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LENGTH-WEIGHT RELATIONSHIPS AND FOOD AND FEEDING HABITS OF SOME CHARACIDS (OSTEICHTHYES: CHARACIDAE) FROM ANAMBRA RIVER, NIGERIA

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ABSTRACT

The length-weight relationship of the commonly caught species of characids; Brycinus leuciscus, Hydrocynus vittatus, Alestes baremoze, and Brycinus macrolepidotus is provided in this study along with their percentage relative frequency of food groups. There were positive estimates for coefficient of correlation (r^2) of length-weight relationship whereas the condition factor (K) values depict overall poor being of these species. The lower condition factor (K) range (0.693 – 1.688) was suggestive of poor ecological conditions due to heavy anthropogenic influence on the River. The feed spectrum showed that the four species consumed oligochaetes, detritus, rice bran, adult insects, larvae of insect, groundnut, maize, beans, bambara nut and cassava tuber.

Keywords: Characidae, Anambra River, Tropics, Food reserve, Ecosystem

INTRODUCTION

The members of the family Characidae are only found in African and South American continents, which once existed as parts of a single continental mass termed Gondwana. They are widely consumed as they form important food resource for human communities living along the banks of broad tropical rivers (Orti and Meyer, 1997). The Nigerian people with a population of over 120 million are perhaps the largest consumers of characid species in Africa and frequently caught characids in Nigeria (Echi, 2005). The Characidae is one of the dominant fish groups in African freshwater rivers (Ikomi and Sikoki, 2001). Some attempts have been made towards providing information on general biology of the characids in Nigeria (Ikomi and Sikoki, 2001; Echi and Ezenwaji, 2010). Also, the condition factor of the commonly studied characid *Brycinus nurse* has been observed by (Saliu, 2001).

However, comparatively most studies and information on the aspects of biology of characids is relatively concentrated and even ongoing in Latin American region much more than in African region due to so many obvious reasons. For instance, Marco (2010) chronologically appraised some of the records offered in the literature on fecundity aspect of biology in species of Characidae, which typically emphasizes high level of studies of Characidae in neotropical region.

Nevertheless, as part of imbued efforts to improve data deficient situation in Africa and that the information concerning the length-weight relationship of the commonly caught species of characids at Otuocha sampling port, Anambra River is limited. Therefore, the present effort was to provide length-weight relationships information

among the commonly caught specie of characids to bridge the present fissure in information.

Information about length-weight relationship gives insight about state of fish as vital food resource (Rawat *et al.*, 2014). Also, high and low condition factor K for instance, is an expression of overall well being and poor state of the fish respectively (Gupta and Gupta, 2013).

It is understood that fish sampling courses and analyses of fisheries data cannot be complete without comprehensive Length-weight relationship data generation, which is essential set standard in fisheries (Morato *et al.*, 2001; Mendes *et al.*, 2004).

In aquatic science, heavy nature of fish as translated in general suitability of certain length possessed by fish samples relation to living and non living factors influence on fish life and their aquatic ecosystem (Bagenal, 1978; Anene, 2005).

MATERIALS AND METHODS

Fresh samples of commonly caught characids from fishers at Otuocha sampling port in Anambra River Basin, Nigeria were identified according to details in (Leveque *et al.*, 1990; Olaosebikan and Raji, 1998). Based on monthly ecological survey (August 2004 - July 2005) they were measured to obtain each sample's total length (TL), (SL) and body weight (W) to the nearest 0.1 cm and 0.01 g using meter rule and a top loading Mettler balance respectively. Then length-weight relationship using the formula $W = aL^b$, where W is total body weight (g), L the total length (cm), a and b are the coefficients of the functional regression between W and L according the details in Ricker (1973). The 95% confidence interval, CI of b was computed using the equation; $CI = b \pm (1.96 \times SE)$. To confirm whether b values obtained in the linear regressions were significantly different from the isometric value of $\pm 95\%$ CI of b at $\alpha = 0.05$, t-test was applied as expressed by the equation according to Sokal and Rohlf (1987); $t_s = (b-3) / SE$, where t_s is the t-test value, b the slope and SE the standard error of the slope (b). All the statistical analyses were considered at significance level of 5% ($p < 0.05$).

The Fulton's condition factor (Ricker 1975) was calculated using the formula: $k = (W/L^3)100$, where k is the Fulton's condition factor, W is the weight of fish (grams), and L the total length of fish (centimeters). The visceral was opened; the stomach was sectioned into in a Petri dish containing normal saline. Then the stomach of each sample was further cut longitudinally to expose the partly digested food contents (Echi, 2005).

RESULTS

The analyses involving a total 996 samples of four commonly caught characid species', number of specimens, length-weight relationship parameters a and b, 95% confidence interval for b, correlation coefficient (r), condition factor, mean length, mean weight and growth type (negative allometric type) are presented.

The sample size for the fish species varied from 63 *H. vittatus* (female) to 194 *B. leuciscus* (male) whereas the value of b ranged from 1.997 in *B. leuciscus* (Female) to 3.221 in *B. macrolepidotus* (Female). The lowest condition factor (K) (0.693) was recorded in *A. baremoze* (Female) whereas the highest value (1.688) was observed in *B. leuciscus* (male). The values of correlation coefficient (r^2) varied from 0.256 in *B. leuciscus* (Female) to 0.783 in *A. baremoze*. Except *H. vittatus* (male) 0.384, *B. leuciscus* (male) 0.386 and *B. leuciscus* (Female) 0.256 had correlation coefficient (r^2) < 0.5 whereas others had correlation coefficient (r^2) ≥ 0.5 . The t-test showed that all the fish species had negative allometric growth (Table 1).

The River is typically, seasonally flooded during wet seasons with the experience of volume contraction during dry season months. This is a means of incorporating various organic matters into it. The influx of organic materials into the River plays role in the feeding pattern of these characids.

For instance, percentage relative frequency of the food groups consumed by the characids indicated that the following items form their feed: oligochaetes, detritus, rice bran, rice, adult insect, larvae of insect, groundnut, maize, beans, bambara nut, fry, cassava tuber and wheat.

Table 1: Length-weight relationship of the commonly caught characids in Anambra River, Nigeria

Species	N	a	b	95% CI for b	r ²	K	Mean L (cm)	Mean W (g)	Growth Type
<i>Alestes baremoze</i> (Female)	133	-2.778	2.254	2.011- 2.496	0.72	0.693	20.2225	8.955	Negative allometric
<i>Alestes baremoze</i> (Male)	176	-2.583	2.199	2.026 - 2.372	0.783	0.748	19.803	59.542	Negative allometric
<i>Brycinus leuciscus</i> (Female)	109	-0.772	1.505	1.013 - 1.997	0.256	1.648	9.548	14.198	Negative allometric
<i>Brycinus leuciscus</i> (Male)	194	-1.902	2.009	1.648 - 2.370	0.386	1.688	9.475	14.181	Negative allometric
<i>Brycinus macrolepidotus</i> (Female)	113	-3.829	2.734	2.246 - 3.221	0.527	1.053	19.845	87.535	Negative allometric
<i>Brycinus macrolepidotus</i> (Male)	105	-2.662	2.309	1.882 - 2.736	0.527	0.958	19.414	71.068	Negative allometric
<i>Hydrocynus vittatus</i> (Female)	63	-1.696	1.973	1.541- 2.404	0.578	0.971	19.039	66.942	Negative allometric
<i>Hydrocynus vittatus</i> (Male)	103	-0.832	1.654	1.241- 2.067	0.384	0.958	18.743	60.621	Negative allometric

N = Sample size; *a* and *b* = regression coefficient; *CI* = confidence interval; *r*² = correlation coefficient; *K* = condition factor; *L* = total length; *W* = weight

Table 2: Percentage relative frequency of food groups in the commonly caught characids in Anambra River, Nigeria

Food group	<i>Alestes baremoze</i>	<i>Hydrocynus vittatus</i>	<i>Brycinus leuciscus</i>	<i>Brycinus macrolepidotus</i>
Oligochaetes	19.6	13.7	13.3	15.7
Detritus	10.9	7.8	26.7	13.7
Rice bran	17.4	3.9	13.3	5.9
Rice	-	2	2.2	-
Adult insects	13	19.6	-	9.8
Insect larvae	10.9	23.5	-	9.8
Groundnut	10.9	3.9	11.1	11.8
Maize	6.5	2	2.2	3.9
Beans	-	3.9	15.6	15.7
Bambara nut	6.5	2	6.7	5.9
Fry	-	13.7	-	-
Cassava tuber	4.3	3.9	8.9	3.9
Wheat	-	-	-	3.9

The feed spectrum showed that the four species consumed oligochaetes, detritus, rice bran, adult insects, larvae of insect, groundnut, maize, beans, bambara nut and cassava tuber. From the study, only *H. vittatus* consumed fry (13.7%), *B. macrolepidotus* consumed wheat (3.9%), *A. baremoze* did not consumed beans whereas the others consumed beans, *H. vittatus* and *B. leuciscus* consumed rice (2%), (2.2 %) respectively.

Also, larvae of insects and adult insects were consumed by the three other species except *B. leuciscus* (Table 2).

Conclusion: Although, some contribution towards providing data on length-weight relationship of some Nigeria fish in the various aquatic ecosystems have been provided by some authors (Bakare, 1970; Saliu, 2001; Fafioye and Oluajo, 2005;

Agboola and Anetekhai, 2008), thitherto the present study on the commonly caught characids at Otuocha, Anambra River was lacking.

In Nigeria, members of the characids comprise 7 (Seven) genera and 19 (Nineteen) species (Olasebikan and Raji, 1998). This study therefore provides information on the length-weight relationships of the commonly caught characids as plumb line information to study other characid species as individual species occur differentially, relatively or predominantly in different aquatic ecosystems. Also, the differential feed material/s directly or indirectly determines the type of parasitic organisms that associate with them (Echi, 2005). For instance, comparatively, the occurrence of helminthes parasites in *B. macrolepidotus*, *H. vittatus* and *A. baremoze*, as well as *Myxobolus* sp. was high whereas the other parasites in *B. leuciscus* and *B. macrolepidotus* has much lower values (Echi and Ezenwaji, 2010). The condition factors (K) range (0.693 – 1.688) was outside the range (2.9 – 4.8) recommended as suitable for matured freshwater fish by Bagenal and Tesch (1978). This could have been caused by adverse environmental factors (Anene, 2005). Nevertheless, the active anthropogenic influence on the ecological parameters of the River from its bank should be a concern. For instance, complex human activities at the bank have increased influx of mainly organic materials example food materials, human excreta etc into the River and this keeps the pH range (5.5 – 7.0) at fairly constant (Echi and Ezenwaji, 2010). In such aquatic ecosystems the pH range is an indication of predominating high carbonic acid content. Also, this is characteristic of water body that is heavily infested with heavy organic materials from its surroundings resulting in low alkalinity value/s. The carbonic acid formed after dissolution of carbon dioxide gets dissociated into Bicarbonate (HCO_3^-) and Carbonate (CO_3^{2-}) ions (Gupta and Gupta, 2013).

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HAEMATOLOGICAL INDICES OF MALARIA INFECTED RESIDENTS OF ISU COMMUNITY, ONICHA LOCAL GOVERNMENT AREA, EBONYI STATE, NIGERIA

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ABSTRACT

Malaria as a major mosquito-borne public health problem is likely to initiate changes in haematological parameters of its sufferers. This study investigated the changes in haematological indices of malaria infected residents of Isu community in Onicha Local Government Area of Ebonyi State. A two-stage sampling design was adopted in which selection of villages constituted the first stage/Primary Sampling Units (PSUs) where three (3) villages (Isuachara, Agbabor and Mgbala-ukwu) out of the seven villages were selected using simple random sampling. In the second stage, a simple random sample of 240 individuals was taken from the three villages using 95% confidence level and a margin of error of 6.32% with a standard deviation of 0.5. Thick blood smears of venous blood stained with Giemsa were examined microscopically for malaria parasitaemia (MP) and its intensity. Those negative for malaria parasite served as controls. Haematological indices (packed cell volume (PCV)), total leucocyte counts (TLC) and white blood cell differentials of malaria positive and negative individuals were determined using standard procedures. Packed cell volume and monocytes of malaria infected individuals were higher and differed significantly from those of uninfected individuals ($p < 0.05$). Correlation analysis showed significant association between the total leucocyte count, packed cell volume and eosinophil count and intensity of malaria parasites. From the results of this study, intensity of malaria parasite altered the values of haematological indices of the sufferers. It was therefore recommended that the diagnosis of malaria and changes in haematological parameters of patients should go hand in hand in our health institutions for effective management and control of the infection.

Keywords: Malaria, Haematological, Indices, Parasites, Infected, Residents

INTRODUCTION

Malaria is an important infectious protozoan disease and despite intensive worldwide efforts to reduce its transmission, it still remains the

most serious infection of humans. It can also be defined as a typical blood disease that is characterized by fever, anaemia and splenomegaly. It poses a threat to public health with 80 – 90 % of morbidity and mortality

occurring in Africa and affecting both young and old (Ogbodo *et al.*, 2010). According to WHO (2000) about 500 million people are affected by malaria at any time and approximately 2 million of them mostly children die each year (Onyesome and Onyemakonor, 2011).

The global malaria situation continues to show no real improvement. Downward trends in the number of reported cases are maintained in some countries but are counter balanced by increasing trends in others. Although accurate figures are difficult to come by, it is estimated that in Africa alone malaria is responsible for one million death of infants and young children each year (Angyo *et al.*, 1996). Another risk group in endemic areas are pregnant women who become susceptible to severe infection due to diminished cellular and humoral immunity during pregnancy (Okwa, 2003). With regards to morbidity, people in areas of high endemicity usually go through several attacks every year with each attack lasting about 5 to 15 days and often incapacitating the victim.

The four species of the parasite that infect man are *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *Plasmodium falciparum* and *P. vivax* are the most common in the tropics but mixed infection with two or more of the *Plasmodium* species is common. Severe falciparum malaria remains an important cause of mortality in the tropical world with an annual mortality of 1 – 2.7 million people and a mortality rate as high as 15 – 30 % despite effective anti-malarial treatment (WHO, 1990).

The developing trophozoite of malaria parasite depends upon the host for its nutritional requirements. It follows therefore that the development of the parasite must in part depend on successful competition with the host for certain substances required equally by both. Pathology with all malaria species is related to the rupture of infected erythrocytes and the release of parasite materials and metabolites, hemozoin and cellular debris. As parasites of the blood for the majority of their complex life cycle, it is expected that they may induce haematological alterations in humans. Abnormalities such as anaemia, thrombocytopenia splenomegaly, neutropenia, eosinophilia, neutrophilia and monocytosis have

therefore been recorded (Layla *et al.*, 2002, Chandra and Chandra, 2013). Such complications vary with the level of malaria parasite intensity, presence of haemoglobinopathies, nutritional status, demographic factors and level of malaria immunity (Erhart *et al.*, 2004).

Although some studies have been done on the haematological indices of malaria infected individuals in Nigeria, non has been documented Isu community. The aim of this study therefore was to investigate changes in haematologic indices of malaria infected residents of the Isu community.

MATERIALS AND METHODS

Study Area: The study was a community based survey conducted at Isu Community in Onicha Local Government Area of Ebonyi State, South-eastern Nigeria. The villages that make up the community include Agbabor, Isuachara, Mgbaleze, Amanator, Uminiko, Mgbala Ukwu and Obeagu (Figure 1).

