that increasing dietary unsaturated fatty could be beneficial to the animals as it reduces methane production. Though they did not describe the level of unsaturated fatty acid associated with this effect. Though this has been demonstrated in this study, inclusion of unsaturated fatty acid beyond 50 % of the recommended dietary intake should not be considered entirely beneficial as it could adversely affect other important digestive indices especially as it bothers on digestion and absorption at the post ruminal segments of the gastrointestinal tract.

The relative increase in methane production in treatments A is considered wasteful because methane is a high energy compound and its elimination from the body as a waste product represents the loss of about 8 percent of the total digestible energy of the diet (Leek, 2004).

The methane production in treatment C, D and control occur as intermediates. The mechanisms surrounding the suppressive effect of unsaturated fatty acids on rumen bacteria and particularly the methanogenic bacteria has not been clearly studied and this cannot be clearly explained here. However, it may be related to the inhibitory effect of biohydrogenation products or intermediates, which probably exert selective lethal effect on methanogenic bacteria. Ruminal microbes rapidly hydrolyze dietary lipids and using the unsaturated fatty acids as hydrogen acceptors quickly convert most of them to stearic acid. It is probable that methanogenic bacteria have need for hydrogen ions in the rumen and these ions are mopped up at high level of dietary or intraruminal infusion of unsaturated fatty acid by a competitive effect for hydrogen between unsaturated fatty acids and the methanogenic bacteria. When the available hydrogen become depleted and thus not readily available for the unsaturated fatty acids and the mathanogenic bacteria both rate of biohydrogenation and rate of methane formation will be reduced. In this situation, both number and activity of methanogenic bacteria become reduced leading to reduced methane production. Philipson (1970) noted that C 18 unsaturated fatty acids, particularly the polyunsaturated fatty acids, inhibited methanogenic bacteria and that the nature of the inhibition was one of direct toxicity towards the bacteria, resulting from the adsorption of fatty acids on the bacterial surface. Czerkawski *et al*. (1986) found that intraruminal infusion of unesterified C 18 fatty acids, particularly the unsaturated homologues, caused a marked reduction in methane production. They attributed this effect to the inhibition of gram-positive *Methanobactrium ruminatium.* The variations in methane production at different proportions of fatty acids, especially on the decreasing effect of unsaturated fatty acids, agrees with the report of these worker, who stated that the effects of fatty acids on the growth and metabolic activity of microorganisms is dependent not only on the nature of the added fatty acid but also, *interalia,* on the concentration of the added fatty acid in a culture media thus laying credence to the varied effects at different proportions of the unsaturated fatty acids. Except in treatment A (i.e. 70 % saturated fatty acid versus 30 % unsaturated fatty acid), methane production was reduced when compared with the control, thus suggesting optimum level of bacterial tolerance for saturated fatty acids beyond which (treatment D) population and activity decrease. On the other hand, at any dietary or ruminal infusion level of unsaturated fatty acids exceeding 30 % of

recommended fat intake, methanogenic bacterial activity wanes.

In conclusion, this study has demonstrated that the proportion of saturated and unsaturated fatty acids in the ruminant diet is critical to the functional efficiency of the rumen particularly as it concerns rumen fermentation of feed nutrients (carbohydrates, proteins, lipids). It is particularly important therefore, that the proportions of these fatty acids should be carefully regulated in the formulation of fat supplements for the improvement of ruminant milk and meat productions.

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PHYSIOLOGIC EVIDENCES OF GOOD TOLERANCE OF CONCURRENT RUMEN FISTULATION AND DUODENAL CANNULATION IN WEST AFRICAN DWARF SHEEP

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ABSTRACT

Twelve adult West African Dwarf (WAD) sheep of mean age and body weight of 18 ± 1.19 months and 14.69 ± 2.56 kg body weight respectively were used to study the compatibility of concurrent rumen fistulation and duodenal cannulation with normal life. The compatibility with normal life was assessed by differences in body weight, voluntary feed intake, rectal temperature and some haematological and biochemical parameters for a period of eight weeks between test and control groups. During the study period, all the six animals survived the surgery. There was no significant ($p < 0.05$) difference in body weight between the test and control groups for the entire study period (week 1-8). The mean rectal temperature of the test group was significantly ($p < 0.05$) higher than the control group on week 1(44.6 ± 3.15^{0}C), week 2 (43.26 ± 4.14 0C) and week 3 (41.15 ± 6.62^{0}C) after implantation. Packed cell volume (PCV) and erythrocyte count (EC) were significantly reduced in the test group within the first three weeks (33.19 ± 1.41 to 24.45 ± 1.39% and 7.01 ± 1.34 to 4.37 ± 1.58 x 10^{6}µl) respectively. On the contrary, total leukocyte count (TLC), and neutrophils were significantly higher in the test group between weeks 1 to 3 (12.48 ± 4.35 and 31.09 ± 3.67 respectively) compared to the control (8.06 ± 0.95 and 23.41 ± 2.09 respectively. Lymphocytes were insignificantly higher in the test group while voluntary feed intake was insignificantly reduced in the test group throughout the eight weeks. Biochemical analyses revealed that serum creatinine was significantly higher in the test group at week 1 (3.62 ± 1.18) and week 2 (3.08 ± 1.44) after surgery. There was however, no significant difference in serum total proteins during the study period. Serum potassium concentrations were significantly decreased in the test group at week 1 (4.96 ± 1.03 to 1.34 ± 0.04). Sodium was also significantly reduced (146.08 ± 3.78 to 96.03 ± 6.21) but on week 3 post implantation (PI). On the other hand, serum globulin was significantly higher in the test group throughout the study period. Gross observations of incision sites showed adhesions characterized by coalesced tissue granulations. The study has shown that the use of polymetric materials in fabricating rumen fistulae and duodenal cannuale could pave way for increased routine nutritional studies in ruminants particularly in developing countries where the availability of these materials constitute great limitation to research involving rumen studies.

Keywords: Duodenal cannulation, Rumen fistulation, West African Dwarf sheep

INTRODUCTION

Rumenotomy is one of the important and commonest surgical procedures in ruminants (Remi-Adwunmi *et al.,* 2006). In Nigeria, the most significant indication of the procedure in small ruminant is in the relief of rumen impaction especially due to indigestible materials such as polythene bag, pieces of leather, bailing rope, rubber, cloth, metal and glass (Sanni *et al.,* 1998). Other indications include surgical

treatment of toxic indigestion, primarily of rumen origin, relief of obstruction of the rumino-recticular and reticulo-omasal orifices, a prelude to the treatment of omasal and abomasal impactions and removal of neoplasm such as papillomas at the cardia of the rumen (Gyang, 1992). Another very important area where rumenotmy is indicated is in *in-vivo* nutritional studies where it is usually accompanied by implantation of rumen fistula alone or together with intestinal (duodenal) cannula.

In nutritional studies such as rumen degradation of forages, manipulation of fermentative activities, bioengineering of rumen functions and nutrient digestibility trials, rumen fistulation and duodenal cannulation are very essential. This is so because they afford the researcher opportunities to investigate digestive events under unaltered physiologic conditions (Leng, 1993).

Measuring feed fractions degradability within the rumen in usually a difficult task (Hungate, 1966). The difficulty usually arise from post surgical management of surgical complications often associated with rumenotomy, fistualtion and cannualtion, as well as physiologic variation likely to be introduced and which probably may lead to conflicting results. For this reasons *in vitro* studies involving cultures of rumen fluid are usually preferred.

Nevertheless, rumen fistulation and duodenal cannulation, adopted under well-managed conditions and minimal disruption of rumen environment, are becoming popular in digestibility studies in ruminants. However, the problem of unavailability of rumen fistulation and duodenal cannualtion materials in developing countries has limited this area of research in such countries including Nigeria. In other to make remarkable progress in rumen studies in these areas there is need to solve the problem of availability of fistulation and cannulation materials. For this reason, we fabricated rumen fistulae and duodenal cannulae using polymetric materials and consequently used them to perform concurrent rumen fistulation and duodenal cannulation so as to evaluate their compatibility with normal life using the West African Dwarf sheep.

MATERIALS AND METHODS

Fabrication: Rumen fistula was fabricated using a car driving shaft cover made of polypropylene rubber. It was designed in such a way that the portion with larger internal diameter formed the flange, by cutting it to be together with the projected cylindrical neck (Figure 1).

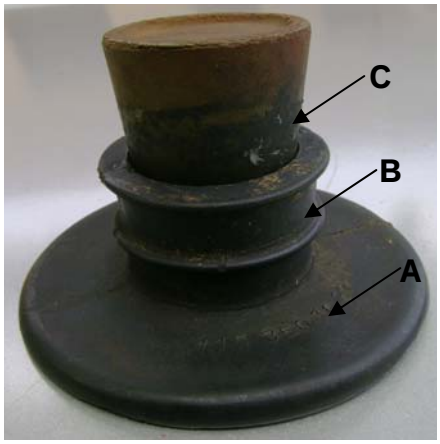

Figure 1: Showing the fabricated rumen fistula using a car driving shaft cover. The rubber bung is also shown. A, Fistula flange. B, projected end of the fistula. C, rubber bung stoppered on the fistula opening for firm closure

A suitable solid rubber bung was used as stopper (Figure 1). The internal diameter of the flange was 8 cm while the internal and external diameter of the cylindrical neck was 4cm and 4.8 cm respectively. A round fistula clamp made of synthetic polypropylene plastic; measuring 0.5 cm thick with a circular hole (about the size of the external diameter of the cylindrical neck of the fistula) in the centre (Figure 2) was used for firm anchorage of the fistula.

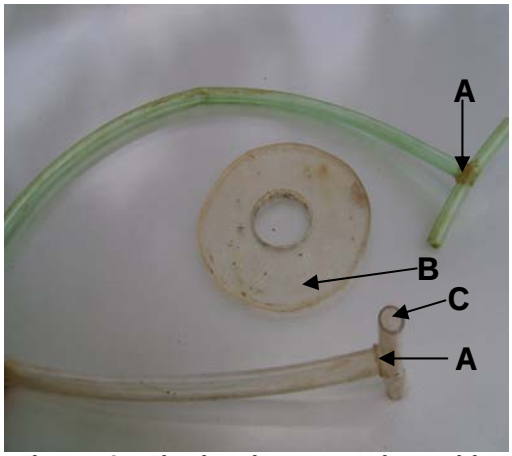

Figure 2: Fistula clamp together with duodenal cannula showing the T end. A, duodenal cannula. B, fistula clamp. C, internal diameter of the T end of the duodenal cannula

The duodenal T-canula (Figure 2) was fabricated using two polypropylene tubes-one measuring 6cm and the other 10 cm in length. A hole, about the size of the external diameter of the 10 cm tube was made at the middle of the 6cm tube (Figure 2). One end of the 10 cm tube was carefully inserted into the hole on the 6 cm tube, thus forming a T-junction (Figure 2).

The insertion was carefully done to avoid blockage of the lumen of the 6 cm tube. The junction was well sealed by heating. The choice of tube diameter was informed by predetermining the diameter of the small intestine of the WAD sheep few minutes after slaughter. All fabricated materials were alternately soaked in 70 % alcohol and Izol® germicide for 24 hours each before use.

Animal: Twelve West African Dwarf Sheep bought from Opi market in Nsukka area of Enugu state, Southeastern Nigeria were used for this study. They were kept at the animal house of the Department of Veterinary Physiology and Pharmacology where they were acclimatized for two months. During this period, they were fed with *Panicum maximum, Pennisetium purpureum* and *Centrosema pubescens.* Occasional supplementation with maize bran was performed. Water was provided *ad libitum* while salt was provided as a lick once (24 hours) a week. During the acclimatization period, ectoparasites and endoparasites were routinely controlled, together with the prescribed vaccination. At the end of the two months acclimatization period, blood samples were obtained via the jugular vein. The samples were examined for haemoparasite, which was found to be negative for all haemoparasites. Likewise, baseline haematological parameters were within normal ranges. Fecal samples were examined for worm eggs by the floatation technique and did not show worm eggs.

Experimental Design: The animals were then divided into two groups of six sheep each. The sheep in group A fitted with rumen fistulae and duodenal cannulae, served as the test group, while those in group B were neither fitted with rumen fistulae nor duodenal cannulae, and therefore served as the control group. The usual pre-surgical protocols were observed. Feed was withdrawn for 24 hours and water was withdrawn for 12 hours prior to surgery. The left Para lumbar area extending from the last rib to the tuber coxae transversely and from the vertebral column down to the ventral abdominal midline longitudinally was prepared for aseptic surgery. Xylazine® HCl administered intramuscularly at a dose rate of 0.01ml/kg was followed with an inverted L field infiltration of 2 % Lingnocain hydrochloride at the paralumbar region. Animals were restrained properly on right lateral recumbence.

Following effective anesthesia, a longitudinal incision (about 8 cm) was made on the left para lumbar region. Upon exposure of the rumen wall, the stomach was retracted until the abomasum was revealed and using the abomasum as a landmark, the duodenum was located and a loop of the proximal duodenum (about 4 – 6 cm) with the shortest mesentery was isolated with sterilized gauze napkins. Then a longitudinal incision about (2 – 4 cm) was made on the isolated loop of the proximal duodenum after retraction of its content proximally and distally. The T end of the sterilized and paraffin treated cannula was carefully inserted into the duodenal lumen starting distally and then ending proximally (Lopukhin, 1976; McGillard, 1982). The incision was then sutured using absorbable chronic catgut (number 3.0). Correct alignment of the duodenal cannula in the duodenum was indicated by a free flow of intestinal content into the exteriorized end of the T-cannula. Following complete retraction of the duodenum and abomasum, stay sutures were then applied on the rumen wall and the abdominal muscle at 3, 6, 9 and 12 o'clock positions. The wall of the stomach was then cut open about the size of the internal diameter of the fistula. The protruding mucosa was griped with a haemostatic clamp at the middle of the incision and pulled upward. The cone thus formed was cut off, creating a circular opening on the stomach. The walls of the stomach (especially the two edges of the incision) were kept raised on hooks and forceps to prevent spillage of rumen content into the peritoneum. Thereafter, the flanges of the rumen fistula were folded and slipped carefully into the stomach through the opening created on the rumen. The ruminal incision was subsequently closed tightly around the neck of the fistula with medium chronic catgut (type C) using two rows of Cushing sutures. Soiled drapes were removed and fresh ones applied. Procaine penicillin (4,000.000 iu) diluted appropriately was sued to lavage the peritoneal cavity prior to closer of the laparotomy.

The peritoneum and abdominal muscles were closed using simple interrupted suture with chromic catgut (number 2). Subcuticular suture was used to appose the subcutaneous tissue using chronic catgut (number 2). The skin was closed using interrupted horizontal mattress suture with silk (number 2). All sutures were made to allow for exteriorization of the duodenal cannula. The fistula clam was used to hold the fistula firmly in place by passing the protruding neck through the central hole on the clamp. Postoperative medication with 5 % dextrose solution at 20ml/kg B.W (iv), procaine penicillin and streptomycin at recommended does were done. Fly repellants, Scabicur® together with Spray plus® (oxytetracycline + Gentian violet) were alternately applied on the surgical would. Skin sutures were removed 10 days post surgery.

Evaluation: After implantation of rumen fistulae and duodenal cannulae, the animals were evaluated weekly. Mean weekly rectal temperature, body weight, and voluntary feed intake, were recorded. Blood samples were collected weekly, from each sheep in a group, for determination of packed cell volume, erythrocyte count, total leukocyte count, lymphocyte and neutrophil count. Blood was sampled, using needle and syringe by jugular venipuncture into duplicate bijou bottles-one containing EDTA, an anticoagulating agent, and the other without EDTA for the hematological and biochemical analyses respectively. Erythrocyte count, packed cell volume, total leukocyte, lymphocyte and neutrophil count were determined using standard methods (Schalm *et al.*, 1975). Serum creatinine was analyzed by the Folin-Wu method (Coles, 1986). Serum total proteins were determined by the biuret method (Coles, 1986). Sodium and potassium levels were determined colorimetrically using Randox® test reagents based on standard protocol (Coles, 1986).

Data Analysis: The data obtained were analyzed statistically using student t-test (Steel and Torrier, 1980).

RESULTS

Figure 1 showed the fabricated rumen fistula. The rubber bung was also shown. Figure 2 showed fistula clamp together with duodenal cannula showing the T end. Figure 3 showed the duodenal cannula well fitted into the duodenal lumen.

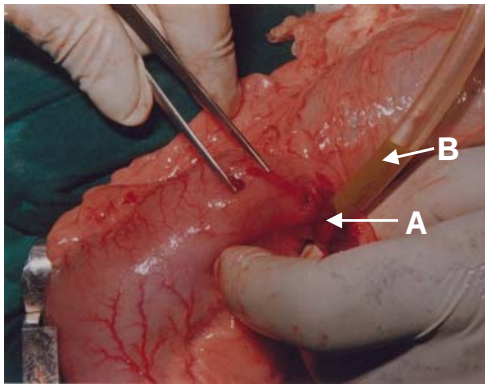

Figure 3: Shows duodenal cannula already implanted into the duodenum. A, point if insertion of the cannula. B, outflow of duodenal content into the cannula indicating correct alignment of the cannula within the duodenal lumen

Figure 4 showed a West African Dwarf sheep carrying the concurrent rumen fistula with clamp and exteriorized duodenal cannula 2 weeks after implantation.

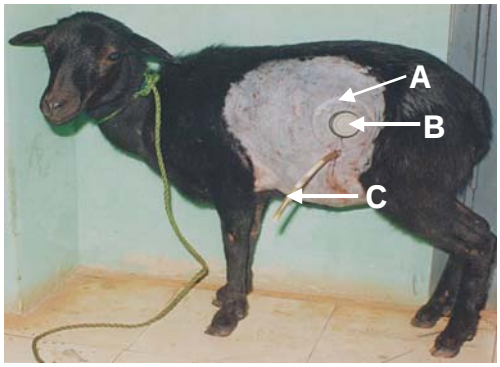

Figure 4: A West African Dwarf sheep carrying concurrent rumen fistula and duodenal cannula fabricated with polymetric materials. (A, rumen fistula with rubber bung for a firm covering, B, fistula clamp for firm anchorage, C, exteriorized duodenal cannula)

Changes in body weight and rectal temperatures in the test and control groups between week 1 and 8 is presented in table 1. There was no significant ($p < 0.05$) difference in body weight in both test and control groups between week 2 and week 8. However, on the first week after implantation, the body weight of the test group significantly decreased compared to the control. Rectal temperature in the test group increased significantly in week 1 and 2 than the control group. From week 3 onwards, no significant difference in rectal temperature was observed in both test and control groups.

Table 2 showed the packed cell volume (PCV) and erythrocyte count (EC) in both test and control groups for the study period. The packed cell volume was significantly decreased in the test group in week, 1, 2 and 3 compared to the control. Likewise, the erythrocyte count followed the same trend as the PCV, though it was significantly reduced in week 3 relative to other weeks including weeks 1 and 2 compared to the control.

Table 3 showed the total leukocyte, lymphocyte and neutrophil count in both test and control groups. Total leukocyte count increased significantly ($p < 0.5$) in the test group in week 1 through week 3. The increase persisted throughout the eight-week period but after week 3 it was not significantly different from the control. In addition, lymphocytes were insignificantly increased in the test group compared to the control throughout the study period. On the contrary, neutrophil in the test group was significantly higher than the control in week 2 and week 3.

Table 4 showed the voluntary feed intake in the test and the control groups. In the test group, voluntary feed intake decreased insignificantly from baseline value in week 0 and compared with the control group for the entire study period.

Table 1: Mean body weight and rectal temperatures in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Body weight (kg)		Rectal temperature (^{0}C)	
	Group A	Group B	Group A	Group B
0	13.88 ± 2.19	14.96 ± 2.66	38.75 ± 1.50	39.94 ± 52.26
1	10.92 ± 1.46^a	14.53 ± 1.62^b	44.64 ± 3.15^a	39.83 ± 1.00^b
2	12.94 ± 1.93^a	14.08 ± 2.61^a	43.26 ± 4.14^a	38.93 ± 2.25^b
3	12.11 ± 0.88^a	13.63 ± 3.11^a	41.15 ± 4.62^b	38.66 ± 1.75^b
4	11.64 ± 2.43^a	13.84 ± 1.69^a	40.05 ± 6.11^b	39.15 ± 2.65^b
5	12.16 ± 1.56^a	14.02 ± 2.36^a	39.65 ± 2.93^b	38.50 ± 1.19^b
6	12.84 ± 3.14^a	14.17 ± 2.15^a	40.15 ± 2.15^b	39.50 ± 2.25^b
7	12.92 ± 1.92^a	14.24 ± 3.0^a	38.91 ± 1.58^b	38.39 ± 3.11^b
8	11.82 ± 1.27^a	13.62 ± 2.51^a	39.74 ± 2.67^b	39.75 ± 4.11^b

Means within row with different superscript are significantly ($p<0.05$) different