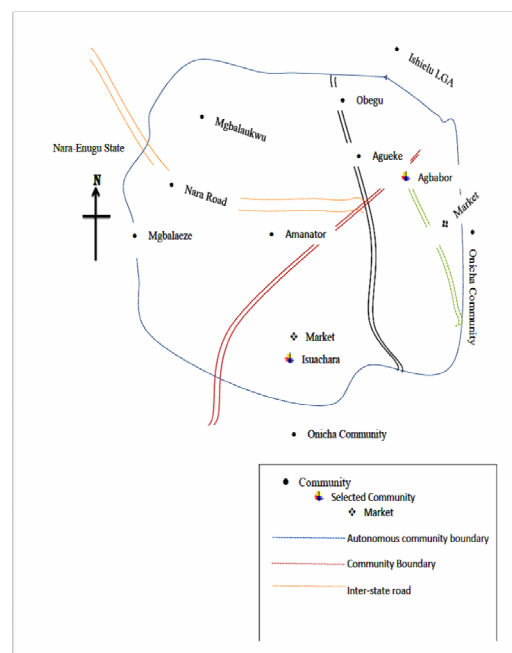


Figure 1: Map of Isu showing the selected villages in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

It is bounded to the West by Nkanu in Enugu State, to the South by Ohaozara, to the North by Ishielu and to the East by Ezza North Local Government Areas of Ebonyi State.

The study area is defined by longitude 8°6' 6" E and latitude 6° 22' 28" N. The vegetation is characteristic of derived savannah with high rainfall intensity, high run-off volumes and high relative humidity and an average rainfall of about 1600mm-2000mm per annum.

The mean daily maximum and minimum temperatures are 32°C and 25°C respectively. The residents are prominently farmers but also engage in trading and crafts as well as public and civil services. A government owned General Hospital is the largest health institution located in the area. There are also some comprehensive health centres that operate under the supervision of medical doctors.

Ethical Consideration: Ethical approval was obtained from the Ethical Committee of the Federal Medical Centre Abakaliki, Ebonyi State. Informed consent of the village heads of the villages and those of the subjects involved in the research were sought and permission granted before the commencement of the study.

Sampling: From seven villages that make up Isu community, a multi-stage sampling technique was used to select three villages. A two-stage sampling design was adopted in which selection of villages constituted the first stage/Primary Sampling Units (PSUs) where three villages (Isuachara, Agbabor and Mgbalakuwu) out of the seven villages were selected using simple random sampling. In the second stage, a simple random sample of 240 individuals was taken from the three villages using 95% confidence level and a margin of error of 6.32% with a standard deviation of 0.5.

Thick smears of venous blood obtained from the 240 individuals were stained with Giemsa and examined microscopically for malaria parasitaemia (MP) and intensity using x100 objectives with oil immersion. Parasitaemia was quantified in thick films by counting parasites against white blood cells (Cheesbrough, 1999) while the intensity of parasitaemia was measured per high power field or microscopic field. Up to 5 – 10 high power fields were examined before intensity was confirmed and the number of parasites per field noted per sample. Level of parasitaemia was in

microliter of blood and expressed as scanty, mild and severe (+, ++ and +++) (Cheesbrough, 2005).

Determination of Haematological Indices

Total leucocyte count (TLC): 0.02 ml of anticoagulated blood and 0.38ml Turk's solution (diluent) were pipetted into a test tube and mixed properly. Then 0.02 ml of the mixed solution was pipetted into an already charged Neubauer machine and allowed to stand for 2-5 minutes. The set up was examined microscopically using x 10 light microscope.

Leucocyte differential count: Thin film of the blood specimen was prepared and allowed to air-dry. This was Giemsa stained and viewed microscopically using x100 oil immersion as described by Cheesbrough, (1998).

Packed cell volume: A special capillary tube was filled with well mixed anticoagulated blood up to 2/3 of its length. The end of the tube was then sealed with plasticine. The filled capillary tube was placed in the grooves of the haematocrit centrifuge head with the sealed end placed away from the centre of the centrifuge. The centrifuge was covered by screwing up the lid adequately and spun for five minutes at 12,000 rpm. The haematocrit tubes were removed as soon as the centrifuge stopped spinning, placed on the haematocrit reader and read. The PCV of each individual was then calculated in percentage thus: $PCV (\%) = \frac{\text{Length of red cell column (mm)} \times 100}{\text{Length of total column (mm)}}$.

Statistical Analysis: Data were entered into a computer and analyzed using SPSS version 20.0 for windows (SPSS Inc. Chicago, IL: USA). Differences and proportions were tested by Chi-square tests and student's t tests for trend or independence as appropriate. Multiple logistic regression analysis was used to show whether there was significant correlation between malaria infection and haematological indices. A probability value of < 0.05 was taken as significant.

Table 1: Haematological parameters of malaria positive and negative individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

Haematological Parameters	Malaria		P value	r value
	Infected individuals (n = 90)	Uninfected individuals (n =150)		
Packed cell volume (%)	39.41 ± 4.62	41.27 ± 3.65	0.001*	0.102
Total leucocytes count (x10 ⁹)/L	5.86±1.38	5.31 ± 0.66	0.001*	0.563
Neutrophils (%)	40.69 ± 9.98	38.15 ± 10.53	0.061 ^{ns}	0.176
Lymphocytes (%)	52.81±12.98	53.41 ± 9.03	0.676 ^{ns}	0.147
Eosinophils (%)	4.40±3.18	2.19 ± 2.70	0.0001*	0.114
Monocytes (%)	3.32±1.84	4.12 ± 2.46	0.008*	0.023
Basophils (%)	0.067±0.03	0.026 ± 0.013	0.209 ^{ns}	0.219

* Significant difference at $p < 0.05$, ns = no significant difference ($p > 0.05$)

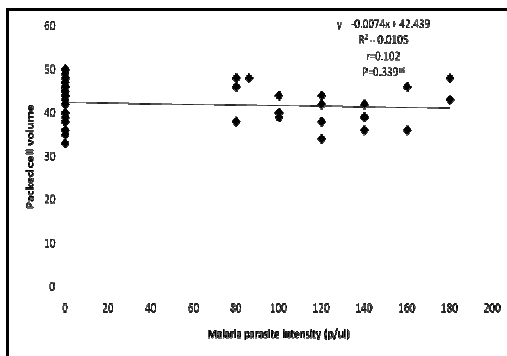


Figure 2: Malaria parasite intensity in relation to packed cell volume among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

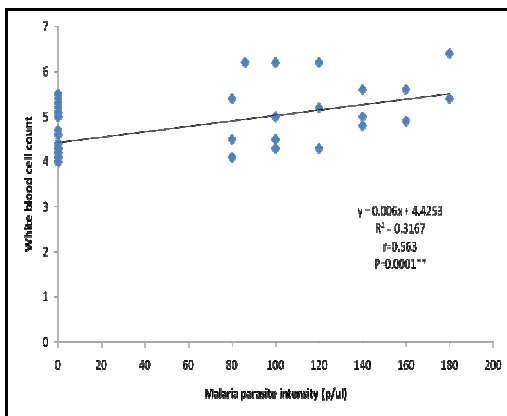


Figure 3: Malaria parasite intensity in relation to white blood cell count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

RESULTS AND DISCUSSION

Haematological Parameters of Malaria Positive and Negative Individuals: The values of packed cell volume, monocyte counts and the lymphocytes of malaria positive individuals were lower than those of negative

individuals while the values of total leucocyte counts, neutrophil counts, basophils and eosinophil counts were higher in malaria positive individuals than the negative ones. The differences were statistically significant in the packed cell volume, total leucocyte counts, eosinophils and monocytes (Table 1).

Correlation analysis of malaria parasitaemia and haematological parameters showed positive but insignificant association except with the total leucocyte counts ($r = 0.563$) (Figures 2 – 5).

Haematological changes have been associated with malaria infection and these have been found to involve red blood cells, leukocytes and thrombocytes (Layla *et al.*, 2002, Ai, 2008, Maina *et al.*, 2010, Imoru *et al.*, 2013). In the present study, significantly lower values of PCV and monocytes were observed in malaria infected individuals compared to the controls. This is in agreement with the findings of previous report of George and Ewelike-Ezeani (2011). The drop in PCV values in malaria positive subjects may have resulted from the mechanical destruction of parasitized red blood cells, reduction in red blood cell production in the bone marrow, phagocytosis of uninfected red blood cells, autoimmune destruction of red blood cells and nutritional status of infected individuals. Maina *et al.* (2010) had earlier reported that malaria-related anaemia is often more severe in areas of intense malaria transmission and affects infants more than older children and adults. The drop in PCV values of malarious individuals could serve as confirmatory symptom of anaemia in malaria patients.

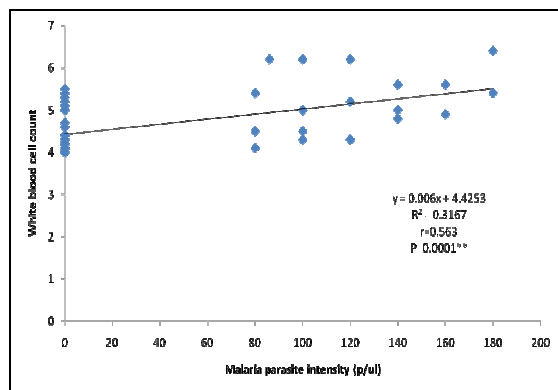


Figure 3: Malaria parasite intensity in relation to white blood cell count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

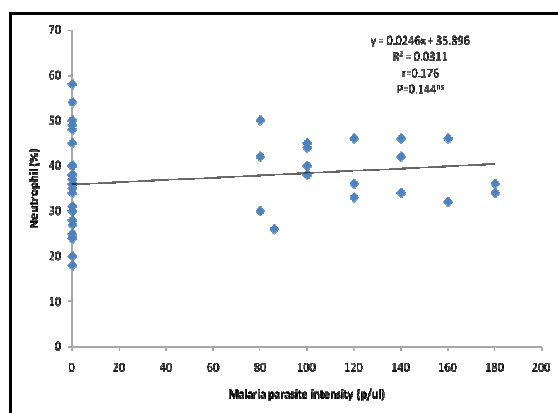


Figure 4: Malaria parasite intensity in relation to neutrophil count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

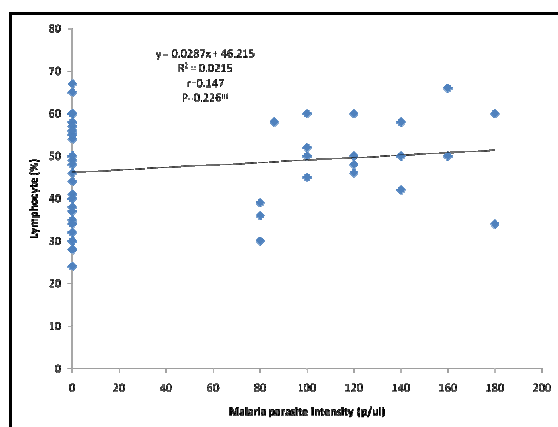


Figure 5: Malaria parasite intensity in relation to lymphocyte count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

Lower values of monocytes were also noticed in infected subjects which corroborated the report of Ladhani *et al.* (2002) which associated low values of monocytes with severe malaria. It was

suggested by Weatherall *et al.* (2002) that the function of monocytes may be inhibited by the action of hemozoin from digestion of haemoglobin of the red blood cells by malaria parasites during merozoite stage of the infection

The total leucocyte counts were higher in infected subjects but the differences were not significant. The association between malaria parasitaemia and the leucocyte counts was however, statistically significant ($r = 0.563$). The significant higher values of total leucocyte count in the infected subjects contradicted the reports of Smita and Harish (2013) and Igbeneghu and Odaibo (2013) which showed significant lower values of total leukocyte count in malaria positive individuals. However, the present study is in agreement with the findings of Ladhani *et al.* (2002). Kayode *et al.* (2011) also reported significant increase in total white blood cell count of malaria and typhoid co-infected patients. They posited that it could have been elicited by increased production of leukocytes at the onset of the infection to ward off malaria and typhoid parasites. Similarly, increase in WBC in malaria patients was also reported by Adesina *et al.* (2009).

With regards to the leucocyte differentials, the values of neutrophils, eosinophils and basophils were higher in the infected subjects than the controls but the differences were only significant in the values of neutrophils ($p < 0.05$). In view of these findings, haematological changes in individuals may serve as predictive values of malaria infection. It is therefore recommended that the diagnosis of malaria and haematological parameters of patients should go hand in hand in our health institutions for effective management and control of the infection.

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FEED INTAKE, GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN GROWING RED SOKOTO BUCKS FED DIETS CONTAINING GRADED LEVELS OF DRIED SWEET ORANGE PEEL MEAL

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ABSTRACT

The study was carried out to evaluate the growth performance, nutrient digestibility and nitrogen intake of growing red sokoto bucks fed graded levels of dried sweet orange (Citrus sinensis) peel meal (DSOPM). Twelve animals aged between 5 – 7 months with average body weight of 9.17 kg were assigned to four dietary treatments T₁ (control), T₂, T₃ and T₄ containing 0%, 2.5%, 5.0% and 7.5% DSOPM, respectively in a completely randomized block design (CRBD) with three goats per treatment. Each animal was fed at 2.5% body weight and provided drinking water ad libitum for a period of 90 days. The results obtained revealed that DSOPM had significant (p<0.05) effect on total feed intake (TFI), average daily feed intake (ADFI), final body weight (FBW), total body weight (TBW) and average daily weight gain (ADWG). Diet containing 0% inclusion level was significantly (p<0.05) best in total feed intake (37.13 ± 2.10kg), total body weight (4.16 ± 0.44kg), average daily weight gain (0.05 ± 0.008kg) and feed conversion ration (8.93 ± 0.11) compared to other treatment diets. Diet with the highest inclusion level of DSOPM (7.5%) recorded the lowest TBW (1.66 ± 0.50kg), average daily weight gain (0.02 ± 0.009kg), total feed intake (27.52 ± 1.02kg) and average daily feed intake (0.31 ± 0.03kg). Dry matter (DM) digestibility significantly (p<0.05) declined across T₁, T₂, T₃ and T₄ (54.24 ± 3.09%, 48.63 ± 4.11%, 44.03 ± 4.01% and 37.78 ± 3.34%), respectively. Crude Protein (CP) digestibility was highest in T₃ (64.67 ± 4.11%) and lowest in T₄ (48.33 ± 3.98%). Crude Fibre (CF) digestibility was highest in T₃ (73.12 ± 12.05%) and lowest in T₄ (20.77 ± 11.40%). Digestibility of ether extract (91.75 ± 15.15%) was highest in diet T₂ and significantly (p<0.05) different from the other treatment diets. Nitrogen intake was highest in T₁ (3.34 ± 0.13%) and lowest in T₄ (2.83 ± 0.07%). It was concluded that diet T₁ (containing 0% level of DSOPM) supported better growth performance than T₂ (2.5%), T₃ (5.0%) and T₄ (7.5%) signifying that inclusion of DSOPM in goat diet significantly (p<0.05) reduced feed intake and improved nutrient digestibility.

Keywords: *Citrus sinensis* peels, Growth performance, Nutrient digestibility, Red sokoto goats

INTRODUCTION

Ruminant animals constitute a very important part of the livestock sub-sector of the Nigerian agricultural economy. The potential of small ruminant production in alleviating the low

animal protein intake by man in developing nations such as in Nigeria has been reported (Fajemisin *et al.*, 2010). Recently, more attention have been paid to small ruminant production in the tropics as their advantages are becoming more understood than ever before,

particularly for their ability to produce meat, milk and skin, even in hostile environments (Konlan *et al.*, 2012; Makun *et al.*, 2013; Okoruwa *et al.*, 2013).