Table 2: Packed cell volume (%) and erythrocyte count (x10^6 µl) in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	PCV (%)		Erythrocyte count (x10^6 µl)	
	Group A	Group B	Group A	Group B
0	33.19 ± 1.41	32.28 ±1.36	7.01 ± 1.34	6.68 ± 1.48
1	27.25 ± 2.62^a	33.46 ± 1.24^b	4.72 ± 0.44^a	6.99 ± 1.53^b
2	25.65 ± 2.87^a	32.61 ± 4.46^b	4.96 ± 1.46^a	7.26 ± 1.56^b
3	25.45 ± 1.39^a	33.65 ± 2.96^b	4.37 ± 1.58^a	7.86 ± 0.81^b
4	28.43 ± 5.56	30.29 ± 3.16	5.88 ± 1.18	6.23 ± 1.48
5	27.86 ± 4.11	31.90 ± 3.66	5.96 ± 0.13	6.84 ± 1.35
6	29.57 ± 1.62	30.45 ± 2.93	6.37 ± 1.77	7.95 ± 1.36
7	30.14 ± 2.63	37.34 ± 3.03	6.17 ± 2.51	6.82 ± 2.33
8	29.45 ± 2.71	34.95 ± 4.62	6.83 ± 3.16	7.69 ± 3.17

** Period of most significant reduction. Means within row different superscript are significantly ($p<0.05$) different*

Table 3: Total leucocyte, lymphocyte and neutrophil count in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Total Leukocyte (x10^3 µl)		Lymphocyte (%)		Neutrophils (%)	
	Group A	Group B	Group A	Group B	Group A	Group B
0	8.36 ± 2.71	7.85 ± 1.38	60.33 ± 4.15	58.93 ± 4.19	23.96 ± 4.11	20.46 ± 2.83
1	11.89 ± 1.94^a	7.64 ± 2.16^b	63.81 ± 3.91	59.84 ± 3.34	28.42 ± 3.28^a	21.35 ± 2.75^b
2	12.17 ± 1.89^a	7.86 ± 1.05^b	62.11 ± 4.11	58.74 ± 9.14	30.94 ± 2.98^a	20.38 ± 3.11^b
3	12.48 ± 1.35^a	8.06 ± 0.95^b	58.44 ± 3.47	59.73 ± 2.81	31.09 ± 4.23^a	23.41 ± 2.09^b
4	11.36 ± 1.41^a	7.58 ± 0.42^a	61.17 ± 4.19	60.03 ± 4.71	26.65 ± 5.11	22.95 ± 2.88
5	10.08 ± 2.56^a	8.01 ± 0.94^a	62.27 ± 2.81	59.73 ± 6.48	24.06 ± 3.56	23.16 ± 2.71
6	9.63 ± 1.42^a	8.44 ± 1.33^a	61.95 ± 5.13	57.44 ± 4.18	25.45 ± 4.31	22.75 ± 3.17
7	8.58 ± 1.56	7.41 ±1.7	59.12 ± 3.67	43.11 ± 2.68	26.82 ± 3.71	24.65 ± 2.81
8	8.93 ± 2.82	6.48 ± 0.92	63.14 ± 2.86	51.78 ± 3.41	29.01 ± 2.43	21.03 ± 3.04

Means within row with different superscript are significantly ($p<0.05$) different

Table 5 showed the serum creatinine and total protein profile in the test and control groups. There was significant ($p < 0.05$) increased in serum creatinine in the test group from week 1 to week 3 compared to the control. However, beyond week 3, the increase persisted but was insignificant up until week 5. There was no significant difference in total protein between the test and control groups fro the entire study period.

Table 6 showed the mean serum globulin, potassium and sodium concentrations in both experimental groups. Serum globulin was significantly higher in the test group throughout the eight weeks. On the other hand, potassium was significantly decreased in week 1. This decrease was restored to near normal almost after this period (i.e. week 2). Serum sodium concentration was normal in week 1 and 2 and only significantly decreased in week three after which an insignificant increase was maintained up till week 5.

DISCUSSION

In the study, we observed that concurrent rumen fistulation and duodenal cannulation is well tolerated by West African dwarf sheep as evidenced by remarkable positive progressive physiological changes within eight weeks after implantation. These observed changes characterized good healing processes, effective defense mechanism against infectious organisms and adequate restoration of electrolyte balance in interstitial fluid.

Table 4: Voluntary feed intake in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Feed intake kg/day	
	Group A	Group B
0	3.86 ± 0.96[a]	3.94 ± 1.71[a]
1	2.93 ± 0.73[a]	3.96 ± 1.86[a]
2	3.16 ± 0.46[a]	3.72 ± 0.66[a]
3	3.46 ± 0.62[a]	3.83 ± 1.17[a]
4	3.53 ± 0.48[a]	3.76 ± 1.31[a]
5	3.66 ± 0.93[a]	3.84 ± 0.81[a]
6	3.71 ± 0.76[a]	3.93 ± 0.73[a]
7	3.16 ± 1.12[a]	3.97 ± 1.02[a]
8	4.11 ± 1.51[a]	4.94 ± 0.49[a]

Means within row with different superscript are significantly ($p<0.05$) different

Table 5: Serum creatinine and total proteins in WAD sheep fitted with concurrent rumen fistulae and duodenal cannula

Expt. Period (Wks)	Biochemical parameters			
	Serum creatinine (mg/100ml)		Total proteins (mg/100ml)	
	Group A	Group B	Group A	Group B
0	1.31 ± 0.04	0.98 ± 0.09	9.01 ± 0.89	7.18 ± 1.44
1	3.62 ± 1.18[a]	1.03 ± 0.06[b]	6.69 ± 1.16[a]	7.21 ± 2.02[a]
2	3.08 ± 1.44[a]	1.14 ± 0.09[b]	6.34 ± 2.04[a]	6.73 ± 1.82[a]
3	2.94 ± 0.33[a]	0.95 ± 0.04[b]	7.16 ± 2.05[a]	6.73 ± 1.84[a]
4	2.09 ± 0.0[a]	0.86 ± 0.07[a]	6.84 ± 1.63[a]	6.28 ± 2.01[a]
5	1.36 ± 0.03[a]	0.91 ± 0.03[a]	7.04 ± 0.99[a]	6.19 ± 1.02[a]
6	1.33 ± 0.04[a]	0.94 ± 0.06[a]	6.96 ± 1.38[a]	7.47 ± 1.92[a]
7	1.55 ± 0.11	0.96 ± 0.43	7.32 ± 1.77	7.19 ± 0.83
8	1.19 ± 0.06	0.82 ± 0.21	6.49 ± 0.09	6.39 ± 0.76

Means within row with different superscript are significantly ($p<0.05$) different

The decrease in body weight which was insignificant during the entire observation period (Table 1) was probably due to reduction in fed intake which was also insignificantly decreased (Table 4) in the test group for the same entire study period . This reduction was probably due to surgical stress that gave rise to inappetance. Stress in general has been shown to reduce appetite and thus feed intake in both humans and animals (William, 2004). Under this experimental condition, the surgical stress in addition to the relative increase in rectal temperature (Table 1) (thermal stress) within the first three weeks post implantation probably gave rise to sustained anorexia which lasted for the entire observation period, thus probably responsible for the decreased body weight. Another factor, which probably was responsible for the suppression of appetite, was the stretching of the rumen and intestine. Stretching of the rumen and the intestine causes anorexia (William, 2004). The rumen fistulae and duodenal cannula (especially considering the materials used) probably produced stretching effect on the rumen and intestine such that intragastric and intra-intestinal pressures were so increased as to send satiation signal via stretch receptors to the feeding centre in the brain, which in turn regulated feeding negatively. These stretching effects probably contributed to anorexia especially few days after implantation. Whatever was the mechanism (s) that resulted in reduced feed intake and body weight, we had the opinion that the reductions were not inimical to health since there variations in the test and control groups were insignificant for the entire observation period.

The decrease in mean packed cell volume and erythrocyte count within the first three weeks suggested anaemia. Post surgical anaemia has been demonstrated few days after surgery (Dougherty, 1981) and is usually associated with the extent of blood loss during surgery (Gyang, 1992; Venugopalan 1997), post surgical complication (Santra and Karim, 2002), post surgical feeding behaviour and quality of feed offered (Ragab, 1989; Appleby and Hughes, 1997). In this study, the surgery that lasted for 56 minutes was characterized by relative high blood loss. This level of blood loss together with the suppressed appetite and decreased feed intake probably accounted for the decrease in PCV and EC at least for the first 3 weeks. The fact that beyond the third week, PCV and EC began to improve led us to conclude that their decrease were surgery related particularly due to degree of blood loss and post-surgical inappetance, which waned with time. Thus, three weeks and beyond was enough for improvement in PCV and EC especially as intake of protein rich legumes, *Centrosema pubescens* and *Stylosanthese gracilis* during the first one week after implantation was assured.

The significant increase in total leukocyte and neutrophil within the first three weeks suggest physiologic response to inflammation and enhanced immune response. Various factors such as time of day, a meal, exercise, epinephrine (endogenous), anesthesia and stress conditions are known to contribute to physiological leukocytosis (William and Melvin, 2004). With some anesthesia, animals may undergo an excitement period that causes epinephrine release, which contributes to a leukocytosis. However, in this study, most of the sheep showed some excitement immediately after administration of anesthetic agent (xylazine). The excitements were very short-lived however. Therefore, it was difficult to relate the observed leucocytosis to epinephrine influence, weeks after surgery. Increase in total leucocytes few weeks after surgery has been earlier reported (Leifer *et al.*, 1983; Buckner, 1995; Abdel-Fattah, 1999). In localized traumatic conditions such as rumenotomy, leucocytosis and neutrophilia has been reported (Weisis, 1984; Hassanein *et al*; 1988; Aka *et al.*,

Table 6: Serum globulin, potassium and sodium levels in WAD sheep fitted with concurrent fistula and duodenal cannula

Expt. Period (Wks)	Serum biochemical parameters					
	Globulin (g/dl)		Potassium (mEq/L)		Sodium (mEq/L)	
	Group A	Group B	Group A	Group B	Group A	Group B
0	2.83 ± 0.36	3.41 ± 0.63	4.96 ± 0.98	5.18 ± 0.98	146.08 ± 4.87	55.14 ± 6.11
1	4.939 ± 0.71	3.08 ± 0.14	1.34 ± 0.06[a]	4.12 ± 0.54[b]	158.46 ± 4.33	63.44 ± 3.83
2	6.86 ± 1.08	3.14 ± 0.61	3.89 ± 0.93	4.62 ± 0.65	67.93 ± 3.78	153.43 ± 2.96
3	6.36 ± 1.42	2.94 ± 0.48	3.66 ± 0.87	4.17 ± 0.84	96.03 ± 4.11[a]	116.37 ± 4.2[b]
4	5.16 ± 1.33	2.88 ± 0.55	4.28 ± 10.06	4.86 ± 0.76	143.78 ± 3.98	156.18 ± 3.22
5	5.98 ± 0.96	3.16 ± 0.43	4.06 ± 1.05	4.82 ± 0.42	157.63 ± 4.81	167.46 ± 3.71
6	5.48 ± 1.17	3.66 ± 0.37	4.71 ± 1.13	4.56 ± 1.11	141.67 ± 3.77	163.83 ± 3.23
7	4.93 ± 0.88	3.61 ± 0.44	4.45 ± 0.54	4.34 ± 0.32	144.23 ± 3.21	171.55 ± 3.88
8	5.33 ± 0.23	3.12 ± 0.43	3.97 ± 1.02	4.63 ± 0.54	146.98 ± 3.65	166.98 ± 4.62

Means within row with different superscript are significantly ($p<0.05$) different

2006). Tissue destruction, irrespective of its cause will produce an increase in the number of circulating neutrophils. Increase in neutrophils occurs in prolonged surgical procedures where there has been considerable tissue damage (Buckner, 1995). These observations, which have been represented in this study, made it apt for us to conclude that the increase in total leucocytes in this study was more of a physiologic relative leucocytosis that resulted from neutrophilia.

The insignificant increase in lymphocytes in this study points to mild proliferative response by the immune cells. This probably was to boost immunity to combat infection by microorganisms. This was particularly corroborated by significant increase in serum globulin level for the same period after surgery (Table 6). Increased serum globulin level with concomitant leucocytosis highlights an improved immune status (Obidike *et al*; 2009).

At first, second and third week after implantation there was almost double increased serum creatinine level in the test group compared to the control. This observation agrees with the report of other workers. Swanson and Wilkinson (1972) observed that trauma associated with surgery and accidents with high degree of muscle damage caused significant rise in serum ceratinine. In this study, serum creatinine level was high in test group at the earlier stages after implantation (week 1 to 3) and was decreased as wound healing progressed as observed from week 4 onwards. Surgical trauma to muscle, or any form of trauma, bruises, fracture and even severe exercise may cause a marked elevation of blood creatinine and serum kinase activity, which may persist for a week or longer (Tietz, 1978). The increase in serum creatinine in this study seems to be a physiologic response to surgical trauma and thus was probably not inimical to the health status of the animals as it waned with time.

Relative changes in electrolyte balance in interstitial fluid have been reported to be characteristic of most surgeries. The significant reduction in serum potassium and sodium concentrations in week 1 and week 3 respectively lays credence to this assertion. Potassium is one of the major cations of fluids of the cell (cytosol) while sodium occurs in small amounts within the cell. In surgical condition, the cells that are usually lost in greater numbers are the erythrocytes and consequently potassium is lost much more than sodium. This probably explains its (potassium) significant reduction (Table 6) within the first week as against sodium that required up to 3 weeks recording a significant fall (Table 6). A change in electrolyte balance affects the osmotic pressure of cells and body fluids. Changes in electrolytes and osmotic pressure of cells and body fluids probably did not develop in this study. This could be due to the sharp fall in potassium level, which was restored in week 2. Restoration of potassium deficiency via diet is usually faster than sodium (Rosa *et al.*, 1992) as most foodstuffs contain considerable potassium, and the kidneys are more capable of excreting this excess than of conserving it. Ruminants have a good ability to vary their urinary excretion rate of potassium to meet wide and rapid changes in intake (William, 2004). Thus, the time taken for the physiologic adjustments of these electrolytes after depletion was not long enough to produce any adverse effects on the health of the animals.

Furthermore, the study showed no significant difference in the total plasma proteins for the entire observation period. This shows that in all groups (test and control), the normal profiles and functions of serum proteins were maintained. Since plasma proteins are chiefly formed in the hepatic cells, it could be said that the surgical technique had no obvious clinical effect on the liver. Again, since there was no obvious change in total protein we also speculate that cellular proteins were not depleted in the prevailing circumstance. This could be so because in situation(s) where cellular proteins are depleted

reduction in plasma protein occurs due to their breakdown into amino acids by the mononuclear phagocytic system (MPS) cells. These amino acids are then used for the formation of cellular protein. Plasma proteins are known to maintain the colloidal osmotic pressure, contribute to viscosity of blood, influence the suspension stability of erythrocytes, and help regulate acid-base balance, transport substances (vitamins, hormones, nutrients etc) and affect solubility of carbohydrates, lipids and other substances held in solution in the plasma. It then follows that in both groups these functions probably were not affected by the surgical technique since no significant difference in total protein was observed – a situation that maintains homeostatic balance between blood and cellular fluids.

Conclusion: In conclusion, we have observed that some physiologic changes do occur in WAD sheep following concurrent rumen fistulation and duodenal cannulation. Most of these changes were thought to be normal (physiologic) consequences of most surgical techniques and were usually restored with 3 to 4 weeks post implantation. On the other hand, those changes, which could compromise normal health if prolonged, did not persist beyond three weeks after implantation. Therefore, it was concluded that the WAD sheep tolerates concurrent rumen fistulation and duodenal cannulation without obvious clinical effects for at least the observation period (eight weeks). In small ruminants, particularly the WAD sheep, the use of local polymetric materials to fabricate rumen fistulae and duodenal cannula could provide more opportunities for *in vivo* nutritional studies.

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THE GENERATION AND PROPERTIES OF SOLID MONODISPERSE AEROSOLS OF STEARIC ACID AND CARNAUBA WAX

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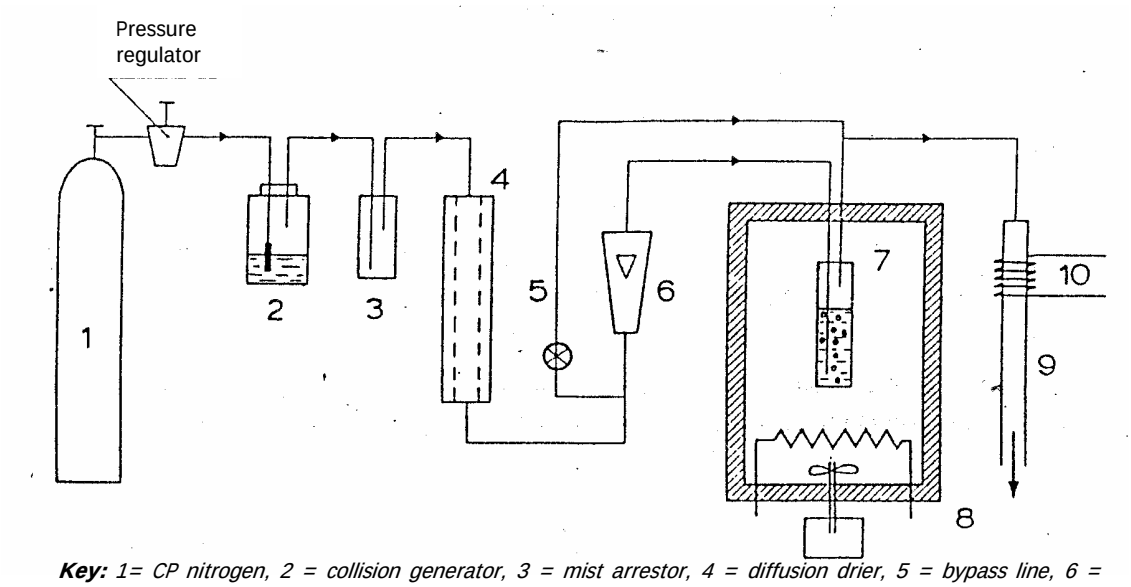
ABSTRACT

A monodisperse aerosol generator (MAGE) was used to generate calibration or monodisperse aerosols containing stearic acid and carnauba wax. Some of the factors affecting the size of aerosol particles generated with the MAGE were determined. The factors include: temperature of operation of the MAGE, type and purity of coating material used. The reproducibility of aerosol particles and stability of the aerosol generated were evaluated. The performance characteristics of the MAGE over an extended period of time of operation were also established. The calibration aerosols generated with the MAGE can be used *in situ* for studies of aerosol deposition measurement equipment.

Keywords: Generation, Calibration aerosols, Monodisperse aerosol generator, Performance characteristics

INTRODUCTION

An important aspect of aerosol technology is the production of test aerosols with which to calibrate instruments used to simulate behaviour on inhalation. These test aerosols are usually monodisperse or practically so. A monodisperse aerosol is defined as one having a particle size distribution with a geometric standard deviation (GSD) less than 1.25 (Fuchs and Sutugin, 1966). The more widely used modern monodisperse aerosol generators are based on three mechanisms: spinning disc, vibrating orifice and controlled condensation. The spinning disc generators are based on the formation of uniform liquid filaments around the lip of a fast rotating disc. These filaments break up into a main drop at the outer end of the filament and into several smaller "satellite" droplets. Air circulation around the "satellite" droplets extracts them on the basis of their smaller inertia leaving only the main monodisperse drops. A large variety of materials can be made into aerosols by this method. Size control is achieved by controlling rotational speed, suspension or solution concentration and feed flow rate. Reproducibility is a problem with spinning disc generators and, often, an independent measurement of aerosol particle size may have to be carried out. Vibrating orifice generators eject a liquid filament under pressure from a calibrated orifice. Superimposition of an appropriate disturbance, like an ultrasonic field, breaks up the filament regularly, producing monodisperse droplets (Fulwyler and Raabe, 1970; Berglund and Lin, 1973; Tu, 1982). Particle coagulation, high particle electrostatic charges and particle size drift due to clogging of orifices are some problems associated with vibrating orifice generators. The condensation aerosol generators are all derived from the LaMer-Sinclair (Sinclair and LaMer, 1949) generator in which a uniform and constant mixture of condensation nuclei is produced and combined with vapours of the desired material in an inert gas. If the cooling is slow, under controlled conditions, each nucleus takes up, by condensation, a constant mass of condensate and uniform liquid or solid particles are formed (at room temperature). Examples of such aerosol generators are the Prodi generator (Prodi, 1972), the falling film generator (JJicolaon *et al.,* 1970), the Tu single stage generator (JJicolaon *et al.,* 1970), the Kogan-Burnasheva generator (Kogan and Burnasheva, 1960) and the Rapaport and Weinstock generator (Rapaport and Weinstock, 1955). A comprehensive investigation into aerosol formation by homogeneous and heterogeneous nucleation using a laminar continuous flow aerosol generator was presented by Nguyen and others (see Tu, 1982). A review of the condenser conditions for practical monodispersity of many examples of these aerosol generators using dimensionless heat and mass transfer groups was given by JJicolaon *et al.* (1970). The objective of this study was generation and characterization of solid, monodisperse aerosol particles suitable for calibrating devices used in deposition experiments.