Goats are the most prolific of all domesticated ruminants under tropical and sub-tropical conditions (Webb and Mamabolo, 2004) and they play a significant role in livelihoods of the rural populace in most developing countries like South Africa. Apart from serving as a vital protein source, goats also provide income for meeting household needs (Peacock *et al.*, 2005). Notwithstanding, the high cost of formulating livestock feed has been a major constraint militating against the increased production of valuable sources of animal protein (Okoruwa *et al.*, 2013) in Nigeria. The shortage in feed supply due to high cost and seasonality, have caused ruminant livestock farmers to search for alternative feed resources that are inexpensive and readily available which are not directly required as component of human dietaries and can economically supplement the feed ingredients in rations without adverse effects on the rumen microbial fermentation and performance of the animals (Oluremi *et al.*, 2007a; Aka *et al.*, 2011; Okoruwa *et al.*, 2013).

In Nigeria, the availability of crop residues and agro-industrial by-products has been highlighted (Onyeonagu and Njoku, 2010). One such by-product is the peels of citrus fruits. Sweet orange (*Citrus sinensis*) peel is an alternative feed resource that is gaining more recognition among small ruminant producers. According to FAO (2004), 140 countries produce citrus fruits and Nigeria production is about 2%. Citrus fruits have been reported to be available throughout the year especially during the peak season of October to December which happens to be the bumper harvest period (Omodamiro and Umekwe, 2013). A good number of by products are derived from sweet orange fruits; these include citrus pulp, citrus molasses, citrus peel oil and citrus peels (Ezejiofor *et al.*, 2011). Studies have shown that *Citrus sinensis* peel is a major source of pectins that is non-digestible carbohydrates that stimulate the growth of probiotic bacteria in the colon which prevent the growth of pathogenic bacteria. As a dietary supplement, sweet orange peel can enhance the

immune system and decrease the risk of contamination of animal meat with pathogenic bacteria (Zohreh *et al.*, 2012).

Sweet orange peels in Nigeria can be easily collected at no cost from sweet orange fruit retailers who peel and sell sweet orange fruits to consumers for direct consumption. Oluremi *et al.* (2007b) reported that sweet orange peels contained 9.30 – 10.96% crude protein, 13.66 – 14.94% crude fibre, 2.33 – 2.90% ether extract, 65.30 – 67.95% nitrogen free extract and 5.07 – 5.56% ash. The composition of citrus peel is similar to that of citrus pulp, except that citrus peel has a higher content of citrus essential oils (CEO). The CEO in citrus peel have antimicrobial and antioxidant properties so citrus peel could act as a preservative, which would be beneficial for long term storage of feed (Nam *et al.*, 2009). Sweet orange peels contain tannin, saponin, oxalate, flavonoid and limonene, although the amounts present, is in such quantities that may not be deleterious to the health of animals. Processing such as sun-drying and fermentation of the sweet orange peels further reduce even the small quantities of these anti-nutrients that are present (Oluremi *et al.*, 2007b).

The peels are available through out the year and are usually noticed on streets and along major roads in Nigeria, because government and orange retailers have no strategic disposal programme, thus, becoming an environmental problem (Oluremi *et al.*, 2006). Rather than discarding the orange peels, suggested that they can be sun-dried and then milled in grinding machine to fine particle to obtain the orange peel meal which can be a potential feed ingredient for ruminants (Silva *et al.*, 1997; Oluremi *et al.*, 2007a; Oyewole *et al.*, 2013). Sweet orange fruit rind (peel) meal has been reported to have both calorie and protein comparable with maize (Oluremi *et al.*, 2006). The peel contains citrus essential oil and the oil is composed of 91 – 94% d-limonene and 2.0-2.1% β -myrcene as a minor constituent. Polymetholated flavones are also a class of compound found in citrus peel and produce no negative side effects in the animals fed polymetholated flavones containing diets (Silva *et al.*, 1997). Ruminants feeding systems based

on locally available by-product feedstuffs are often a practical alternative because the rumen microbial ecosystem can utilize by-product feedstuffs which often contain high levels of structural fibre to meet their nutrient requirements for maintenance, growth, reproduction and milk production (Bampidis and Robinson, 2006). Feeding citrus peels to small ruminants could be a practice that would diminish dependence on grains and contribute to reducing the environmental problems linked to their elimination (Abdel Gawad *et al.*, 2013).

The general objectives of this study was to evaluate the nutritional potential of sun-dried 48 hours sweet orange (*Citrus sinensis*) peel meal in the diets of growing bucks. While the specific objectives were to evaluate the growth performance and nutrient digestibility of growing red sokoto bucks fed diets containing graded levels of dried sweet orange peel meal (DSOPM).

MATERIALS AND METHODS

Location of the Study: The study was conducted at the Teaching and Research Farm, of the Department of Animal Science, Ahmadu Bello University, Samaru, Zaria, Nigeria. The area is geographically situated between Latitude 11° 12'N and Longitude 7° 37'E at an altitude of 670 metres above sea level. Vegetationally, it is located in the Northern Guinea Savanna zone of Ngeria. Detailed description of Samaru climate had been documented (Malau-Aduli and Abubakar, 1992).

Experimental Design: The experimental design used was Completely Randomized Block Design (CRBD) comprising of four treatments (blocks) replicated thrice with each replicate having three animals.

Experimental Deits: Groundnut and cowpea haulms were sourced from the harvested haulms produced for practical purposes by students of the Faculty of Agriculture, Ahmadu Bello University, Zaria. Fresh sweet orange (*Citrus sinensis*) peels of mixed varieties were collected from orange fruit retailers from different locations around Zaria metropolis in

Kaduna State of Nigeria. They were sun-dried on concrete floors for 48 hours, when it became crispy; it was milled to obtain sweet orange peel meal and stored in synthetic bags before incorporation in the diets (Oyewole *et al.*, 2012; Oyewole *et al.*, 2013; Oloche *et al.*, 2015). Four diets were formulated containing maize offal, rice bran, cotton seed cake, groundnut haulms, cowpea haulms, bone meal and common salt (Table 1). Dried sweet orange peel meal (DSOPM) substituted maize offal at 0%, 2.5%, 5.0% and 7.5% inclusion levels in the experimental diets. Twelve bucks were randomly assigned to the four dietary treatments (0%, 2.5%, 5.0% and 7.5%) comprising of three animals per treatment.

Experimental Animals: The experimental animals were locally sourced from Anchau market in Ikara Local Government Area of Kaduna State. Thirty-six weaned red sokoto bucks aged between 5 – 7 months with average weight of 9.17kg were used. The animals were vaccinated against pestes des petits ruminants (PPR), using PPR vaccine and dewormed using albendazole suspension (Sambezole) administered orally at about 1ml/10kg body weight. Ecto-parasites were checked using Ivermectin (Ivomec) at 2ml/10kg body weight. Two weeks to the arrival of the animals to the experimental site, 12 pens were thoroughly washed and cleaned using disinfectant (Izal) and allowed to dry. On arrival, the animals were weighed and randomly distributed into four treatment groups of three animals per replicate, in a Completely Randomized Block Design (CRBD). The animals were housed in an open-sided, well-ventilated pens which was bedded with wood shavings to serve both as litter materials and beddings and equipped with feed and water troughs. Animals were given a weighed amount of the experimental diet containing varying levels of DSOPM (0%, 2.5%, 5.0% and 7.5%) between 0:700 and 09:00 hour daily. They were allowed 3 hours to feed on the concentrate afterwhich the groundnut and cowpea haulms were served *ad libitum*. Clean fresh drinking water was served to the animals daily *ad libitum*.

Table 1: Percentage dietary ingredients and proximate compositions of varied levels of dried sweet orange peel meal in experimental diets fed to growing red Sokoto goats

Feed Ingredients	DSOPM	Experimental Diets			
		0% DSOPM	2.5% DSOPM	5.0% DSOPM	7.5% DSOPM
Maize Offal	-	42.50	40.00	37.50	35.00
Rice Bran	-	9.00	9.00	9.00	9.00
Cotton Seed Cake	-	14.00	14.00	14.00	14.00
Groundnut Haulms	-	15.00	15.00	15.00	15.00
Cowpea Haulms	-	15.00	15.00	15.00	15.00
DSOPM	-	0.00	2.50	5.00	7.50
Bone Meal	-	3.00	3.00	3.00	3.00
Common Salt	-	1.50	1.50	1.50	1.50
Total	-	100.00	100.00	100.00	100.00
Analyzed nutrients (%)					
Dry Matter	89.60	93.48	93.42	92.25	93.64
Crude Protein	7.00	16.38	15.00	13.19	14.13
Crude Fibre	13.50	16.13	15.13	17.03	16.00
Ether Extract	2.40	9.41	10.14	10.39	10.68
Nitrogen Free Extract	65.38	64.36	53.48	70.31	65.30
Ash	6.90	15.33	17.65	10.44	18.58

Key: DSOPM = dried sweet orange peel meal

The feeding trial lasted for 90 days after an acclimatization period of seven (7) days.

Performance Indices: The goats were weighed at the beginning of the experiment and subsequently on a weekly basis to evaluate average weight changes. Data on feed intake and body weight gain were determined. Feed intake was obtained by subtracting left-over feeds from the quantity offered each week to obtain weekly feed intake per replicate. The goats were weighed at the beginning, fortnightly and at the end of the study. Weight gain was computed by subtracting initial weight from final weight. Average daily weight gain (ADWG) was determined by dividing weight gain by the number of goats and the number of days of the feeding trial.

Digestibility and Nitrogen Balance: At the end of the growth study, all the animals were weighed and transferred to individual metabolic crates fitted with facilities for separate collection of voided faeces and urine. Experimental diets fed were the same as those used in the growth study. An adjustment period of 7 days was allowed before the faecal and urine samples were measured for subsequent 7 days. Faeces voided daily was collected separately from

animals in each treatment and were pooled, thoroughly mixed and sub-samples taken. Feed intake was measured by finding the difference between the amount of feed offered and the amount leftover. Nitrogen loss from urine due to bacterial infestation and growth were prevented by introducing the urine into a well-labelled urine collection bottle containing 5ml 0.1M tetraoxosulphate (vi) acid (H₂SO₄) and stored in a refrigerator for laboratory subsequent analysis. Feed and faecal samples were oven dried at 65^o C to constant weight, milled and stored in air tight containers, until required for nutrient analysis. Apparent digestibility of the diets was calculated as the difference between nutrient intake and nutrient in faeces, expressed as a percentage of the nutrient intake using the formula: Apparent Nutrient Digestibility = Nutrient intake – Nutrients in faeces / Nutrient intake x 100 (Marshal, 2001; Aduku, 2004; Okoruwa *et al.* 2012; Bello and Tsado, 2013).

Chemical Analysis: Ten percent representative of each feed offered and refused was sampled every day and combined for the entire collection period on each replicate basis using air tight containers. Sub-sampling was performed on the aggregated feed materials for both offered and refused and kept in the

refrigerator for analysis. Samples of feeds and faeces were weighed and oven dried at 105°C for 48 hours to constant weight. Both feed and faecal samples were ground using a hammer mill to pass through a sieve of 1 mm diameter and were analyzed for dry matter (DM), crude protein (CP) was calculated as $N \times 6.25$, crude fibre (CF) and ether extract (EE). Nitrogen free extract (NFE) was determined by difference while ash content was determined by combusting samples at 550° C overnight according to procedure described by AOAC (2001). The nitrogen content of the urine was determined by the Kjeldahl method according to AOAC (2001) procedure.

Statistical Analysis: All data generated were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedures of Statistical Analysis System version 9.0 (SAS, 2002). Significant differences at $p < 0.05$ among means were separated using Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSIONS

The dietary composition of the experimental diets fed to the growing red sokoto bucks indicated differences nutrients arising for substitution of maize meal with dried sweet orange peel meal (DSOPM) (Table 1). The effect of DSOPM on growth performance of the goats indicated that the initial body weights of animals were not significantly different ($p > 0.05$), indicating that similar weight of animals were used at the commencement of the study. Final body weights of growing goats were significantly ($p < 0.05$) influenced by the dietary treatments, with animals on 0% inclusion having higher mean body weight ($13.33 \pm 0.25\text{kg}$), while the lowest mean body weight ($10.83 \pm 0.10\text{kg}$) was recorded for goats receiving 7.5% DSOPM inclusion (Table 2). The observed highest mean final body weight and mean total body weight gain values for goats fed diet having 0% DSOPM inclusion could be as a result of the ability of the goats to properly utilize nutrients in the diets without DSOPM than in diets with DSOPM. The result of this study on weight gain is in agreement with the report of

Nkoku and Evbuomwan (2014) that efficient utilization of nutrients in diets that supply adequate energy and protein is required for optimum growth performance of ruminants. Mean body weights (MBW) of the animals were 4.16 ± 0.44 , 2.83 ± 0.63 , 2.66 ± 0.36 and $1.66 \pm 0.50\text{kg}$ for 0%, 2.5%, 5.0% and 7.5% inclusions of DSOPM, respectively. Animals on 0% DSOPM had significantly ($p < 0.05$) higher body weights followed by 2.5%, 5.0% and 7.5% DSOPM inclusions. This result is in agreement with the result of Oyewole *et al.* (2012) who reported that substituting maize with sweet orange fruit peel meal significantly ($p < 0.05$) reduced live weight of growing pullets. However, the MTBW values obtained in this study, were comparable to the range of values (2.73 – 3.51kg) and (2.07 – 5.32kg) reported by Okoruwa *et al.* (2013) for West African Dwarf (WAD) sheep and Okoruwa *et al.* (2015) for WAD male sheep respectively. Feed intake followed a similar pattern of variation as observed in MTBW, with their values ranging from 27.52kg (at 7.5% inclusion) to 37.13kg (at 0% inclusion). However, the highest total feed intake value obtained at 0% inclusion level might be as a result of palatability of the diet, nature of diet preparation and the nutrient content of the diet which make the goats to consume more to meet up with their energy and protein requirement (Okoruwa *et al.*, 2015). The highest average daily weight gain (0.05kg) and average daily feed intake (0.41kg) values recorded for animals at 0% inclusion level were consistent with the reports of Ososanya *et al.* (2013) and Okoruwa *et al.* (2015). Furthermore, Ososanya (2010) and Okoruwa and Adewumi, (2010) reported that feed intake is an important factor in the utilization of feed by livestock and is also a critical determinant of energy and protein as well as performance in small ruminant. There was no significant ($p > 0.05$) difference in the average daily weight gain (ADWG) between animals on 2.5% ($0.03 \pm 0.005\text{kg}$) and 5.0% ($0.03 \pm 0.003\text{kg}$) but 0% ($0.05 \pm 0.008\text{kg}$) was significantly ($p < 0.05$) higher than goats on 2.5% and 5.0% inclusion levels. Feed conversion ratio (FCR) that is measured by feed intake per unit weight gain was significantly ($p < 0.05$) higher in goats at

Table 2: Performance response of growing red Sokoto goats fed varied levels of dried sweet orange peel meal in the diets

Parameters	Experimental Diets			
	0% DSOPM	2.5% DSOPM	5.0% DSOPM	7.5% DSOPM
Initial body weight (kg)	9.17±0.71	9.17±0.18	9.17±0.10	9.17±0.09
Final body weight (kg)	13.33±0.25 ^a	12.00±0.21 ^{ab}	11.83±0.08 ^{ab}	10.83±0.10 ^b
Total body weight (kg)	4.16±0.44 ^a	2.83±0.63 ^b	2.66±0.36 ^b	1.66±0.50 ^c
ADWG (kg/day)	0.05±0.008 ^a	0.03±0.005 ^{ab}	0.03±0.003 ^{ab}	0.02±0.009 ^b
Total feed intake (kg)	37.13±2.01 ^a	32.65±3.20 ^a	30.65±2.03 ^{bc}	27.52±1.02 ^c
ADFI (kg)	0.41±0.01	0.36±0.01	0.34±0.05	0.31±0.03
Feed conversion ratio	8.93±0.11 ^c	11.54±1.91 ^b	11.52±0.81 ^b	16.57±0.10 ^a

Key: ^{abc} Means in the same row having different superscripts are significantly different ($p < 0.05$), ADWG = Average daily weight gain; ADFI = Average daily feed intake; DSOPM = Dried sweet orange peel meal

Table 3: Apparent nutrient digestibility (%DM) of growing red Sokoto goats fed varied levels of dried sweet orange peel meal in the diets

Parameters (%)	Experimental Diets			
	0% DSOPM	2.5% DSOPM	5.0% DSOPM	7.5% DSOPM
Dry Matter	54.24±3.09 ^a	48.63±4.11 ^a	44.03±4.01 ^{ab}	37.78±3.34 ^b
Crude Protein	53.53±4.08 ^b	62.94±4.00 ^a	64.67±4.11 ^a	48.33±3.98 ^c
Crude Fibre	43.45±13.08 ^b	34.26±11.04 ^b	73.12±12.05 ^a	20.77±11.40 ^{bc}
Ether Extract	69.49±22.10 ^{ab}	91.75±15.15 ^a	70.61±9.00 ^{ab}	45.02±4.95 ^b
Nitrogen Free Extract	74.18±1.26	78.79±1.00	73.73±1.11	75.32±1.29
Ash	37.92±5.40 ^b	89.09±7.10 ^a	32.91±4.00 ^c	40.27±4.40 ^b

Key: ^{abc} Means in the same row having different superscripts are significantly different ($p < 0.05$), DSOPM = Dried sweet orange peel meal

Table 4: Nitrogen utilization (g/day) of growing red Sokoto goats fed varied levels of dried sweet orange peel meal in the diets

Parameters	Experimental Diets			
	0% DSOPM	2.5% DSOPM	5.0% DSOPM	7.5% DSOPM
Nitrogen intake	3.34±0.13 ^a	3.12±0.12 ^a	2.98±0.06 ^b	2.83±0.07 ^b
Faecal Nitrogen	1.97±0.11 ^a	1.80±0.09 ^a	1.60±0.07 ^{ab}	1.51±0.02 ^b
Urinary Nitrogen	0.62±0.01	0.62±0.01	0.62±0.01	0.61±0.01
Digested Nitrogen	0.98±0.37 ^a	0.75±0.31 ^{ab}	0.63±0.19 ^b	0.61±0.21 ^b
Nitrogen retention	29.34±2.01 ^a	24.04±0.20 ^b	21.14±0.11 ^c	21.55±0.08 ^d

Key: ^{abc} Means in the same row having different superscripts are significantly different ($p < 0.05$), DSOPM = Dried sweet orange peel meal

7.5% inclusion level (16.57 ± 0.10) and lowest in those on 0% (8.93 ± 0.11). This implies that, the efficiency at which goats converted feeds for their body weight gain in diet at 0% inclusion level is lowest, indicating a better feed conversion ratio of the feed. Moreover, the positive response between average daily weight gain and better feed conversion ratio obtained at 0% inclusion level could be probably used to further attest the superiority of goats on the control diet (0%) in terms of nutrient utilization for body weight gain over others.

The apparent nutrient digestibility (% DM) of growing goats fed experimental diets indicated significant difference ($p < 0.05$) in the dry matter digestibility with diet at 0% (54.24%) being the highest and diet at 7.5% (37.78%) the lowest (Table 3). Nutrient digestibility in animals is the classical and direct method for estimating feed digestion by ruminants; hence studies on digestibility of ruminant feeds are very important as they allow for the estimation of nutrients actually available for ruminant nutrition (Okoruwa *et al.*, 2012).

This difference could probably explain nutrient accumulation rate in the diets. This was contrary from those reported by Oloche *et al.* (2013) and Okoruwa *et al.* (2013) who reported that there was no significant ($p>0.05$) difference between treatment groups. Dry matter range values of 37.78 – 54.24% were lower as compared with range values of 93.29 – 94.01% reported by Oloche *et al.* (2013) for WAD goats fed diets containing graded levels of sweet orange peel meal.

Crude protein digestibility range from 48.33 in 7.5% to 64.67% in 5.0% inclusion levels respectively. The higher significant ($p<0.05$) difference observed in 5.0% compared to 0%, 2.5% and 7.5% could be as a result of the presence of tannin and saponin in the test diets which reduced protein degradation in the rumen so that appreciable quantity was available post-ruminally for digestion. This is in agreement with earlier report of Nkoku and Evbuomwan (2014) that the presence of tannin and saponin lowers the solubility of proteins entering the abomasum and small intestine for digestion. It has been reported that sweet orange peel meal contains saponin and tannins (Oyewole 2011). Crude fibre digestibility was significantly highest at 5.0% inclusion level ($73.12 \pm 12.05\%$) and low at 0% ($43.45 \pm 13.08\%$) followed by 2.5% ($34.26 \pm 11.04\%$) before 7.5% ($20.77 \pm 11.40\%$) which was the lowest. The low crude fibre digestibility observed was probably because the rumen micro-organisms were not able to effectively digest the nature of fibre in the diets. While the highest CF digestibility at 5.0% inclusion level ($73.12 \pm 12.05\%$) might be due to changes in the rate of ingesta from the rumen. This observation was different from range values of 79.5 – 82.11% and 69.43 – 80.23% reported by Oloche *et al.* (2013) and Okoruwa *et al.* (2013) for WAD goats and sheep, respectively. They observed that the high digestibility of CF is a reflection of longer retention of feeds in the digestive system. Ether extract digestibility showed significant ($p<0.05$) difference between 2.5% ($91.75 \pm 15.15\%$) and 5.0% ($70.61 \pm 9.00\%$) before 0% ($69.49 \pm 22.10\%$). 7.5% inclusion level ($45.02 \pm 4.95\%$) was significantly ($p<0.05$) lower than 0%, 2.5% and 5.0%.

The highest ether extract digestibility value recorded in animals on 2.5% and 5.0% inclusion levels confirmed the reports of Okoruwa *et al.* (2013) that the diets were more effective in improving the utilization of ether extract. Nitrogen free extract digestibility values were 78.79 ± 1.00 , 75.32 ± 1.29 , 74.18 ± 1.26 and $73.73 \pm 1.11\%$ for 2.5%, 7.5%, 0% and 5.0% inclusion levels respectively, with no significant ($p>0.05$) differences between treatment groups. The observed range values were higher than 50.81% – 57.93% reported by Oloche *et al.* (2013) for WAD goats fed graded levels of sweet orange peel meal and was also higher than 64.89% – 68.37% reported by Okoruwa *et al.* (2013) for WAD sheep fed graded levels of orange and pineapple pulps. More so, the high NFE digestibility reported in this study implies that substantial amounts of fermentable carbohydrates were digested. Suggesting better proportion of energy for improving rumen fermentation that provides appropriate balance of nutrient to the animals for absorption. This agreed with the reports of Okoruwa and Njidda (2012) that nutrient digestibility among other factors will depend on differential level of a ration and vary level of nutrient composition in the diet taken by animal.

The nitrogen metabolism result indicated significant ($p<0.05$) variations in digested nitrogen (g/day) across the dietary treatments (Table 4). The control diet 0% (0.98 ± 0.37 g/day) had the highest digested nitrogen followed by 2.5% (0.75 ± 0.31 g/day), while 5.0% and 7.5% inclusion levels were not significantly ($p>0.05$) different. The goats on 0% inclusion digested significantly ($p<0.05$) higher values of nitrogen per day than those on 2.5%, 5.0% and 7.5%. This may be as a result of higher nitrogen intake by animals on 0%. The nitrogen retention (NR) in 0% and 2.5% inclusion levels were 29.34 ± 2.01 and 24.04 ± 0.20 g/day respectively which were significantly ($p<0.05$) higher than those in 5.0% and 7.5% (21.14 ± 0.11 and 21.55 ± 0.08 g/day), respectively. This result is similar to the report of Aye and Adegun (2010) that the N-retention for diets with high protein levels tend to be higher ($p<0.05$) compared to low protein level diets.

The treatment effects on nitrogen balance were not significantly ($p>0.05$) different. Nitrogen balance values for goats on 0%, 2.5%, 5.0% and 7.5% were 0.98 ± 0.37 , 0.75 ± 0.31 , 0.63 ± 0.19 and 0.61 ± 0.21 g/day respectively. This is contrary to 1.12 – 5.35 g/day reported by Aye and Adegun (2010) for WAD sheep fed gliricidia based multinutrient block supplements suggesting that N-intake significantly ($p<0.05$) influenced N-balance.

Conclusion: It was concluded that goats fed the control diet (0%) performed better than other treatment diets signifying that incorporation of dried sweet orange peel meal in goat diet significantly ($p<0.05$) reduced feed intake while at the same time improved the digestibility of crude protein and crude fibre at 5.0% level of inclusion. Dried sweet orange peel meal could be used as feed for growing goats without any detrimental effect.

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BIRD SPECIES OF MOUAU WITH SPECIAL EMPHASIS ON FORAGING BEHAVIOR OF THE NORTHERN GREY-HEADED SPARROW (*PASSER GRISEUS*)

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ABSTRACT

*Ten different bird species were peculiar to the Umudike environment and of these eight were regular thus closely observed and identified. The other two species were scarcely available and may be regarded as visiting birds. The eight species identified were either Passerine or Non-Passerine. The northern grey-headed sparrow (*Passer griseus*) was one of the Passerine species encountered. The amount of time spent by the birds foraging varied significantly with group size. Pecking rate reduced with increased scanning time. Pecking rate of individuals increased with group size and reduced with increasing group size. Birds in fewer groups will gather food and move away quickly than with smaller groups, the movement characterized by small walks or hops. Scanning rate reduced with increasing group size and increased with reduced group size. Group size was the most determinant factor in determining the relationships between vigilance, hopping and feeding rates.*

Keywords: Passerine, Foraging, Pecking rate, Scanning, Hops, Vigilance, Group size

INTRODUCTION

Birds are endothermic reptile-like vertebrates that belong to the class Aves. They are abundant in nature and engage in fascinating behaviors. In Nigeria, a total of 940 species of birds are resident, of which four are endemic and five are rare or accidental (Wikipedia, 2013). They are either passerine or non-passerine birds. Some passerine species known to occur in Nigeria include the following bird species; Swallow, Greenbul, Akalat, Ant thrush, Warbler, Cisticola, Flycatcher, Sunbird, Pied crow, Pin-tailed Whydah, Finch, Sparrows, Seed eater, Weaver birds, among many others (Burrow and Domey, 2001). The Northern Grey-headed Sparrow (*Passer griseus*) is one of the common *Passer* species in Nigeria.

All birds employ foraging behaviour to survive in the ecosystem (Liker and Barta,

2002). This behaviour exhibits how they manipulate their immediate habitat in search for food. To a great extent the kind of food available determines the feeding behavior of bird species. A particular food may exist in many different situations requiring different feeding techniques (Giraldeau and Caraco, 2000). Many birds steal food from other birds, and some bird species rely on klepto-parasitism tactics for locating and capturing food. Some land birds form mutualistic food-searching association either with conspecifics or other bird species (Cueto and Lopez de Casenove, 2000). Studies on the foraging behavior of birds have shown that they can be influenced by some biological factors, such as, habitat, group size and anti-predator vigilance (Beck and George, 2000; Bednekoff and Lima, 2002).

Habitat selections have important implications on foraging behavior. Old and

recent studies have investigated bird foraging behavior to understand microhabitat selection processes. Most of these studies demonstrated that microhabitat selection differs among species, within species and between individuals exposed to different habitats (Beck and George, 2000). A few bird species have been studied in Kainji Lake area, Hadejia-Nguru wetlands and Jos Plateau in Nigeria (Borrow and Domey, 2001). The report showed that differences in microhabitat selection and foraging behavior based on their foraging tactics reflect differences in the use of food resources (Borrow and Domey, 2001; Gabbe *et al.*, 2002). This accounts for the fact that habitat selection may be determined in part by the availability of suitable food substrates. Several other studies have supported the view that the abundance, distribution and availability of food are believed to be the principle factor influencing habitat suitability for birds (Strong and Sherry, 2000; Fayat, 2003).

Vigilance, another behaviour shown by birds, has been predicted to decrease with group size due to increased predator detection and dilution of predator risk in larger groups (Krause and Ruxton, 2002). Group size is an imperative factor in determining an individual's behavioral actions and this applies to all organisms (Krause and Ruxton, 2002). A reduction in individual vigilance with an increase in group size is one of the reported relationships in the study of animal behavior (Ale and Brown, 2007). Animals often interrupt feeding bouts to scan their surroundings. Scanning is referred to as vigilance and may serve several purposes, including; detection of threats and assessment of within-group competition (Krause and Ruxton, 2002). Due to the risk of predation, birds will usually try to forage in areas near dense vegetation that can provide safety.

Vegetation structure is a feature of habitat which provides opportunities and constraints that determine how and where birds detect and capture prey (Whelan, 2001). Such vegetation structures include; leaf morphology, foliage architecture and heights. These structures present in the habitats have a strong influence on bird foraging behavior (Whelan, 2001). They spend less time watching for

threats, because with cover nearby, birds are able to flee to safety (Clemens *et al.*, 2001). Studies have shown that distances of just one meter away from cover is risk enough to cause some birds to avoid feeding (Barta *et al.*, 2004).

The influence of foraging behaviour, vigilance and scanning determines passerine bird species choice of field and habitat for foraging. Schulenberg (2010) reported that the pin-tailed whydah (*Vidua macroura*) prefers disturbed grasslands with patches of bare soil for feeding. The house sparrows on the other hand are adapted to forage wherever they find preferable food, such as, in farms, fields, parks, lodges and other human habitations where they eat food scraps from humans (Esteban *et al.*, 2008). There are usually large feeding groups at areas where food is plentiful, usually forming flocks greater than 50 (Arnaiz-Villena *et al.*, 2009) and this aids in increasing the amount of time spent foraging in that particular microhabitat. Solitary birds will often use the chirrup call to attract conspecifics to a feeding site, to reduce the amount of time spent alone and the risk of predation (Johnson *et al.*, 2001).

Bird species in Michael Okpara University of Agriculture, Umudike (MOUUAU) environment have scarcely been studied. This research was thus aimed at identifying bird species in the MOUUAU environment and to describe the foraging behavior of Northern grey-headed sparrow (*Passer griseus*) in near primeval conditions, using indices such as; microhabitat selection, group size and anti-predator vigilance.

MATERIALS AND METHODS

The study was conducted within the campus of Michael Okpara University of Agriculture, Umudike (MOUUAU), Abia State, Nigeria. Field work was carried out in three selected sites within the campus. Two of the sites were fields characterized with a variety of native grasses, which contain weed seeds and grass seeds. These grasses also harbor insect life, most especially at the inflorescence. One of the fields, characterized with dense vegetation (small shading trees) is not more than one metre away from the feeding site. The vegetation served as

cover to the birds. In the second field, there were no trees closed by for cover. The third site was close to human habitation, a field located in one of the hostels within the university campus. Leftovers of food from the occupants of the hostel characterized this field.

Observations of the bird to ascertain their species and the activities they carried out was on a daily basis at the three different sites. This was done in the morning and evening hours. Morning observations were carried out between 6.45 am and 11.00 am, while evening observations were carried out between 4.00 pm and 6.00 pm. A pair of 8 × 40 binoculars was used to observe the birds. Other handy materials included; a digital camera, a stop watch and a field guide. The following data were recorded: time of the day, type of microhabitat, and minutes devoted to foraging, vigilance (number of heads-up scan and duration of scanning), number of hops and number of pecks. Recording and observation ended when at most 5 birds were absent from the feeding site for more than 3 minutes.