Key: *1= CP nitrogen, 2 = collision generator, 3 = mist arrestor, 4 = diffusion drier, 5 = bypass line, 6 = bubbler line, 7 = bubbler, 8 = forced air circulation thermostat, 9 = reheater, 10 = heating tape*

Figure 1: MAGE a modified Sinclair-LaMer generator, with a forced air circulation thermostat and bypass - which allows rapid size adjustments during aerosol generation

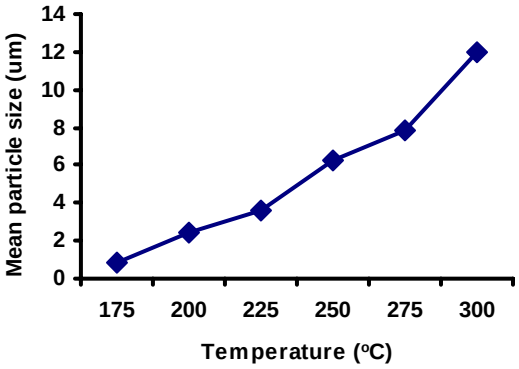


Figure 2: Effect of temperature on aerosol particle size

Monodisperse aerosol particles in the size range 1 - 12 μm, of high concentration output rates, are required for this purpose.

MATERIALS AND METHODS

Aerosol Generator: The MAGE is custom built equipment and its performance characteristics have not been fully ascertained. There is no published information on the factors affecting the performance of the MAGE outside the claims of the manufacturers. The successful generation of aerosols in our laboratory at King's College, London was the primary objective of this work. This success, after years of effort, necessitated the determination and validation of some of the important factors affecting the performance of the MAGE. MAGE is a modified Sinclair-LaMer Generator, with a forced air circulation thermostat and bypass - which allows rapid size adjustments.

Essentially, a dilute solution or colloidal suspension is atomized by pressurised compressed, forcibly recirculated air as a working fluid and a Proportional-Integral, Proportional-Derivative temperature control unit, with an overall stability within ± 0.5°C. At the outlet of the bubbler, the vapours condense on the nuclei, producing an aerosol with a narrow size distribution. A reheater, as the final stage, repeats the evaporation-condensation cycle producing better size distribution control. A by-pass around the bubbler allows the vapour flow rate to be decreased without affecting the nuclei flow rate; thus, at constant temperature, a fine and rapid size adjustment is possible, when the whole stream is again processed through the reheater.

The performance of the generator can be estimated by appropriate assessment of the ensuing aerosol particles. The system takes about 1 hour to warm up from a cold start, but if the oven is left on, the time taken for a useful, stable aerosol to be produced is about 30 minutes after the nebulizer is turned on. After equilibrium has taken place, diameter stabilisation takes less than 1 minute. The generator can be operated with only slight adjustments to give solid stearic acid particles for over 3 hours. The length of time MAGE generates aerosols is temperature-dependent. The higher the temperature setting, the shorter the performance time.

The following performance characteristics of the MAGE were established and validated: effect of coating material type on aerosol particle size, effect of purity of coating material on aerosol particle size, effect of aerosol particle size on Malvern

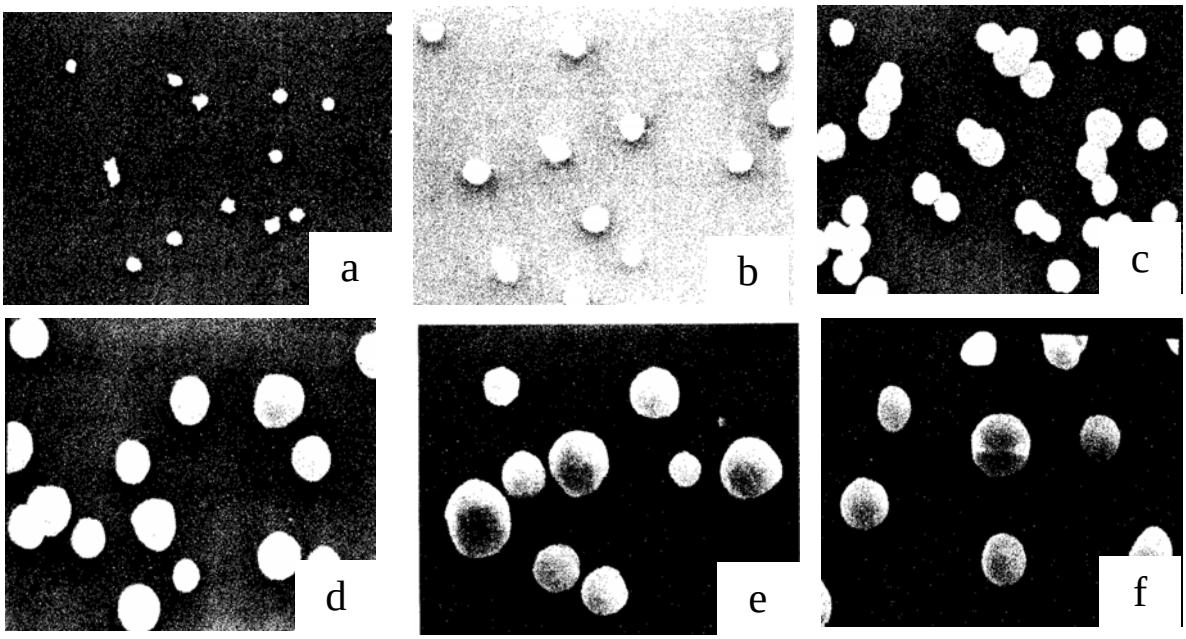


Figure 3: Scanning electron micrograph of stearic acid aerosols generated with MAGE showing the effect of temperature on aerosol particles size. Aerosol in the micrographs have the same magnification and were generated at a) 174, b) 200, c) 225, d) 250, e) 275 and f) 300 °C

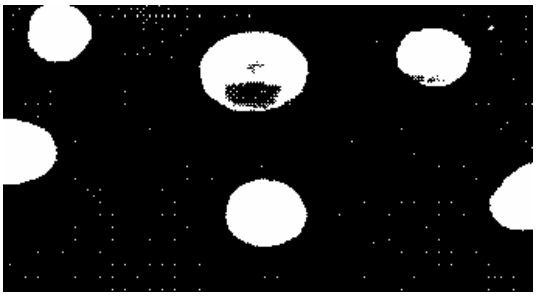


Figure 4: Scanning electron micrograph of stearic acid aerosols generated at 300 °C

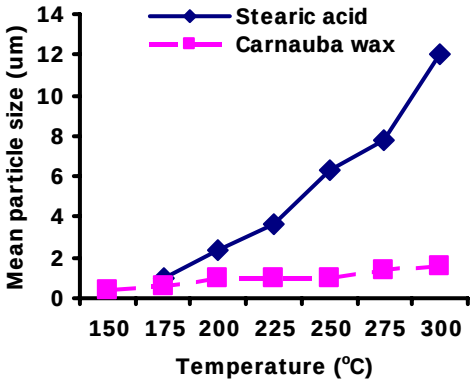


Figure 5: Effect of coating material type on aerosol particle size

analysis mode, reproducibility of the properties of aerosol particles generated, effect of carrier gas flow rate on aerosol properties, the stability of the aerosols generated from the MAGE, effect of by-pass flow control of the MAGE on aerosol properties and the performance characteristics of the MAGE over an extended period of time.

Condensate Materials: Common solid organic materials of great purity were investigated with the requirements of low melting points and low vapour diffusion coefficients. Two solid condensate materials were chosen, stearic acid and carnauba wax, with specific gravities of 0.847 and 0.997 respectively. After several studies showing excellent monodispersity, it became obvious that carnauba wax was unsuitable for use, because at the highest operating temperatures, a maximum particle diameter of only 2 μm was attainable. Stearic acid is readily available in very pure form and is non-toxic. It has a low thermal conductivity and needs to be chilled quickly in droplet form or the particles will grow into non-spherical crystalline structures. The air in the system was found to be adequate to solidify the particles into spheres, suitable for calibration experiments. When stearic acid aerosol particles were sampled onto microscope slides, the spheres began to crystallise into non-spherical particles after 12 hours or longer at room temperature. The aerosol particles should therefore always be used within 12 hours of generation and in these studies, they were used immediately.

Vapour-Nuclei Generation: The nebulizer of the MAGE conforms to the classic six-jet Collision nebulizer (British Standard 1984).

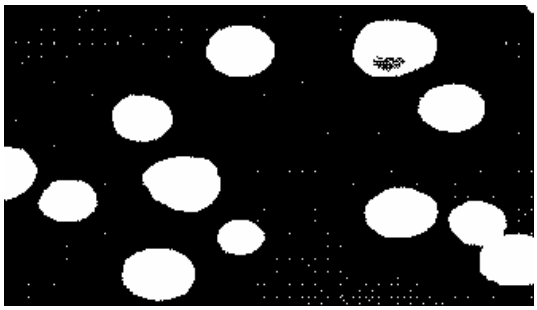


Figure 6: Scanning electron micrograph of stearic acid aerosols generated at 250 °C

Figure 7: Scanning electron micrograph of stearic acid aerosols generated at 300 °C

Figure 8: Scanning electron micrograph of stearic acid aerosols generated at 275 °C

Its glass and metal design allows it to withstand high temperatures. The nuclei are produced by atomization of a very dilute solution of NaCl in water by means of the collision atomizer, using pressurised compressed nitrogen. The droplets are dried and the condensation nuclei are sent to the bubbler filled with stearic acid (see Figure 1). The particles are produced at a high concentration, are practically neutral and start from sizes as low as 0.1 μm.

Aerosol Sizing Methods: The aerosols were measured by two different methods, using a commercial Malvern Series 2600 Particle Sizer (Malvern Instruments Limited, Malvern, Worcestershire, England) and scanning electron microscopy (SEM). No detailed comparison of these two methods of size measurement was made in these experiments and data reported are those obtained with the Malvern Series Particle Sizer. The aerosols were characterised by determining volume mean diameter with associated GSD. The Malvern Particle Sizer is capable of several modes of particle size analysis. The log-normal distribution has proved useful in many types of particle size analysis problems, including the sizing of aerosols. 'The log-normal distribution particle size analysis mode was used in this work. The particle size results given by the Malvern were accepted when obscuration was between 10 – 30 % and the log difference in the analysed set of data was below 5.

RESULTS AND DISCUSSION

Effect of MAGE Temperature on the Particle Size of Aerosols Generated: The manufacturer's claim is that the particle size of aerosols generated with the MAGE depends on the temperature of operation of the MAGE. The MAGE was therefore operated at different temperatures and the particle sizes of aerosols issuing from the equipment at these temperatures were characterized. The results obtained are shown in Figure 2. It can be seen that particle size of aerosols generated increased with increase in the temperature at which the MAGE was operated. The scanning electron micrographs (SEMs) of stearic acid aerosols generated at different temperatures are shown in Figures 3 and 4. It can be seen that the higher the temperature of operation of the MAGE, the larger the particle size achieved. The higher temperatures result in higher vapour concentrations at the outlet of the bubbler and a greater amount of material to each condensation nucleus. A size range 1 - 12 μm was achieved within the operational temperatures possible with the MAGE. This result is however specific for stearic acid.

Effect of Aerosol Material Type on Aerosol Particle Size: Two aerosol materials, carnauba wax and stearic acid were investigated for use in the calibration experiments. Figure 5 shows the particle size data obtained with carnauba wax and stearic acid. Carnauba wax gave excellent monodisperse aerosol particles but the particle size obtained at the highest MAGE operational temperature was inadequate. A maximum 2 μm particle diameter was obtained. The type of aerosol material was therefore shown to influence the resultant aerosol particle size. Several other materials are capable of being used in the generation of solid aerosol particles. Such materials include di-2-ethylhexyl sebacate (DBS) and triphenyl phosphate. The shape and surface characteristics of the aerosol particles were ascertained by scanning electron microscopy. Figures 6, 7 and 8 are the micrographs for stearic acid and carnauba wax microspheres, respectively.

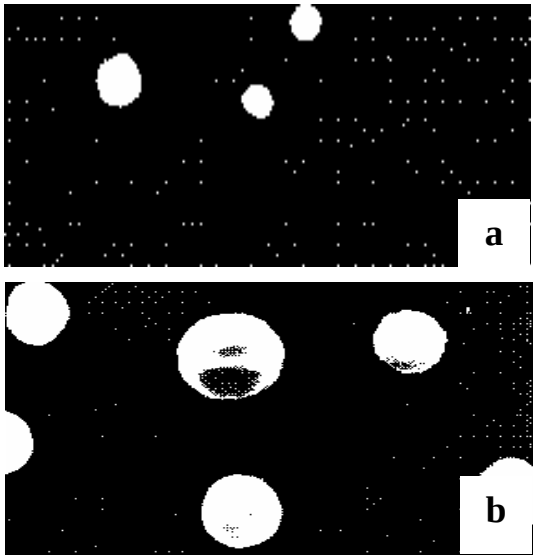


Figure 9: Effect of type of aerosol material on aerosol particle size. Scanning electron micrographs are for a, carnauba wax and b, stearic acid generated at 300 °C

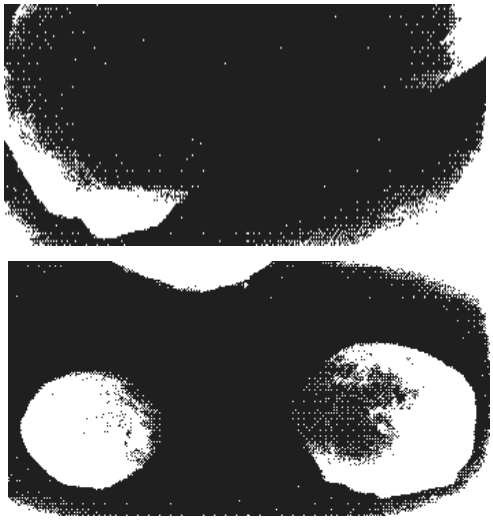


Figure 10: Effect of purity of coating materials on particle size of aerosols generated at 300 oC. Scanning electron micrographs at the top are for 99 % purity stearic acid and those below are for 90 % purity stearic acid

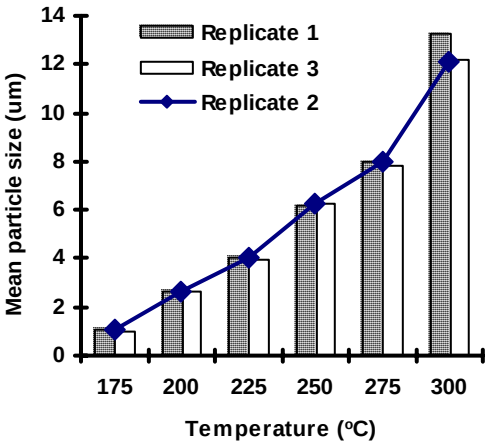


Figure 11: Reproducibility of the characteristics of aerosols generated

The particles are spherical and fairly ideal. Instruments used for the particle size analysis of aerosols encounter both ideal and non-ideal particles in practice. Both classes of aerosol particles are used in their calibration. It is important therefore to determine the aerodynamic properties of such particles prior to use.

Effect of Purity of Aerosol Material: The effect of purity of stearic acid on particle size was determined by selecting two different grades of 90 % and 99 % purity for study. There was little difference in the particle size range of the particles produced from the different grades of stearic acid. SEMs of aerosols produced from 90 % purity stearic acid at 300 °C are shown in Figure 9. SEMs of aerosols generated with 99 % purity stearic acid at 300 °C are shown in Figure 10. It is possible that impurities alter the vapour pressure of the stearic acid and induce the larger particle size of the aerosol particles at higher temperatures. It was thought desirable to use high purity grades of stearic acid, since crystallisation, which occurs on the storage of stearic acid particles, is likely to be less of a problem. The sample of stearic acid used in this work was therefore 99 % pure.

Reproducibility of Properties of Aerosol Particles Generated by MAGE: Particles were generated at the same temperatures on three separate occasions. Other variables, such as concentration of NaCl solution, by-pass control, sampling regimen and particle size analysis mode, were maintained constant. Figure 11 shows the results obtained and on the basis of which, it was concluded that the particle sizes of the generated aerosol particles were reproducible. The stability of the aerosol particles issuing from the MAGE was determined over a 3 hour period when the MAGE operational temperature was below 250 °C.

Particle size analyses were performed at 60 minutes intervals. Over the three hours of the experiment, the particle size remained constant. In calibration experiments, the generation of data from a single calibration point seldom lasts 1 hour or more; hence, it can be concluded that the reproducibility of the aerosols generated by MAGE is sufficient for the purposes of the experimentation.

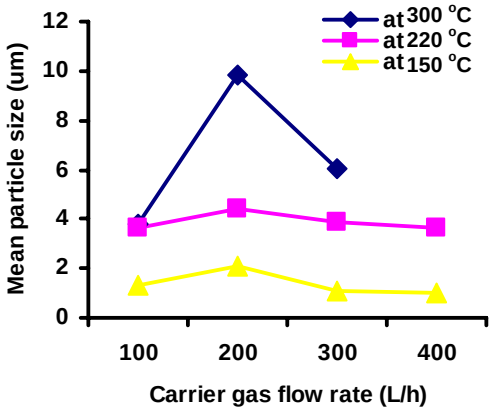


Figure 12: Effect of carrier gas flow rate on aerosol particle size

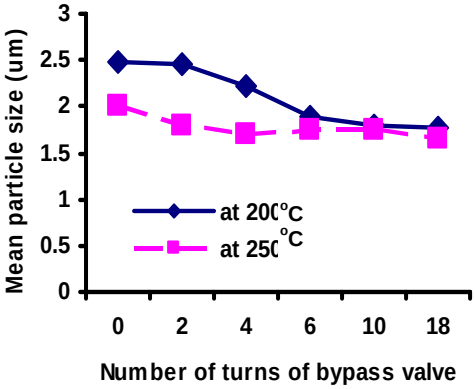


Figure 13: Effect of bypass flow control on aerosol particle size

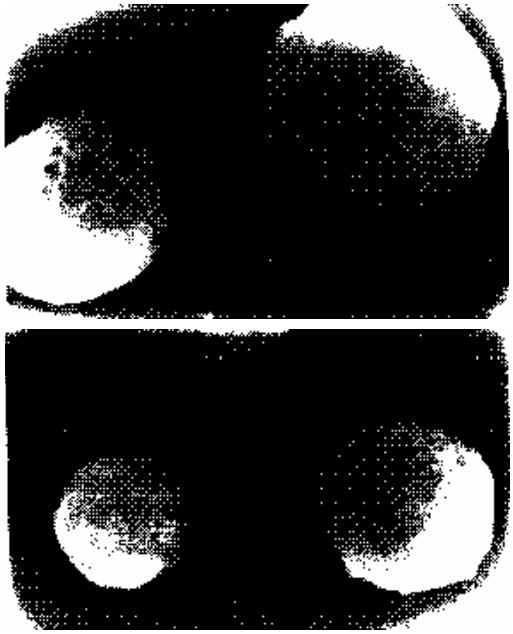


Figure 14: Typical scanning electron micrographs of stearic acid containing aerosols generated with the MAGE at 225 °C after storage for six hours on a glass slide at room temperature of 25 °C and 50 % relative humidity

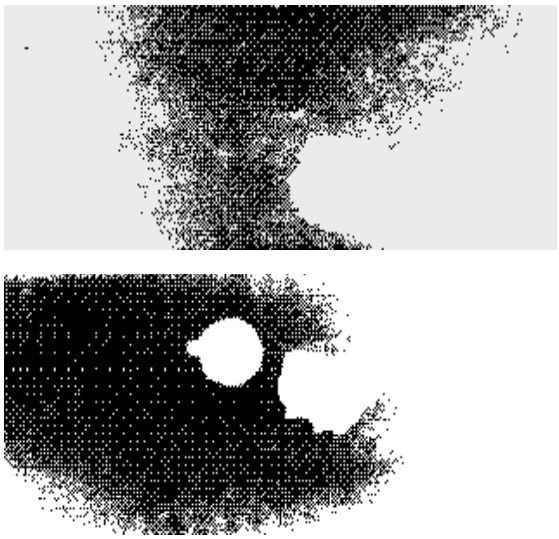


Figure 15: Typical scanning electronic micrographs showing the stability of stearic acid containing aerosols generated with the MAGE at 300 °C (above) and 174 °C (below). The aerosols were stored overnight for 12 hours on a glass slide at ambient conditions (room temperature of 25 °C and 50 % relative humidity)

Effect of Carrier Gas Flow Rate: The MAGE uses compressed nitrogen as the carrier gas. The carrier gas flow rate influences nuclei supply for condensation. Various carrier gas flow rates were used to determine their effect on aerosol particle size. In Figure 12 it can be seen that carrier gas flow rate affects particle size of aerosol generated. The carrier gas flow rate in the MAGE is accurately controlled. Any variation in the flow rate is related to temperature that can be digitally set. A by-pass flow allows a fine and prompt particle size decrease since the by-pass flow contributes to the condensation nuclei effectively decreasing the mass available to each nucleus. The effect of by-pass flow control is shown in Figure 13. At 0 setting, the by-pass flow is completely turned off. After 18 turns, the bypass flow is fully open. The effect of bypass flow control is more noticeable at higher temperatures and it is important that the flow be properly adjusted before sampling for particle size analysis. Any change from a fixed setting affects particle size thereafter.