Data analysis: The data were analyzed for the differences in proportion using chi-square test. Statistical significance was determined using analysis of variance (ANOVA) and the relationships between the variables were established using correlations statistics.

RESULTS

Ten different bird species were encountered in the field in the course of this study. Figures 1 – 5 shows some of the frequently encountered birds. Of these species, eight were regular thus they were closely observed and identified. The other two species were scarcely available and may be regarded as visiting birds. The eight species identified were either passerine or non-passerine (Table 1).

Foraging Behavior of *Passer griseus* and its Relationship with Microhabitat, Group size and Vigilance: The northern grey-headed sparrow (*Passer griseus*) fed more in the evening between 17.00 and 18.30 hours (Tables 2).

In each of the three sites, the group size varied and was the most determinant factor in determining the relationships between the vigilance, hopping and feeding rates. The mean number of pecks in the evening was higher than in the morning hours. Also, the mean number of pecks was higher the field 1 than in the other selected sites. This may be due to the large group size of the species there. In field 1, the group size was closely related to the feeding rate; they pecked more with increasing group size thus significant difference was recorded between the group size and feeding/pecking rate ($r = 0.95$; $p = 0.00$). It should be noted however that the presence of other species of birds in the field often reduced the pecking rate of *Passer griseus*. In field 2, significant difference was not recorded between any of the parameters. However a close relationship between these parameters was recorded.

Number of Scans: Scanning reduced with increased group size. The mean value of heads-up scans was low during the morning and evening vigilance in field 1; though the mean value recorded for time spent scanning was higher in this same field (Table 2). In field 2, though the mean value of heads-up scans recorded was high, it was lower than the mean scanning time in field 1. This may be related to the nearness of field 1 to vegetation.

Pecking and Vigilance: There was a relationship between pecking and vigilance as low scanning rate lead to increased pecking rate. In field 1, the significant effect of vigilance on pecking rate was not a surprise vigilance ($n = 10$, $p = 0.002 < 0.01$) (Table 3). In field 2, a significant effect of vigilance on pecking rate was recorded ($n = 10$, $p = 0.007 < 0.01$). Significance was recorded in the correlations between number of scan and pecks in the evening (Table 3). In field 3 (around human environment), there was no significance ($n = 10$, $r = 0.129$, $p = 0.723$).

Group size and Number of Scans Recorded in the Selected Sites: Microhabitat had no significant effect on the group size of the birds.



Figure 1: Group of Northern Grey-headed Sparrow foraging in one of the fields



Figure 2: Northern Grey-headed Sparrow foraging in the same field as the Pin-tailed Whydah (R-L)



Figure 3: *Passer griseus* feeding on food scraps in a bare patch around field 3



Figure 4: White breasted Negro finch foraging on a grass in nearby field



Figure 5: *Passer griseus* in a tree cover close to their feeding site

Table 1: Bird species encountered in fields in Michael Okpara University of Agriculture, Umudike (MOUUAU), Umuahia, Abia State, Nigeria

Passerine species

- Pied crow (*Corvus albus*)
- Parasitic weaver (*Anomalospiza imberbis*)
- White-breasted Negrofinch (*Nigrita fusconota*)
- Pin-tailed Whydah (*Vidua macroura*)
- Northern grey-headed sparrow (*Passer griseus*)

Non-Passerine species

- Cattle Egret (*Bubulcus ibis*)
- Eagle (*Aquila spilogaster*)
- Vulture (*Torgos tracheliotus*)

The group size was often higher in the sites close to vegetation which served as cover for the birds (Table 4). The amount of time spent foraging varied significantly with group size.

DISCUSSION

From this study, the Umudike environment just like any other stable ecosystem, houses various bird species. The Northern grey-headed sparrows actively foraged on the various selected fields. Depending on the size of the group analyzed and the microhabitat, different

trends and statistical significant results were obtained. The birds never fed for more than 50 cumulative minutes in any of the observation period. Pecking rate increased with group size. When in group *Passer griseus* engaged more in active feeding rather than spending their time standing and scanning. Bertram (1980), found out that although individual birds may scan more than birds in a group, the overall proportion of time an ostrich in a flock uses in scanning increases with group size. Since ostrich raise their heads at random times, it makes it impossible for lion to predict the appropriate time to strike (Bertram, 1980). This is not likely to occur in *Passer griseus*, because of their small body size. *Passer griseus* spent less time scanning and exhibited a little heads-up scan as its group size increased, while their pecking rate increased. Solitary birds or birds in pairs are at greater risk of predation, which is why they spend more time scanning. In groups however, responsibility is shared among individuals within the group thus there is reduced rate of scanning by an individual and pecking rate increases. Scanning rate may also be influenced by the microhabitat.

Table 2: Correlation between group size and number of pecks in the various fields

Number of pecks		Field 1		Field 2	
		Morning	Evening	Morning	Evening
Group size	Morning				
	Pearson correlation	0.632	0.212	0.141	0.113
	Significant (2-tailed)	0.050	0.556	0.698	0.756
	N	10	10	10	10
	Evening				
	Pearson correlation	0.053	0.950	0.310	0.400
	Significant (2-tailed)	0.885	0.000	0.384	0.253
	N	10	10	10	10

Correlation is significant at 0.01 levels (2 tailed)

Table 3: Correlation between number of pecks and number of scans in the various fields

Number of pecks		Field 1		Field 2	
		Morning	Evening	Morning	Evening
Number of scans	Morning				
	Pearson correlation	0.665	0.147	0.328	0.198
	Significant (2-tailed)	0.036	0.685	0.355	0.584
	N	10	10	10	10
	Evening				
	Pearson correlation	0.005	0.852	0.318	0.787
	Significant (2-tailed)	0.988	0.002	0.370	0.007
	N	10	10	10	10

Correlation is significant at the 0.01 level (2-tailed)

Table 4: Correlation between group size and number of scans in the various fields

Number of scans		Field 1		Field 2	
		Morning	Evening	Morning	Evening
Group size	Morning				
	Pearson correlation	0.655	0.066	0.730	0.115
	Significant (2-tailed)	0.029	0.556	0.017	0.752
	N	10	10	10	10
	Evening				
	Pearson correlation	0.334	0.794	0.051	0.949
	Significant (2-tailed)	0.346	0.006	0.888	0.000
	N	10	10	10	10

Correlation is significant at the 0.01 level (2-tailed)

The presence of cover at the feeding site, close enough for the birds to fly to for protection in case of danger made for increased feeding. Contrary to Barta *et al.* (2004), *Passer griseus* does not avoid feeding in fields that are more than a meter away from cover. They rather increase their rate of scanning. This result confirms the existence of a scanning-group size effect in flock of Northern grey-headed sparrows similar to what had been previously reported by Schmaltz (2001). Habitat selection by birds is mostly based on the nature of the feeding site and availability of food.

This is consistent with the findings of Strong and Sherry (2000) and Fayat (2003), who recorded that the distribution and availability of food is a principle factor influencing habitat selection. Although risk predation is sometimes considered, it can be diluted by the presence of a large group size. The relationship between group size and vigilance is very useful in the study of the behavior of birds. As the number of birds per site increased, the rate of vigilance reduced, while the feeding rate increased.

In the course of the study, *Passer griseus* was observed to often form flocks with other passerine bird species, such as the Pin-

tailed Whydah (*Vidua macroura*) and a species of the finches' family. They foraged peacefully with the finches, but the pin-tailed whydah will often intrude and interrupt the sparrows' feeding. The Pin-tailed whydah will either chase the sparrows off a patch or off the site completely. The pin-tailed Whydah is known by many agriculturists and field biologists to be very pugnacious towards other species, chasing all manner of birds, both small and large (Schulenberg, 2010). By doing this, they reduced the amount of time spent by the sparrows on feeding.

Conclusion: This study has shown that group size contributes to the foraging behavior of the Northern grey-headed sparrow in fields. It also has shown that scanning-group size affects foraging behavior. However, group size and vigilance are the major parameters that influence the foraging behavior of Northern grey-headed sparrows. Microhabitat selection is not much of a factor influencing their foraging behavior rather they forage in areas having food resource of choice.

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PREVALENCE, KNOWLEDGE ATTITUDE AND PRACTICES ASSOCIATED WITH ONCHOCERCIASIS IN ENUGU EAST AND NKANU WEST LOCAL GOVERNMENT AREAS OF ENUGU STATE, NIGERIA

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ABSTRACT

Onchocerciasis is still endemic in Enugu East and Nkanu West Local Government Areas of Enugu State. In this survey, the Knowledge, Attitude and Practice (KAP) and prevalence of onchocerciasis were evaluated. For the KAP aspect of this survey, Interviewers administered questionnaire was used as instrument for collecting information from Respondents who participated in the skin snipping exercise. Skin snips were collected for the prevalence survey. A total of 149 persons participated in the prevalence survey out of which 88 participated in the KAP survey. Correct knowledge of causative agent, mode of transmission and treatment of the disease were 21.60%, 21.60%, 22.72% respectively, which is quite low; while vector control and intake of ivermectin were 77.27% and 37.50% respectively. The result of the microscopic analysis of skin snips showed zero prevalence for both communities. However, palpitation exercise conducted just before skin snipping revealed the presence of nodules on some of the participants. Therefore a community based health education intervention on vector control, cause, mode of transmission and treatment of the disease to improved KAP on onchocerciasis as well as confirmatory analysis of skin snips using Polymerase Chain Reaction (PCR) is recommended.

Keywords: Onchocerciasis, *Onchocerca volvulus*, Mode of transmission, Disease treatment, Ivermectin, Knowledge Attitude and Practices (KAP), Skin snips

INTRODUCTION

Onchocerciasis is a chronic parasitic disease caused by the filarial worm, *Onchocerca volvulus*. The disease is transmitted from man to man through the bites of the blackfly *Simulium* of the family *Simuliidae* (Eezzuduemhoi and Wilson, 2013). The disease is increasingly recognized as one of the most endemic diseases in Sub-Saharan Africa, particularly in West Africa. In Nigeria, onchocerciasis is widespread and a cause of blindness in most rural communities (Opara *et al.*, 2008). Onchocerciasis has a focal distribution in Africa,

Yemen and Central America. It is endemic in West Africa, in equatorial and East Africa and in Sudan. One of the largest endemic regions occurs in the Volta River Basin area. About 99% of Onchocerciasis cases occur in Africa and most of these cases occur in Nigeria (Adetokunbo and Herbert, 2003). The disease has been shown to be endemic in Oji Local Government of Enugu State, situated in the rain forest belt of Nigeria (Manafa and Isamah, 2013).

Nigeria has the largest number of persons with onchocerciasis, accounting for about a third of the global prevalence. As one of the largest countries in West Africa, she has

been reported to have a high incidence of onchocerciasis infection with 7 million persons infected with the disease and 40 million at risk. Onchocerciasis, also known as river blindness remains a major cause of blindness with about 270, 000 blinded and approximately half a million persons visually impaired due to the disease (WHOa, 2013). The fear of blindness resulted in depopulation of fertile river valleys, thereby making Onchocerciasis a major obstacle to socio-economic development in the savannah regions of West Africa. The fertility of riverine lands and associated high blindness rate are opposing forces which respectively attract and repel human settlement along fast flowing rivers near vector breeding sites. The opposing forces would account for a relatively high human density in hypo-endemic zones where the advantage of fertile land outweighs the risk of infection (WHO/APOC, 2013).

Although onchocerciasis has existed in Nigeria for centuries, it was not until 1908 that the first report was published. Since then, various authors (WHO, 2013; WHO, 2012; Hoerauf *et al.*, 2003) have contributed to the existing knowledge of its natural endemicity and the socio-economic importance of the disease (Nwoke, 2013). However, a study conducted by Manafa and Isamah, showed knowledge about the cause, prevention and complications of onchocerciasis among members of a community to be low and has resulted in misconceptions about the disease among the people. In developing countries such as Nigeria, there are complex set of beliefs and cultural values associated with health and illness. People's attitude to the cause, clinical manifestation, treatment and various preventive measures of a disease are influenced by their knowledge and perception of their conditions (Manafa *et al.*, 2002).

Taking into cognizance the public health significance of Onchocerciasis, such beliefs and values which affect their knowledge, attitude and practice need to be carefully examined. This will possibly, help to check the effectiveness of the onchocerciasis control programme in the area. This study was therefore carried out to determine the prevalence and KAP on onchocerciasis in the study area and to observe

the relationship pattern between both parameters. This will in turn help to check the effectiveness of and progress towards the sustainability of the Community Directed Treatment with Ivermectin (CDTI) programme.

MATERIALS AND METHODS

Study Areas

Nkanu West and Enugu East Local Government Areas are situated in Enugu State, popularly known as the Coal City State. It is a mainland state in south eastern Nigeria. Its capital is Enugu from which the State created from the old Anambra in 1991 derives its name. It covers an area of 7,161km² and has a population of 5,590,513 (NPC, 2006). The area is predominantly agricultural, with yam tubers, palm produce and rice being their main produce. Nkanu West LGA has its headquarters in the town of Agbani. It has an area of 225 km² and a population of 146,695 from the 2006 census (NPC, 2006). Enugu East has its headquarters in the town of Nkwo Nike. It covers an area of 383 km² and has a population of 279,089 from the 2006 census (Wikipedia, 2013).

Study Design

This study is a prevalence/cross-sectional study. The study was carried out in two phases - the pre-survey and the main survey - in Enugu East and Nkanu West local government areas of Enugu state. Using multistage sampling, Ugwogho Nike and Ndiagu-Akpugo communities were selected from Enugu East and Nkanu West LGAs respectively. Skin snippings were carried out in each community. Also, questionnaires on the knowledge, attitude and practice (KAP) on onchocerciasis were administered by interviewers to respondents in each of the selected communities.

Cross-Sectional Study

Study population: The study population was made up of adult males and females aged

above 5 years who are resident in the study areas.

Data collection method: Data collection for the KAP was done using questionnaires. The questionnaires were interviewer-administered with the help of trained interviewers who were members of the research team as well as trained health workers from the study communities.

Ethical considerations: Ethical clearance was sought from the Ethical Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State for the KAP study and the Enugu State Ministry of Health. Consent of each participant was sought and obtained verbally after explaining to them the nature and importance of the study.

Pre-survey: The pre-survey visits were made to the two communities chosen for the study. The nature and importance of the survey was explained to the traditional leaders and leaders of the farm settlements. The public health importance of the survey was explained to them and a date was fixed for the survey.

Main survey: In the main survey, participants first presented themselves for palpitation in which the upper parts of their bodies were felt for presence of nodules. Afterwards, skin snips were collected from the left and right iliac crest of each participant twice with the use of corneoscleral punch and placed in the wells (containing normal saline solution) microtitre plates, blood was also collected on filter paper for macroscopic analysis. Each microtitre plate contains 96 wells. Each well was carefully numbered, and a pair of wells was labeled for skin snips taken from the left and right sides of each participant. The microtitre plates were then covered and sealed with transparent tape in order to prevent a spill over during movement. Skin snips were viewed under the microscope, after some hours, for presence of microfilariae. Questionnaires on KAP on onchocerciasis were also administered in both English and Igbo (native dialect) by the research team, assisted by health workers from

the community health centers to those who participated in the skin snipping exercise.

Data Analysis: Skin snips were analyzed using the microscopy. Using micro pipette, part of the saline solution containing skin snip was taken from each well of the microtitre plates and placed on carefully numbered clean slides, covered with cover slips and then viewed under a light microscope at magnifications of x40 and x100. Data collected on KAP from the survey were analyzed manually.

RESULTS

Samples of skin snips were collected from a total of 149 samples were analyzed. The result of samples analyzed showed that all samples analyzed were negative for microfilariae. Thus, the result of the microscopic analysis showed zero prevalence for both communities.