Stability of Aerosols Generated with the MAGE: The stability of the aerosol generated with the MAGE was monitored overnight. Measurement involving calibration aerosols are usually made *in-situ* at the time of generation of the aerosols. There is need however to determine aerosol characteristics over a 24 hour period. Aerosol particles containing stearic acid were deposited on microscopic slides which were left to stand on the laboratory bench at ambient conditions (25 °C and 50 % RH) overnight. SEMs of

the storage aerosol particles were obtained after 6 and 12 hours. Figure 14 shows result obtained with stearic acid aerosol generated at 225 ^{o}C. It can be seen that the aerosol particles lost their smooth surface characteristics over the 6 – 12 hours storage period. Crystallization of the stearic acid occurred from the aerosol samples deposited on the glass slides. It is normal practice to measure the particle size of aerosols as they issue from aerosol generators and use them, during calibration experiments. The aerosols are generated, characterized and used immediately without the need for storage.

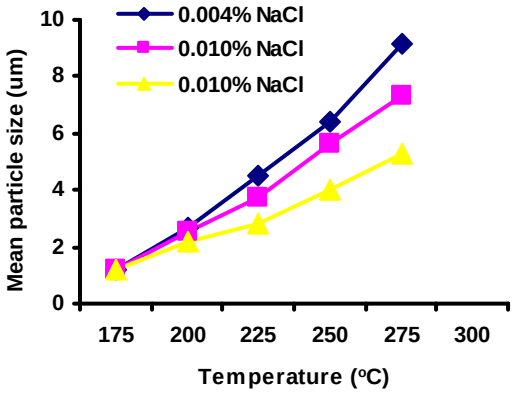


Figure 16: Effect of concentration of core material solution on aerosol size

Effect of Concentration of Core Material Solution an Aerosol Particle Size: The MAGE is a condensation aerosol generator which works by the generation of uniform and constant mixture of condensation nuclei that combine with vapours of the desired coating material (stearic acid or carnauba wax, in this study) in an inert gas atmosphere. It was desirable to assess the effect of concentration of core material solution on the particle size of aerosols generated with the MAGE since the MAGE is dedicated equipment and not much is published about the factors affecting its performance. Figure 15 shows the effect of concentration of core material solution on aerosols particle size. The concentration of core material solution does not have any effect on aerosol particle size at low temperatures (175 – 200 ^{0}C) (Figure 16). At higher temperatures (225 – 300 ^{0}C) differences are noticeable, but there is no consistent trend in these differences (Figure 16). The same concentration of core material solution was therefore used in the generation of aerosols used in our calibration studies.

Conclusion: The monodisperse aerosol generator, MAGE, a condensation aerosol generator, has been used to generate solid, monodisperse calibration aerosols of 1 - 12 μm particle size range. Stearic acid and carnauba wax were used to produce the aerosol particles which contained sodium chloride nuclei. Scanning electron microscopy was used to characterise the aerosol particles. A Malvern Series Particle Size Analyser was used for determination of aerosol size. The aerosol particles can be assayed for their NaCl content by a flame photometric method after extraction by ultrasonication or heating in aqueous solution for 2 minutes. The aerosol particles generated with the MAGE are currently being used in the calibration of the Andersen ICFM Ambient Sampler with pre-separator and other impactors.

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THE USE OF RECTAL TEMPERATURE FLUCTUATIONS IN THE STUDY OF CIRCADIAN RHYTHM IN THREE ADULT VERTEBRATE SPECIES IN AWKA, NIGERIA

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ABSTRACT

Biological clocks allow organisms to assess and respond to the oscillating environmental rhythms that result from the earth's movements via the generation of biological rhythms. Circadian clocks are also assumed to enhance survival and reproductive fitness in part by promoting optional timing of behaviour and physiology in relation to regular cycles in the environment. Temperature fluctuations were studied in three different vertebrate species – Sylvilagus floridanus, Rattus norvegicus and Columba livia in relation to the rhythmicity of rectal temperature. Clinical digital thermometer was used to record the core temperature by inserting it 2 – 3 cm deep from the anal sphincter before taking readings 3 minutes after. The highest recorded mean rectal temperature for R. norvegicus and S. floridanus were 38.85 ± 0.40 ^{0}C and 39.83 ± 0.32 ^{0}C respectively with the lowest being 36.58 ± 0.74 ^{0}C and 36.63 ± 0.18 ^{0}C respectively. C. livia failed to exhibit core temperature fluctuation. There were not significant differences in the mean rectal temperature for both sexes for S. floridanus in relation to time of day. Differences in the circadian temperature fluctuations were traced to variation among the animals, of preferred temperature arising from differential behavioural and physiological regulation, in relation to environmental cues. It is also possible that daily changes in illumination were secondary to C. livia. The results also suggest that circadian rhythmicity persists even in artificially imposed selective environment. Rectal temperatures are adequate for monitoring the biorhythms, with each species exhibiting endogenous peculiarities in the various circadian phases.

Keywords: Circadian temperature, Rectal temperature, Vertebrate species, Physiological regulation

INTRODUCTION

Circadian clocks are assumed to enhance survival and reproductive fitness in part by promoting optional timing of behaviour and physiology with respect to regular cycles in the environment. Unlike other sensory modalities, the clock does not respond to any form of energy from the environment. It measures the passage of time internally, and gives the non-directional physical time a direction ("arrow") by virtue of being in a living organism (Frazer, 1996). The clock is, thus, an internal representation of time, a cognitive temporal map analogous to the cognitive spatial maps. This allows the organism to perform internal time measurement, distinction between, before and after, measurement of duration of events, and learning of the local time of day (Shettleworth, 1998). The rising and setting of the sun provides a precise and reliable signal for regulating the phase of the circadian clock, and it is therefore not surprising that light is a powerful, if not dominant 'zengeber' (entraining stimulus) for most species. However, for many species, daily changes in illumination may be secondary to what is really important, namely, the activity patterns of other denizens of the greater social community, the potential mates, competitors, predators, prey and parasites with which they must share time and space. It may therefore by hypothesize that circadian clock in some, if not all species have evolved a capacity for phase resetting in response to significant interactions with other organism. There is ample evidence from field work to support the general view that animals do coordinate their behaviour in time with that of other animals, of the same or different species, but the mechanisms by which this is achieved are little studied (Frazer, 1996). Studies on circadian rhythmicity and metabolism in mammals and birds include those of Kramm (1975), Daan and Pittendrigh (1976),

Decoursey (1986, 1990), Pohl (1992) and Underwood *et al.* (1997) with specific studies on circadian rhythm of body temperature (CBT) including those of Gordon (1990), Refinetti and Menaker (1992), Krauchi (2002) and Kumar (2004).

In related studies, the data for domestic fowl indicate that the body temperature does fluctuate over a 24-hr period and in the brain the temperature fluctuations are maintained even under constant condition (Winget *et al.,* 1965; Aschoff *et al.,* 1973). Likewise mammals such as humans, hamsters tree shrews, and golden hamsters exhibit robust circadian temperature fluctuations under light and constant light conditions (Colin *et al.* 1968; Refinetti and Menaker, 1992). The present study tackles a similar problem but using rectal temperature of *Sylvilagus floridenus* (cotton tail/rabbits), *Rattus norvegicus* (albino rats) and *Columba livia* (homing pigeons) to study circadian temperature fluctuations over a 24 hours period in other to ascertain the trend in their fluctuation, in the species. The aim was to compare the fluctuation in the anal temperature and to ascertain any differences between species and consistency in relation to time of the day in the Nigerian environment.

MATERIALS AND METHODS

This work was carried out at Awka, Anambra State of Nigeria in month of June 2005. The experimental animals used in this study include, adult *Sylvilagus floridanus* which were purchased from Head bridge market Onitsha while *Rattus norvegicus* (also adults) which were also purchased from College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria. The adult *Columba livia* were purchased from Eke Awka Main Market, Awka, Anambra State. They were appropriately caged, fed and reared. Such pre-rearing was necessary for the acclimatization of the species before the initiation of taking of readings. Each cage was covered with gauze to prevent escape of specimens and to allow adequate ventilation for the animals.

Ten specimens each were used for the study with four replicates made. Equal number of males and females were used for *S. floridanus*, with only males used for *R. norvegicus* and *C. livia* and to allow ventilation for the animals.

Digital thermometer was used take the rectal temperature of the specimens hourly for twenty-four hour period. In taking the measurement the bulb of the clinical digital thermometer was inserted into the rectum to a depth of 2 – 3 cm from the anal margin or sphincter. The temperature was read from the thermometer 3 minutes after insertion. The readings were taken for two consecutive days after which further measurements and observations from the replicates were terminated to reduce any stress on the animals. The data obtained from the study were subjected to paired Students t-test, to test whether statistical differences exist between mean rectal temperatures of species; in addition the data was subjected to Levene's test for equality of variances (Levene, 1960).

RESULTS

In the collection of the data for the temperature fluctuation of the three vertebrate species studied which lasted for 24 hours period, on each day, the readings from the replicates were pulled together. The highest recorded mean rectal temperatures in degrees Celsius (^{o}C) for *R. norvegicus* and *S. floridanus* were 38.85 ± 0.40 ^{o}C and 39.9.83 ± 0.32 ^{o}C respectively while the lowest temperatures were 36.58 ± 0.74 ^{o}C and 36.63 ± 0.18 ^{o}C respectively. There was no temperature fluctuation for *C. livia* (Table 1).

Furthermore, the mean rectal temperatures for *R. norvegicus* and *S. floridanus* were 37.9 ± 0.06 ^{o}C and 39.11 ± 0.09 ^{o}C respectively (Table 2), were significantly different using t-test ($p \leq 0.05$). Day 1 mean temperature (38.35 ± 0.11 ^{o}C) and those of Day 2 (38.68 ± 0.11 ^{0}C) were significantly different ($p \leq 0.05$). In both cases the F values obtained from Levene's test for equality of variances were greater than 0.5 (Table 2). There were however no significant differences in the rectal temperature variation for both sexes of *S. floridanus* in relation to time of day from the Analysis of Variance (ANOVA) carried out (F ratio = 6.00).

The mean rectal temperature of the male *R. norvegicus* more or less exhibited regularity in fluctuation patterns in relation to time of day but for a slight drop in temperature of 1 ^{o}C at 20.00 hours on Day 2 (Figure 1). The pattern of fluctuation of mean rectal temperature for *S. floridanus* for the 24 hours period was more consistent except for the sharp slight rise for Day 1 and a simultaneous drop (in each case 2 ^{0}C) at 6.00 hours on both days (Figure 2). No temperature fluctuation was however recorded for the mean rectal temperature of male *C. livia* during the 24 hours periods of study, with the mean and temperature maintained at 42 ^{0}C.