The result of the data analysis showed that out of 88 persons who participated in the KAP survey 16(18.18%) and 17(19.32%) were within the age brackets of 15-24 and 35-44 respectively. Contributions by other age groups were 25 – 34 years 14(15.91%); 45 – 54 years 12(13.64%), 55 – 64 years 14(15.91%) and above 64 years 15(17.04%). The male respondents were 42(47.73%), while the female respondents were 46(52.27%). A total of 40 (45.45%) had primary education; 2(2.27%) had tertiary education while 26(29.55%) were non-educated. Fifty eight respondents representing 68.91% were mostly subsistent farmers, 63(71.60%) were married, while 18(20.45%) were single.

Only 21(24.0%) were aware that onchocerciasis (*isi anya ocha* in Igbo) is a disease and 54(61.40%) claimed that they have not heard of the disease before. A total of 19 persons (21.60%) could identify filarial worm as the causative agent of the disease, while 19(21.60%) of the respondents know that the disease is transmitted through the bite of an infected black fly. Twenty of the respondents representing 22.72% could identify Ivermectin (Mectizan) as the drug of choice for the treatment of the disease.

Moreover, only 27(30.68%) of the respondents claimed that they had at one time or the other participated in the CDTI programme and a smaller number, 33(37.50%), of them have at one time or the other received ivermectin from the Community Directed Distributors (CDDs). However, 50(56.80%) admitted the importance of regular intake of the drug i.e. twice every year for 15 years. About 21(23.86%) claimed that black flies interferes with their occupation and 68(77.27%) claim to control the vector by protecting themselves from the bites of black flies when they go into the bush or near fast flowing streams or rivers. Only 33(37.50%) actually take the drug, ivermectin (Mectizan), but 40(45.45%) claimed to have not missed any dosing round for Mectizan.

Table 1: Demographics of respondents

Characteristics	Number	Frequency %
Age		
15 – 24	16	18.18
25 – 34	14	15.91
35 – 44	17	19.32
45 – 54	12	13.64
55 – 64	14	15.91
Above 64 years	15	17.04
Educational Status		
Non-formal	26	29.55
Primary	40	45.45
Secondary	20	22.73
Tertiary	2	2.27
Sex		
Female	46	52.27
Male	42	47.73
Occupation		
Farming	58	65.91
Civil Servant	1	1.14
Student	12	13.64
Others	9	10.23
None	3	3.40
Trading	5	5.68
Marital Status		
Single	18	20.45
Married	63	71.60
Non response	7	7.95
Total	88	100

DISCUSSION

This study examined the prevalence and KAP on onchocerciasis among residents of Ugwogho

Nike farm settlements Enugu East LGA and Ndiagu-Akpugo in Nkanu West LGA. Majority of the respondents in this study are farmers 58 (65.91%) and their educational status is mostly at the primary school level 40 (45.45%). Observations made from this study revealed that the knowledge of respondents on the disease tends to increase as their educational status increases. Knowledge on onchocerciasis is relatively low in the study areas. This is in line with a study carried out in South western Nigeria in which knowledge of the disease among respondents is extremely low (Richards *et al.*, 1995).

The findings from another study in Oji River also revealed knowledge on onchocerciasis to be low among respondents (Adeoye *et al.*, 2010), which supports the findings from this study.

The result of this study showed that majority of the respondents do not take ivermectin 50(56.82%) and only 27(30.68%) claimed to have participated in the CDTI programme. However, a larger percentage of the Respondents 68(77.27%) said that they control the disease by protecting themselves from the bites of blackflies in that they put on clothes like shirts and blouses with long sleeves, long robes and trousers that cover their arms and legs. This practice can give us a clue as to the reason for the negative results from the microscopic analysis despite the low intake of ivermectin in the area. Although 50(56.82%) admitted the importance of regular intake of ivermectin twice every year for 15 years (Mbanefo *et al.*, 2010), the actual intake of ivermectin was very low (37.50%) among respondents. In addition, only 33(37.50%) took Mectizan, but 40(45.45%) claimed to have not missed any dosing round for Mectizan. Major reasons given by respondents for non-intake of Mectizan is that there are not enough drugs to go round. Also, a general belief which seems to pervade the communities is that the drug is only for people with eye problems. This may also be the reason why although more than average claimed not to have missed any dosing round, only a few take the drug. This may mean that about 17.05% of the respondents probably receive the drug from CDDs during the annual

Table 2: Responses on prevalence, knowledge attitude and practices associated with onchocerciasis in Enugu East and Nkanu West Local Government Areas of Enugu state, Nigeria

Variable	Frequency	%
Awareness of Onchocerciasis as a disease		
Yes	21	24.00
No	67	76.00
Heard of Onchocerciasis Before?		
Yes	54	61.40
No	34	38.60
Causative agent of Onchocerciasis		
Filarial worm	19	21.60
Drinking contaminated water	7	7.95
Bad blood from mosquitoes	10	11.36
Strange object in the body	1	1.14
Witchcraft	3	3.41
No idea	48	54.54
Mode of transmission		
Person to person contact	7	7.95
Bite of black fly	19	21.60
Bite of mosquito	11	12.50
Cannot be transmitted	5	5.68
Drinking contaminated water	5	5.68
No idea	41	46.59
Prevention		
Protection from bite of black flies	11	12.50
Protection from mosquito bites	12	13.64
Avoid contact with infected persons	7	7.95
Chains and concoctions	2	2.27
Drinking clean water	3	3.40
No idea	48	54.54
Treatment		
Malaria drug	8	9.10
Mectizan (Ivermectin)	20	22.72
Herbs and concoctions	8	9.10
Blood tonic	2	2.27
No treatment	4	4.54
No idea	46	52.27
Participation in CDTI programme		
Yes	27	30.68
No	57	64.77
Non response	4	4.54
Intake of Ivermectin (Mectizan)		
Yes	33	37.50
No	50	56.82
Non response	5	5.68
Importance of Regular Uptake		
Yes	50	56.80
No	38	43.18
Missed any Dosing Round?		
Yes	48	54.55
No	40	45.45
Control of black flies		
Yes	68	77.27
No	15	17.0
Non response	5	5.68

dosing with ivermectin without actually taking it. An interesting finding in the course of this research is a belief in the ability of ivermectin to cure rheumatism and conjunctivitis. Some of the respondents admitted taking the drug for relief of pains due to rheumatism. However, this belief needs to be carefully investigated in order to ascertain the validity of such claims. On the other hand, it was observed in this survey that majority of the respondents in Ndiagu-Akpugo are aware of onchocerciasis, but their knowledge of aetiology, treatment and control of the disease is quite low. Since peoples' attitude to the cause of, clinical manifestations, treatment and various preventive measures of a disease are influenced by their knowledge of their condition (Manafa *et al.*, 2002), and considering the low level of knowledge of onchocerciasis among respondents in this study, residents of the study areas needed to be exposed to health education regarding issues of disease causation, treatment, vector biology and control (Mbanefo *et al.*, 2010). Health education has been recommended as a way of influencing peoples' knowledge about onchocerciasis (Adeoye *et al.*, 2010). This will in turn influence their attitude and practice on the disease. This survey revealed that in both communities, some of the respondents complained of body itching, and palpitation

done before skin snipping showed the presence of nodules. Although the result of the microscopic analysis showed zero prevalence for onchocerciasis in the communities, the relatively low sensitivity and specificity of the microscopic analysis to polymerase chain reaction (PCR) analysis suggests the need for further confirmatory analysis using PCR technique (Fink *et al.*, 2011).

Conclusion: This study showed the KAP of respondents to be low. Although palpitations done just before skin snipping showed presence of nodules on some of the participants, microscopic analysis of skin snips gave zero prevalence for the disease. We therefore recommend that a confirmatory PCR analysis be carried out on the samples which were stored in ethanol and on the blood smears collected on filter paper. Also, health education intervention needs to be carried out in the study areas so as to improve the residents' knowledge on onchocerciasis. This will in turn improve their attitude and practice on the disease and also improve the health seeking behavior of Residents of the communities.

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HOUSEFLY-BORNE HELMINTH PARASITES OF MOUAU AND ITS PUBLIC HEALTH IMPLICATION FOR THE UNIVERSITY COMMUNITY

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ABSTRACT

*The parasitic load on houseflies (*Musca domestica*) in Michael Okpara University of Agriculture Umudike was investigated, with the view to finding out the public health implications for the university community. A total of 544 houseflies were captured and examined for parasitic loads, using concentration/floating technique for detection of parasites. The highest fly abundance recorded was 302, captured from the farm centre, followed by 219 captured from the hostel; the lowest was 23 captured from the canteen. Parasite species found were all helminthes as represented by *Ascaris lumbricoides*, *Necator americanus* and *Fasciola hepatica*, all in ova form. *Ascaris lumbricoides* had the highest percentage prevalence of 54.54%, followed by *Necator americanus* 42.42%, and *Fasciola hepatica* 3.03%. A simple chi square test was carried out and the results indicated a significance difference in the prevalence of flies and parasites recovered from the sites. Based on this, it is therefore recommended that health education -on the dangers of being infected, mode of transmission of these parasites and prevention-should be intensified within the university to avert possible disease outbreak.*

Keywords: Houseflies, Parasitic load, Public health, Helminths, Health education

INTRODUCTION

The housefly (*Musca domestica*) is a fly of the suborder Cyclorrhapha. It is the most common of all domestic flies, accounting for about 90% of all flies in human habitation all over the world (Nmorsi *et al.*, 2006); and indeed one of the most widely distributed insects, found all over the world. It is considered a pest that can transmit serious diseases. According to Service (2004), about 170 genera and 4200 species in the family Muscidae are recognized, some of which are medically important including the housefly, *M. domestica*. It is a typical example of synanthropic animal, one that lives in association with humans (Subejo, 2010). It is considered one of the most important pests which cause health problems in the environment

as it accompanies human during their daily activity everywhere, on work site or in rest places causing disturbances to them (Howard, 2011). Housefly imposes itself on human and all what is available, food and waste and is considered as very dangerous to public health and causes economic problems to farm animals (Service, 1980). House flies move around mostly during the day and like warm places and showing preference for direct sunshine. Their filthy habits, culminating in their indiscriminate movements between filth and food and defecation while feeding, make houseflies efficient transmitters of germs (Olsen, 1998). The role of house flies in the transmission of helminth eggs, that is, *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Toxocara canis* and *Strongyloides stercoralis*,

protozoan cysts and trophozoites such as *Entamoeba histolytica*, *Giardia* species, *Trichomonas* species, *Taenia* species, *Hymenolepsis* species, *Dipylidium* species, *Diphyllobothrium* species and bacteria such as *Shigella* species, *Escherichia coli* is well documented (Graczyk *et al.*, 1999; Mullen and Durden, 2002).

Besides contaminating food with eggs and maggots, flies can carry bacteria that cause intestinal diseases. Flies can travel from faecal materials to our food very easily, carrying bacteria with them on body hairs or the sticky pads on their feet. When feeding, flies expel saliva and faeces that may also contain bacteria. Sometimes flies lay eggs or maggots on the flesh or wounds of man and animals. Since housefly feed on contaminated substances such as human and animal excreta, sputum, excretion from wound, the flies can carry pathogens on their spongy mouthpart, body, and leg hairs, which is directly transmitted to the next visited surface e.g. human food (Manzon and Sanchoz, 1997).

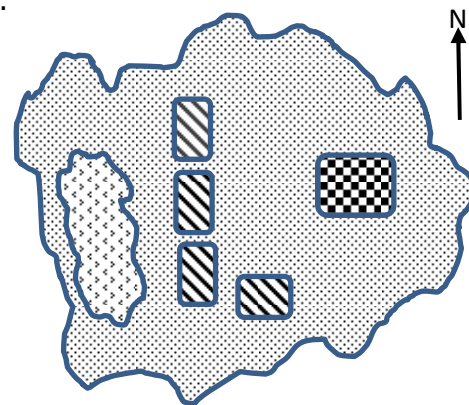
The abundance of housefly causes important nuisance by disturbing people during work and at leisure. It has a negative psychological impact because their presence is considered a sign of unhygienic conditions. Houseflies spread diseases because they feed freely on human food and filthy matter alike. The fly picks up disease-causing organisms while crawling and feeding, they contaminate food material; water, kitchen utensil, animal feed etc, humans and animal are infected by eating contaminated food. These contaminated food materials cause bacterial diseases like typhoid, cholera, dysentery, and viral diseases like poliomyelitis, viral hepatitis.

Despite the abundance of house flies in Michael Okpara University of Agriculture Umudike-necessitated by the recent increase in number of students and staff,-no scientific information exists on the parasitic load on house flies within the University and the potentialities they hold in transmission of pathogenic organisms- capable of causing serious public health problems to entire University community.

MATERIALS AND METHODS

The study was conducted in Michael Okpara University of Agriculture, Umudike. Umudike is a community in Abia State, Nigeria and about 10 kilometers of Southeast of Umuahia the state capital. Umudike is located on latitude 5° 28' 33"N and longitude 7° 32' 66" E. Monthly temperature ranges between 25 – 32 °C. Total annual rainfall ranges from 1700 to 2100 mm (Nwokocha *et al.*, 2006).

Housefly Collection: The houseflies were collected from the different synanthropic spots within the university. These sites include the female hostel, farm centre and canteen (Figure 1).





Keys:  Hostel  Canteens  Farm centre

Figure 1: Houseflies collection sites in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Houseflies were captured using trapping method, the traps were made with a cylindrical container and a cone shaped paper cover. Fresh meat and fish were put into the trap to serve as bait that attracted the flies that were captured afterwards. The collection process was done for five weeks (between October and November, 2015) and carried out between 9 am and 4 pm. The houseflies were collected at intervals of one hour for each of the traps, which were located around the hostels, farm center and canteen. The captured flies were taken to the laboratory of Zoology and Environmental Biology Department, Michael Okpara University of Agriculture, Umudike for analysis of helminth associated with house fly.

Preparation and Technique: Formaldehyde was poured on sample (houseflies) to prevent decay after capturing. Concentration/floating technique for detection of parasites and ova was used. The flies were washed with the formaldehyde so as to obtain the parasites on their exoskeleton (body), which was decanted afterwards. 1 ml of the decanted solution was put in a test tube and was filled with Willis Solution (common salt solution). A cover slip was placed on the bream of the test tube. The principle behind this technique is that, the Willis solution reduces the density of the parasites enabling them to float to the bream of the test tube, which is collected by a cover slip placed on a glass slide containing iodine and was viewed under the microscope using the oil immersion of the microscope. Identifications were made using color atlas of parasitology by Sullivan (2009). A simple chi square test was used to test if there was a significant difference in the prevalence of the parasites species on the houseflies, based on locations.

RESULTS

The results gleaned from the research incriminate *Musca domestica* as the carrier of some of the pathogens within the university. A total number of 544 flies were captured and the highest number of flies was recorded from the farm centre (n = 302). This is followed by the numbers recorded from the hostel and canteen (n = 219, and 23 respectively) as listed in Table 1.

Table 1: Overall percentage abundance of houseflies per sampling sites in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (n=544)

Site	Number of flies trapped	Percentage abundance per site
Farm center	302	55.51
Hostel	219	40.26
Canteen	23	4.23
Total	544	100%

A total of 3 species of parasites were obtained on examination of the flies to establish their parasitic load; *Ascaris lumbricoides*, hookworm

(*Necator americanus*), and *Fasciola hepatica* these are listed in Table 2. These were observed as eggs, as no adult stages were recovered.

Table 2: Parasites stages recovered from houseflies in sampling sites in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Parasite organism	Phylum	Form seen
<i>Ascaris lumbricoides</i>	Nematoda	Egg
<i>Fasciola hepatica</i>	Platyhelminths	Egg
<i>Necator americanus</i>	Nematoda	Egg

Ascaris lumbricoides has the highest percentage prevalence with parasitic load of 54.54 %, Hookworm (*Necator americanus*) 42.42 % and *Fasciola hepatica*, 3.03% as shown in Table 3.

The overall percentage abundance of parasites on vector per site was recorded as follows: farm center has a total of 5.95%, the hostel was 5.94 % and the canteen had 8.70 % as listed in Table 4.

Table 5 showed the percentage abundance of each parasite on vector per site. A simple chi square test was used to test if there was a significant difference in the prevalence of the parasites species on the houseflies, based on locations and it was found that there was a significant difference in the prevalence of the parasite species based on location. It appeared that the prevalence of the parasites depended on the breeding sites of vectors. The same was done on the prevalence of houseflies; and there was a significant difference in the prevalence of houseflies based on location, as was the case in the parasites; showing that the prevalence of flies depended on the breeding sites. The values for the percentage weekly abundance of flies per location are shown in Table 6. Week 2 had the highest weekly abundance of 78.77, while week 5 had the lowest weekly abundance of 33.39.

DISCUSSION

The study showed that houseflies carry some parasites on their body. Ova of three parasites

Table 3: Prevalence of all parasites collected from different sites and the number of parasites found in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Site	<i>Ascaris lumbricoides</i>	<i>Fasciola hepatica</i>	<i>Necator americanus</i>	Total number of parasite
Farm center	12(36.36)	0(0)	6 (18.18)	18(54.54)
Hostel	5(15.15)	0(0)	8 (24.24)	13 (39.39)
Canteen	1(3.03)	1(3.03)	0(0)	2(6.06)
Total	18(54.54)	1(3.03)	14(42.42)	33(100)

n = 33, Number in parenthesis = percentage

Table 4: Overall percentage abundance of parasites on vectors per sampled site in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Site	Number of flies examined	Number of parasites	Percentage of parasites on vectors
Farm center	302	18	5.96
Hostel	219	13	5.94
Canteen	23	2	8.70
Total	544	33	6.07

Table 5: Percentage abundance of each parasite on housefly by site in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Sites	Number of flies examined	<i>Ascaris lumbricoides</i>	<i>Necator americanus</i>	<i>Fasciola hepatica</i>
Farm center	302	12(3.97)	6 (1.99)	0 (0.00)
Hostel	219	5(2.28)	8 (3.65)	0 (0.00)
Canteen	23	1(4.35)	0 (0.00)	1 (4.35)
Total	544	18(3.31)	14 (2.57)	1(0.18)

Number in parenthesis = percentage

Table 6: Weekly percentage abundance of houseflies per sampled site in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Period	Sampled site			Total
	Farm Centre	Hostel	Canteen	
Week 1	30 (9.93)	25(11.42)	10(43.)	64.2
Week 2	102(33.7)	70(31.96)	3(13.0)	78.77
Week 3	55 (18.21)	49(22.37)	5(21.7)	62.32
Week 4	94 (21.12)	31(14.16)	4(17.3)	52.7
Week 5	21 (6.95)	44(20.09)	1 (4.35)	33.9
Total	302(55.5)	219(40.2)	23(4.2)	100

Number in parenthesis = percentage

were found associated with house fly were *Ascaris lumbricoides*, hookworm (*Necator americanus*) and *Fasciola hepatica*, which was in agreement with earlier reports of Ajero and Nwoke (2007) and Wanna *et al.* (2008), where they reported the presence of these parasites in houseflies. The implication of status of houseflies in the transmission of helminth eggs is of serious public health concern to the University community, since houseflies are known to live in close association with human

beings. Houseflies are common around the household, in garbage and in human and animal excreta; they are vectors of pathogens (Getacherv *et al.*, 2007).

Among the parasites that were recovered from captured flies, *Ascaris lumbricoides* had the highest percentage prevalence of 54.54 %, followed by hookworm (*Necator americanus*) 42.42 % and *Fasciola hepatica* 3.03 %. *Ascaris lumbricoides* is a species of roundworm associated with

ascariasis. *Ascaris* is the most common roundworm infection. According to the WHO (2012), as many as one billion people were infected by *Ascaris lumbricoides* worldwide, this figure was alarming and confirmed the large number seen in this study. Ascariasis is highly prevalent in places without modern sanitation like the sites where this study was carried out. According to the Center for Disease Control, hookworm infections occur in an estimated 576 to 740 million people worldwide (CDC, 2010). It mainly affects people in developing nations in the tropics and subtropics due to poor sanitation (CDC, 2010). The poor sanitary conditions of the farm centre and hostel which yielded the highest number of hookworm confirmed earlier reports (Getacherv *et al*, 2007; CDC, 2010; WHO, 2012).

Fasciola hepatica which causes fasciolosis is now recognized as an emerging human disease. WHO (2009) had estimated 2.4 million people infected with *Fasciola*, and a further 180 million were at risk of infection. This number was comparatively low and in line with the small number of *Fasciola hepatica* (3.03 %) obtained in this study.

The percentage abundance of parasites on flies per site showed that increased vector abundance does not necessarily indicate the increased parasite abundance. For instance the total number of flies captured from the farm center was 302 while, percentage of parasite on them was 5.96%, the hostel was 219 with a parasites percentage of 5.94% and total of 23 flies were captured in the canteen with parasites percentage of 8.70%. This therefore means that the location where the vectors were captured determined the parasitic load. The flies captured around the hostel and canteens showed more parasites prevalence irrespective of the fewer number of flies, this may be as a result of improper disposal of waste, making the surrounding unhygienic.

The abundance of flies was more in the farm center than the other locations because cattle have a distinct smell and flies get attracted to it (Bursell, 1998). Houseflies are numerous in areas with large animal population due to the presence of animal fecal matter. The flies are attracted to the hostels due to the

decomposing trash and other food waste. The inability to maintain good sanitation leads to an increase in population of houseflies especially in warm tropical countries.

This study confirmed that housefly (*Musca domestica*) is a vector that transmits parasites to humans in Michael Okpara University of Agriculture, Umudike. Prevention and control of the morbidity and possible mortality associated with these housefly and parasitic infections and reduction can be based on chemotherapy, environmental sanitation, health education (WHO, 1998). Therefore, trash should be properly disposed into sealed containers; dumpers should be emptied regularly and kept as far away from buildings. Manure and other decaying animal materials should be promptly removed.

Conclusion and Recommendations:

Houseflies (*M. domestica*) have a negative psychological impact as they are considered as nuisance and a sign of unhygienic conditions. Houseflies spread diseases because they feed freely on human food and filthy matter alike. The flies pick up disease causing organisms while crawling, feeding and thereby contaminate food and drinks while feeding. These contaminated food materials cause bacterial disease like typhoid, cholera, dysentery and virus diseases like viral hepatitis.

Subsequently, the following measures may be taken to check the population of houseflies within the university and reduce the spread of disease causing organisms which they transmit and forestall possible disease outbreak. The recommendation includes (i) Sanitation or cultural control: Good sanitation is the basic step in any fly management program, (ii) The use of traps: They can be killed using an electrocuting grid, (iii) Biological control: Using biological preys like muscidifurax raptor wasp which feed on a fly puparium, thereby reducing the population, (iv) Integrated fly control: The use of insecticides against adult flies and (v) Health education on the dangers of being infected, mode of transmission of these parasites and prevention should be intensified in communities through the health centres. (vi) People can be told the importance of washing

hands after going to the toilet, (vii) Basic social amenities such as clean portable water, culturally acceptable means of disposal and treatment of human wastes and faeces from the principles vehicles of dissemination of the infective agents and (viii) Chemotherapy can also go a long way to take care of infection and so should be employed based on medical advice.

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MODULATION OF ENZYME ACTIVITIES FOLLOWING THE CO-ADMINISTRATION OF POTASSIUM BROMATE AND CHLOROQUINE IN SELECTED TISSUES AND SERUM OF ALBINO RATS

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ABSTRACT

The effect of administration of potassium bromate, chloroquine (membrane labilizers) and their co-administration on some cellular enzymes was investigated. The levels of activity of these enzymes were measured in the liver, kidney and serum 24 hours after 21 days of daily oral administration of potassium bromate, chloroquine and the co-administration of potassium bromate and chloroquine. The experimental animals were divided into four groups randomly. Group 1 rats were administered with distilled water and served as control. Group 2 were rats administered with 25 mg/kg body weight of potassium bromate. Group 3 were rats administered with 25 mg/kg body weight chloroquine, while rats in group 4 were co-administered with 25 mg/kg body weight of potassium bromate and chloroquine. This study investigated the effects of repeated administration of two potent membrane disruptors either alone or in combination on liver and kidney cellular enzymes and serum enzymes. Rats administered with potassium bromate exhibited some physical observations which include rapid breathing, diarrhoea and difficulty in movement, while rats administered with chloroquine exhibited hyperactivities. Results of enzyme activity determination showed significant decreases ($p < 0.05$) in activity of kidney and liver dehydrogenases (lactate and glutamate) as well as in the transaminases (ALT and ASP) when potassium bromate and chloroquine were separately administered. Similarly, alkaline phosphatase activity was significantly reduced ($p < 0.05$) in both tissues. Nonetheless, acid phosphatase (ACP) activity was not appreciably affected in both tissues. Corresponding significant increases ($p < 0.05$) in activity of these enzymes in the serum was observed. However, unexpected high values of enzyme activities in both tissues when both potassium bromate and chloroquine were co-administered were observed. The elevated level of enzyme activities in serum confirmed further the properties of potassium bromate and chloroquine as membrane labilizers causing the cellular enzymes to leak into the blood. Furthermore the results obtained pointed to a probable synergy in the properties of the two compounds when they were administered concurrently, thereby creating a kind of modulatory effect on the enzymes, hence the observed increases in enzymes activity in the tissues studied. It could be inferred from the results therefore that the intrinsic properties of chemical substances could be modulated or modified intracellularly when in interaction with other compounds and even with the cell system.

Keywords: Food additives, Chloroquine, Potassium bromate, Co-administration, Enzyme activity, Modulation

INTRODUCTION

In the food industry, food (chemical) additives are added purposely to enhance quality (Abdulmumeen *et al.*, 2012). While some are intentionally added, some others become part of food unintentionally occurring only in trace amount due to food packaging, storage and other handlings (Cavanaugh, 2002). Potassium bromate (KBrO₃), a white crystalline salt, soluble in water and only slightly soluble in alcohol but insoluble in ether (Kurokawa *et al.*, 1990) is a chemical food additive intentionally used in food industries, for baking and in confectionaries for improved product quality (Achukwu *et al.*, 2009; Abdulmumeen *et al.*, 2012). Desirable as it may, as a flour improver, there are many reported cases of toxicity involving potassium bromate. Being a strong oxidizing agent, potassium bromate is reported to cause disruption of the plasma membrane of cells (Akanji *et al.*, 2008). Also Kazeem (2009) reported the toxicity of acute oral administration of potassium bromate thereby supporting the work of (Akanji *et al.*, 2008) who reported the general organ toxicity of this compound. Mechanistic studies have also proposed that exposure to bromate causes renal toxicity in man and experimental animals (Uchida *et al.*, 2006) through peroxidation of membrane lipids and DNA damage (Adekoya *et al.*, 2011). Several other studies have established that potassium bromate is capable of causing damage to the plasma membrane of cells thereby causing such cells to release their internal contents to the extracellular environment (Akanji *et al.*, 2008; Olajide *et al.*, 2015).

Due to its common use, relatively large numbers of people are exposed to the compound just as incident of occupational exposure to potassium bromate may occur during its production and its use as food additive (Dennis *et al.*, 1994). From dietary exposure survey on potassium bromate in retail bread samples the presence of bromate was revealed in breads selected for such analysis (Denies *et al.*, 1994; Achukwu *et al.*, 2009).

Chloroquine is an antimalarial agent widely accepted all over the world (Sharma and Mishra, 1999; Izunya *et al.*, 2010; WHO, 2012;

Swagata *et al.*, 2014). It is also indicated in the treatment of rheumatoid arthritis and systemic lupus erythematosus (Dubois, 1978; Ducharme and Farinotti, 1996). Available data have shown that chloroquine is usually concentrated in some tissues such as the liver and kidney, following its oral administration (Adelusi and Salako, 1982; Ajani *et al.*, 2009; Izunya *et al.*, 2010). In toxic doses chloroquine had been reported to cause appreciable cellular damage to liver, kidney and heart muscle (Ngaha and Akanji, 1982; Izunya *et al.*, 2010), thereby affecting the activities of the tissues cellular enzymes (Malomo *et al.*, 1993).

Experimental reports have shown the toxic effect of chloroquine on kidney function when taken either during treatment or prophylaxis of malaria and even when administered acutely or chronically to rats. This is an action suggested to be probably due to its accumulation in kidney cells (Musabayane *et al.*, 1993; 2000a; Cooper and Magwere, 2008) or due to its deposition in the adrenal glands which may indirectly affect the kidney functions through modulation of the secretion patterns of aldosterone causing a reduction in tubular Na⁺ handling (Cooper and Magwere, 2008). The co-administration of chloroquine with other drugs or chemicals have been investigated and have been found to result in adverse effect to the kidney (Musabayane *et al.*, 2000b). For example, concurrent administration of chloroquine and ethanol was discovered to induce extensive damage to the proximal tubule and collective duct cells of the kidney (Musabayane *et al.*, 2000b; Cooper and Magwere, 2008)

The paucity of information on the effects of co-administration of bromate and chloroquine on liver and kidney enzymes aroused our interest in this study. Therefore, the study investigated some of the biochemical implications of the co-administration of bromate and chloroquine on rat tissues because the two compounds are often encountered in Africa sub regions in particular as component of processed food and as anti-malarial respectively.

This study therefore investigated the activities of some important enzymes (phosphatases, transaminases and

dehydrogenases) in diagnosis of organ functions following the co-administration of potassium bromate and chloroquine to rats. The liver tissues play vital role in drug metabolism because it houses most of the drug metabolizing enzymes while the kidney functions in maintenance of cellular homeostasis. The serum is the physiological fluid in which lost substances of tissue origin due to damage are deposited.

MATERIALS AND METHODS

Animals: Twenty male white albino rats (*Rattus norvegicus*) of wistar strain with an average weight of 200 ± 5.0 grams were purchased from the Small Animal House of the University of Nigeria, Nsukka, Nigeria, and used for the study. The animals were kept in separate aluminium made metabolic cages in a well-ventilated room and were subjected to 12 hours light/12 hours darkness with relative humidity 45 – 60 % at temperature of $26 \pm 3^{\circ}$ C. They were allowed free access to feed (Vital Feeds Nigerian Limited) and good drinking water on which they adapted to the environment for three weeks. The rats were handled and used in accordance with guidelines of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe, 1986).

Assay kits: The assay kits for Lactate and glutamate dehydrogenases, alkaline and acid phosphatases, aspartate and alanine aminotransferases were obtained from Labkits - South Africa, Chemelex - Poland and Canovelles - Barcelona Spain. The potassium bromate used was a product of Labtech Chemicals Nigeria Limited, Lagos, CAS No. 7758-01-2, while the chloroquine used is a product of Evans Medical PLC, Agbara, Nigeria. All other reagents used were of analytical grade and were prepared in glass distilled water and stored in reagent bottles until required for use.