DISCUSSION

The results of the study show that biological clocks or circadian clocks are endogenous, inherited timing devices which control rhythms of many physiological

Table 1: Mean rectal temperature fluctuation over a 24 hour period in adult *Sylvilagus floridanus, Rattus norvegicus and Calumba livia*

Time of the Day	*Rattus* Day I	*norvegicus* Day II	*Sylvilagus* Day I	*floridamus* Day II	*Columba* Day I	*livia* Day II
01.00	38.38 ± 0.20	38.3 ± 0.20	33.63 ± 0.18	39.45 ± 0.07	42.00	42.00
02.00	37.6 ± 0.46	38.58 ± 0.28	38.03 ± 0.75	39.48 ± 0.13	42.00	42.00
03.00	37.38 ± 0.30	38.60 ± 0.48	37.08 ± 0.18	39.35 ± 0.14	42.00	42.00
04.00	37.3 ± 0.56	37.65 ± 0.40	38.65 ± 0.48	39.98 ± 0.31	42.00	42.00
05.00	37.60 ± 0.12	38.63 ± 0.20	38.70 ± 0.29	39.03 ± 0.40	42.00	42.00
06.00	36.58 ± 0.74	38.60 ± 0.35	38.30 ± 0.46	39.03 ± 0.38	42.00	42.00
07.00	37.08 ± 0.57	38.0 ± 0.28	38.10 ± 0.69	38.93 ± 0.28	42.00	42.00
08.00	37.63 ± 0.26	37.85 ± 0.14	38.15 ± 0.56	39.00 ± 0.37	42.00	42.00
09.00	37.63 ± 0.25	37.80 ± 0.26	38.68 ± 0.42	39.53 ± 0.36	42.00	42.00
10.00	37.63 ± 0.14	37.55 ± 0.05	38.95 ± 0.16	39.53 ± 0.03	42.00	42.00
11.00	38.00 ± 0.15	37.88 ± 0.36	39.05 ± 0.16	39.53 ± 0.18	42.00	42.00
12.00	37.73 ± 0.10	37.90 ± 0.22	39.05 ± 0.26	39.83 ± 0.32	42.00	42.00
13.00	37.68 ± 0.24	37.70 ± 0.04	38.93 ± 0.31	39.70 ± 0.09	42.00	42.00
14.00	37.73 ± 0.14	37.55 ± 0.20	39.02 ± 0.08	39.73 ± 0.31	42.00	42.00
15.00	38.20 ± 0.23	37.93 ± 0.83	38.25 ± 0.20	39.65 ± 0.17	42.00	42.00
16.00	38.23 ± 0.43	38.08 ± 0.41	39.39 ± 0.18	39.63 ± 0.18	42.00	42.00
17.00	38.48 ± 0.44	38.00 ± 0.38	38.38 ± 0.23	39.53 ± 0.13	42.00	42.00
18.00	37.80 ± 0.44	37.73 ± 0.34	39.40 ± 0.10	39.50 ± 0.17	42.00	42.00
19.00	38.30 ± 0.12	37.83 ± 0.16	39.40 ± 0.16	39.30 ± 0.10	42.00	42.00
20.00	38.10 ± 0.15	38.20 ± 0.05	39.48 ± 0.23	38.50 ± 0.43	42.00	42.00
21.00	38.05 ± 0.28	38.05 ± 0.31	39.48 ± 0.20	39.53 ± 0.37	42.00	42.00
22.00	38.35 ± 0.19	38.15 ± 0.28	39.58 ± 0.20	39.40 ± 0.15	42.00	42.00
23.00	38.53 ± 0.06	38.85 ± 0.40	39.45 ± 0.16	39.33 ± 0.13	42.00	42.00
24.00	38.45 ± 0.10	38.48 ± 0.08	39.40 ± 0.11	39.38 ± 0.22	42.00	42.00

Mean temperatures (^{0}C) obtained in Day I and II

Table 2: Variation in mean rectal temperatures of adult *Rattus norvegicus* and *Sylvilagus floridanus* for each for Day 1 and Day 2, over 24 hour period

Parameter	Mean	Probability values for associated t-test		F values for Levene's test for equality of Variances
		Unequal S^2	Equal S^2	
Rattus norvegicus	37.93 ± 0.06	11.019* (p = 0.000)	11.019* (p=0.000)	2.482 $^{n.s}$ (p=0.119)
Sylvilagus floridanus	39.11 ± 0.09			
Day one mean	38.35 ± 0.11			
Day two mean	38.68 ± 0.11	2.09* (p=0.039)	2.094* (p=0.039)	0.516$^{n.s}$ (p=0.474)

Note: *S = variance, S.E. standard error of mean; ns = not significant, * = significant at 0.05 probability level*

and behavioural functions (Decoursey *et al.,* 2000). These results are in line with the observations of Bligh and Johnson, 1973 who recorded difference among animals of preferred body temperature arising from variability in their behavioural and physiological regulation, and in relation to environmental cues. This no doubt is reflected in the differences in the temperature regimes obtained for the three vertebrate species with at those of *C. livia* being highest followed by *S. floridanus* and then *R. norvegius* (Table 1).

The statistical differences obtained in the comparison of the mean rectal temperatures of *R norvegicus* and those of *S floridanus*, (Table 2) is also a reflection of the differences in the preferred temperature of these species (Bligh and Johnson 1973), and supported by the existence of nonhomogeneity of variance implicit in Levene's (1960) test carried out (Table 1).

Temperature rhythm was exhibited by the three vertebrates species studied with each rhythm exhibiting peculiarities, with the circadian temperature fluctuation of the *S. floridanus* being sharper than that of *R. norvegicus* (Figures 1 and 2). These observations are in line with those of Refinetti and Menaker who reported robust circadian temperature fluctuations, even under light and light conditions. For the studied vertebrate species. Nuesslein-Hildeshelm *et al.* (1995) also observed even among juveniles a high level of core temperature for rabbits and pronounced core temperature rhythm in several strains of rats. There is also evidence from the study of temperature rhythm of *S. floridanus* that when behaviours are

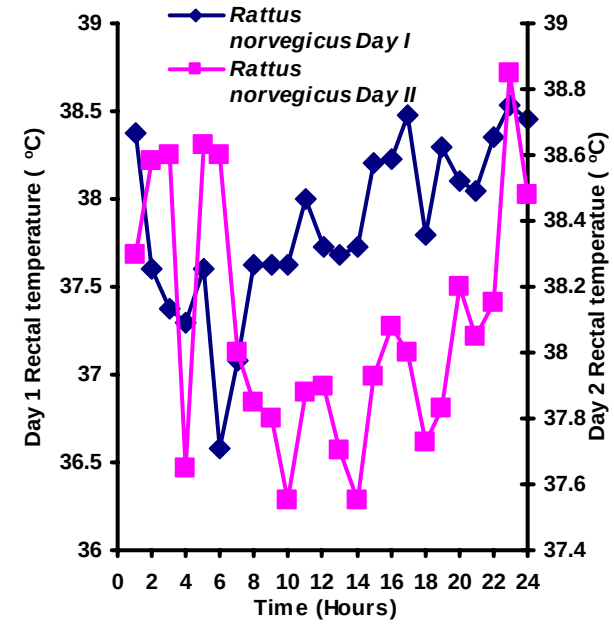

Figure 1: Rectal temperature fluctuation for days 1 and 2 over a 24 hour period in adult *Rattus norvegicus*

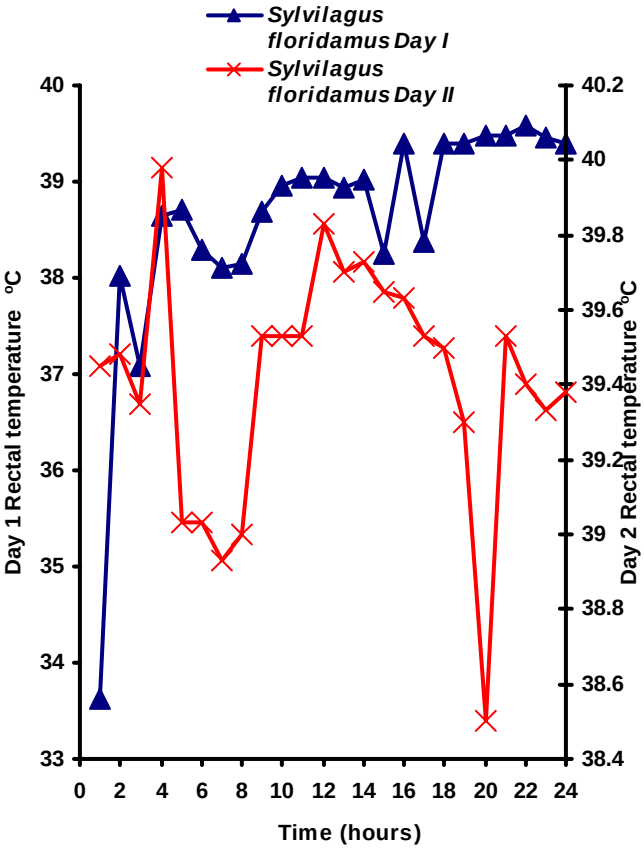

Figure 2: Rectal temperature fluctuation for days 1 and 2 over a 24 hour period in adult *Sylvilagus floridanus*

elicited outside the usual circadian phase of activity, the stimulus is considered to mask true circadian phase (Figure 1) (Aschoff, 1998). The slight drops in the core body temperature of the rats might be attributed to physiological thermoregulatory systems which have been implicated in infant rats (Sullivan *et al.,* 1988) as well as in these adult rats. It is also possible that the constancy of rectal temperature fluctuation observed for *C. livia* is peculiar to the species. Evidently it is possible that daily changes in illumination were secondary to these bird species (Decoursey, 1990) with these birds moderating to unique response to dusk and day in contrast to the other animal species. Earlier results on the domestic fowl by Aschoff *et al.* (1973) are also in line with these observations, since they reported that body temperature failed to fluctuate over a 24 hour period with the result that brain and body temperature fluctuations were maintained under constant conditions.

In the long run evidence abound from the study that circadian rhythmicity does not disappear even in artificially imposed selective environment as obtained from similar studies on animal groups other than vertebrates (Sheeba *et al.*, 1999). It can also be concluded that rectal temperatures are adequate for monitoring biorhythms, with each species showing uniformity in their response to environmental cues. There is also evidence that even though these clocks are organic structures, and not absolutely precise, they have also evolved as means by which external timing cues like dawn and dusk can entrain the phase of the clock to the local time (Decoursey, 1990).

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