Bioassay: The rats used for this study were randomly divided into four groups, replicated thrice with five rats per replicate. Potassium bromate and chloroquine solutions were

prepared in sterile distilled water to obtain the concentrations required and the solutions were given orally into rat groups as single daily dose as indicated below. Group 1 rats were administered with 1.0 ml water only and represent the control. Group 2 rats were administered with 1.0 ml solution 25 mg/kg body weight of potassium bromate. Group 3 rats were given 1.0 ml solution 25 mg/kg body weight chloroquine (CHQ) and group 4 rats were concurrently administered 1.0 ml each of 25 mg/kg body weight potassium bromate and 25 mg/kg body weight chloroquine. Administration of solutions of the compounds lasted for 21 days after which the rats from each groups were sacrificed 24 hours after the last dose.

Preparation of Serum and Tissue Homogenates:

After 21 days post doses administration, rats were fasted overnight and when due for sacrifice they were anaesthetized in desiccator containing cotton wool soaked in ether. The rats were quickly brought out of the desiccator and dissected. Blood was then withdrawn into clean and dried sample bottles by cardiac punctures. The blood was allowed to clot for 10 minutes at room temperature and thereafter centrifuged at 4000 rpm for 30 minutes (Yakubu *et al.*, 2005; Akanji *et al.*, 2008) using Heraeus-Christ GMBH Osterode refrigerated centrifuge. Sera were collected by aspiration into clean, dry sample bottles using Pasteur pipette. This was stored frozen until required for use (within 12 hours of preparation) (Yakubu and Musa, 2012). Thereafter, the rats were dissected and the organs of interest (liver and kidney) were excised into beakers containing ice-cold 0.25M sucrose solution.

Known weight (1.0 g) of the liver and kidney were respectively chopped into small pieces and then homogenised in 0.25 M sucrose solution using Tissues Tearor homogenizer Model 985370-375. The homogenates were diluted with 0.25 M sucrose solution and stored frozen until required.

Determination of Biochemical Parameters:

Enzymes that were assayed include lactate

dehydrogenase (Pesce, 1984), glutamate dehydrogenase (Delma, 1970), aspartate and alanine aminotransferases (Murray, 1984), alkaline phosphatase (Wenger *et al.*, 1984) and acid phosphatase (Abbott *et al.*, 1984).

Statistical Analysis: Data collected were analysed for their central tendencies and subjected to one way analysis of variance (ANOVA). The data were expressed as mean \pm standard error of mean. Graph Pad InStat (Data set 1. SD) was used for all analysis. The Duncan Multiple Range Test (DMRT) was used to separate significant differences among treatment means at 95 % level of confidence. For all the tests, values with $p < 0.05$ were considered to be of statistically significant (Mahajan, 1997; Yakubu and Musa, 2012).

RESULTS

The data on the effects of oral administration of 25 mg/kg body weight of potassium bromate ($KBrO_3$), chloroquine (CHQ) and their combination on activities of some enzymes in the liver, kidney and serum of rats are presented in Tables 1, 2 and 3, respectively.

Following the administration of potassium bromate to rats, both liver and kidney recorded significant decreases ($p < 0.05$) in lactate dehydrogenase (LDH) activities 60.19 % and 33.38 % respectively with corresponding significant increase ($p < 0.05$) (49.37 %) in the serum compared with the control. Similarly the oral administration of chloroquine produced significant decreases ($p < 0.05$) in activity of LDH in both tissues when compared with the control (Table 1).

Administration of chloroquine to rats also led to significant decrease in all the enzyme activities. However, when potassium bromate and chloroquine were co-administered the activity of LDH though lower than the control values but became insignificantly different in the liver when compared with the control but the activity was significantly reduced in the kidney (Table 1). The activities of glutamate dehydrogenase (GDH) provided a similar trend as LDH in both tissues and the serum (Tables 1, 2 and 3). However, the extent of loss recorded

in GDH activity in the tissues was not as pronounced as for LDH. The GDH activity values were almost comparable to the control value in the kidney tissues (Table 2).

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities recorded significant reduction ($p < 0.05$) in both tissues (>50 %) in all cases, following the separate administration of potassium bromate and chloroquine. However with the co-administration of the two compounds increased, enzyme activity was elicited by the compounds when the values were compared results obtained following the administration of bromate and chloroquine alone. The activity values for both enzymes were higher than the control value in the liver but lower in the kidney (Table 1 and 2). The serum values of all enzyme activities were significantly elevated in all the groups ($p < 0.05$).

Alkaline phosphatase recorded significant decreases ($p < 0.05$) in activities in both the liver and the kidney. However, the activity of acid phosphatase was not appreciably affected in both tissues in all the groups following all categories of test administrations (Tables 1, 2 and 3).

DISCUSSION

Upon acute or chronic administration of chemical substances such as drugs and other xenobiotics to experimental animals, both observable physical changes and non-observable biochemical changes manifest usually in the form of alterations in the normal cell functions of tissues, particularly those involved in active cellular metabolism. Such alterations in cell functions could eventually lead to altered structural integrity of the cells, thereby resulting to loss of the cell components to the extra cellular environment. Therefore enzyme activity changes in the liver, kidney and serum of albino rats was followed conventionally in this work to assess the effect of administration of bromate, chloroquine and their co-administration on the tissues. Enzyme assay is a rapid and revolutionary biochemical parameter for obtaining information about

Table 1: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected liver enzymes in albino rats

Activities in IU/L	LDH	GDH	AST	ALT	ALP	ACP
Groups						
Control	15.45±0.09 ^a	39.20±0.06 ^a	4.18±0.17 ^a	6.01±0.35 ^a	3.80±0.04 ^a	19.50±0.04 ^a
KBrO ₃	6.15±0.04 ^d	24.86±0.11 ^d	2.36±0.16 ^c	1.67±0.18 ^b	1.25±0.12 ^b	19.15±0.12 ^a
CHQ	8.15±0.03 ^c	28.22±0.02 ^c	3.60±0.33 ^c	2.40±0.12 ^b	1.65±0.10 ^b	10.80±0.06 ^b
KBrO ₃ + CHQ	13.85±0.05 ^a	35.24±0.23 ^b	5.53±0.14 ^b	6.95±0.23 ^d	3.57±0.03 ^a	19.30±0.01 ^a

Test values with different superscripts across the column are significantly different ($p < 0.05$). KBrO₃ = Potassium bromate; CHQ = Chloroquine

Table 2: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected kidney enzymes in albino rats

Activities in IU/L	LDH	GDH	AST	ALT	ALP	ACP
Groups						
Control	120.30±0.46 ^a	32.25±0.15 ^a	2.74±0.25 ^a	5.60±0.42 ^a	21.35±0.03 ^a	28.50±0.04 ^b
KBrO ₃	80.24±0.22 ^c	33.60±0.11 ^a	1.05±0.10 ^b	2.60±0.28 ^c	18.30±0.06 ^b	34.20±0.21 ^a
CHQ	87.25±0.25 ^b	30.85±0.03 ^b	0.50±0.06 ^c	2.15±0.13 ^c	10.96±0.10 ^c	22.46±0.62 ^c
KBrO ₃ + CHQ	42.60±0.11 ^d	32.05±0.09 ^a	1.20±0.13 ^b	4.50±0.36 ^b	8.71±0.44 ^d	26.73±0.22 ^c

Test values with different superscripts across the column are significantly different ($p < 0.05$). KBrO₃ = Potassium bromate; CHQ = Chloroquine

Table 3: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected serum enzymes in albino rats

Activities in IU/L	LDH	GDH	AST	ALT	ALP	ACP
Groups						
Control	8.44±0.07 ^c	0.23±0.008 ^b	5.96±0.25 ^b	6.37±1.04 ^b	0.17±0.02 ^b	0.15±0.02 ^a
KBrO ₃	12.60±0.14 ^a	0.30±0.016 ^a	14.76±0.80 ^a	15.08±0.50 ^a	0.34±0.03 ^a	0.15±0.06 ^a
CHQ	10.24±0.27 ^b	0.32±0.013 ^a	13.74±0.99 ^a	7.30±2.05 ^b	0.36±0.04 ^a	0.17±0.04 ^a
KBrO ₃ + CHQ	10.36±0.93 ^b	0.34±0.013 ^a	14.46±0.61 ^a	5.89±1.59 ^c	0.36±0.08 ^a	0.16±0.07 ^a

Test values with different superscripts across the column are significantly different ($p < 0.05$). KBrO₃ = Potassium bromate; CHQ = Chloroquine

tissues cellular integrity and it also plays significant role in disease investigation and diagnosis (Malomo, 2000; Yakubu *et al.*, 2003; Nnodin, 2012).

In relatively high doses both potassium bromate and chloroquine employed in this work have been reported to cause appreciable cellular damage to body tissues (Kukoyi *et al.*, 2000; Ajani *et al.*, 2009; Izunya *et al.*, 2010; Olajide *et al.*, 2014).

The significant level of decreases ($p < 0.05$) observed in the activity of alkaline phosphatase, ALP; (a membrane-localised enzyme) lactate dehydrogenase, LDH; aspartate and alanine amino-transferases (cytosol enzymes) in the organs following administration

of potassium bromate pre-supposes damage to the organised membrane structure of cells of the tissues under study. This is in support of the work of (Akanji *et al.*, 2008). The observation established possible damage to the cells structures and hence may be responsible for the loss by leakage of the enzymes to the extra cellular environment or its inhibition. However, the corresponding significant increase in activity of these enzymes in the serum supports loss of enzyme molecules from these tissues rather than inhibition of enzymes activities.

We observed similarly that when chloroquine was administered alone to the rats, the activities of the enzymes when compared with the control were significantly reduced in

the tissues, but with the level of reduction being more pronounced with administration of potassium bromate alone. Earlier loss in some of tissue enzymes was reported when chloroquine was co-administered with insulin to rats (Ajani *et al.*, 2009).

In most cases as reflected in this work, we observed that the liver enzymes were relatively more affected than the kidney following the administration of these compounds either alone or when in co-administration. However, acid phosphatase was by the least affected among the enzyme when potassium bromate and chloroquine phosphate were administered separately. The serum value of acid phosphatase (ACP) showed no significant difference ($p > 0.05$) compared with the control. This observation may be explained by the possession of individual organelle membrane by the lysosomes (Wright *et al.*, 1979; Akanji *et al.*, 2008), the organelle which houses the enzyme, or acid phosphatase due to the lysosomotropic status of chloroquine resulting in increase in size and number of liver lysosomes (Alfonso *et al.*, 1980; Greenspan and Dong, 1989; Zahid and Abidi, 2003; Andrey *et al.*, 2014).

The toxicity of potassium bromate and chloroquine and their abilities to cause damage to both cell membranes and tissues have been severally reported (Savarino *et al.*, 2006; Akanji *et al.*, 2008; Ajani *et al.*, 2009; Olajide *et al.*, 2014). The magnitude of loss of activity of glutamate dehydrogenase (GDH), a mitochondrial indicator of structural or membrane integrity (Yakubu *et al.*, 2003) vis-à-vis the loss of alanine and aspartate aminotransferases (cytosolic enzymes) may imply a reduction in the amount of energy made available to the cells (Akanji *et al.*, 2008) as well as impaired amino acid/protein metabolism. Chloroquine has been reported to cause decrease in activities of some cellular enzymes such as cytochrome a_3 and b , by acting as an uncoupler of oxidative phosphorylation. This role may adversely affect that of the mitochondria in energy transduction (Cooper and Magwere, 2008).

In the present study, we expected based on the individual intrinsic role of the two

compounds as membrane labilizers, an attendant significant decreases in enzyme activities in the tissues following the co-administration of the two compounds to rats.

However, contrary to our expectation when the two compounds were co-administered, it was of striking interest, our observation of relatively significant increase ($p < 0.05$) in values of enzyme activities in the tissues. These values were even higher than when each of potassium bromate and chloroquine was administered alone and this observation was more conspicuous in the liver than the kidney. This observation nullified the expected negative synergy effect of the co-administration of the two compounds. Furthermore, we observed that despite the high values of enzyme activities in the tissues, the activity of the enzymes are still relatively high in the serum. From the results of this study, since the serum enzyme activities were convincingly raised to the level that may suggest the leakage of enzymes from the tissues, due to damage, the following may be the most probable mechanisms to explain the observations in the tissues when both compounds were administered concurrently.

i. The two compounds may probably through coupled action caused the activation of these enzymes in the tissues resulting in the observed high values when compared with the control.

ii. Chloroquine while acting in its capacity may have enhanced the increased secretion of these enzymes. Chloroquine is known to impair receptor recycling and impairments of receptor recycling favours secretion (Alfonso *et al.*, 1980).

iii. A probable modulatory role exerted on the enzymes as a result of synergy effect of the co-administration through the generation of metabolite that may act as effector molecules or by providing a medium in which the binding of one molecule facilitates the binding of another substrate molecule leading to high activity of the enzymes in the tissues.

iv. Possible production of intermediate by the interaction of the two drugs that may favour the induction or denovo synthesis of the enzymes.

In our opinion and from the trend of the results obtained we conclude by considering a probable synergistic action of the two compounds (when co-administered). Such action possibly could have created a form of positive homotropic response (Conn and Stumpf, 1989; Richard and Dennis, 2011; Weil, 2013) causing the binding of one substrate molecule to probably influence or facilitate the binding of the next molecule by increasing the affinities of the vacant binding sites or by the direct effects of the chemical substances combined.

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HAEMATOLOGICAL PROFILE OF THE DOMESTIC PIGEON (*COLUMBA LIVIA DOMESTICA*) IN NSUKKA AGRO-ECOLOGICAL ZONE, ENUGU STATE, NIGERIA

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ABSTRACT

This study evaluated the haematological profile of the domestic pigeon (Columba livia domestica). Seventy five pigeons were acquired for the study from three locations (Ibagwa, Orba and Enugu-Ezike) where pigeons are raised in Nsukka agro-ecological zone, Enugu State, Nigeria, but after two weeks of acclimatization 64 of the pigeons (34 females and 30 males) that were considered apparently healthy were used for the study. One ml of blood was collected from each pigeon by jugular venipuncture for evaluation of the haematological parameters. The haematological characteristics of the pigeons were determined using standard methods. The overall mean values obtained for the haematological parameters were as follows: packed cell volume (PCV) – $44.54 \pm 4.73\%$; haemoglobin concentration (Hb) – 12.89 ± 1.55 g/dl; red blood cell (RBC) count – 3.34 ± 0.38 (10^6 /ul); mean corpuscular volume (MCV) – 133.86 ± 19.37 fl; mean corpuscular haemoglobin (MCH) – 38.67 ± 5.34 pg; mean corpuscular hemoglobin concentration (MCHC) – 28.97 ± 2.59 g/dl; leukocyte counts (10^3 /ul): total leukocyte – 23.36 ± 7.06 ; lymphocyte – 10.66 ± 3.49 , heterophil – 7.80 ± 2.89 , monocyte count – 2.32 ± 0.93 , eosinophil count – 2.25 ± 0.89 , and basophil – 0.24 ± 0.30 . There were no significant differences ($p>0.05$) between the males and females in all the haematological parameters evaluated and the body weights. There were however significant variations ($p<0.05$) in the PCV, absolute heterophil, monocyte and basophil counts, percentage lymphocyte, eosinophil and basophil counts of the pigeons from the three locations.

Keywords: Domestic pigeons, *Columba livia domestica*, Haematology

INTRODUCTION

The domestic pigeon (*Columba livia domestica*) was in ancient times raised as a source of meat, manure (fertilizer) and feather products and also was used for navigation and carrying messages during wars, but currently, pigeons are mostly used as pets, for sports, religious, social, ceremonial and ritual purposes and as laboratory animal models (Levi, 1974; Aggrey and Cheng, 1992; Vogel *et al.*, 1994). Pigeons also stand as a symbol of peace, love, purity,

innocence, the Holy Spirit and the soul of the deceased (Fakhri *et al.*, 2013). The use of pigeons during various national ceremonies as a symbol of peace is conspicuous, and the shift of attention from keeping pet dogs and/or cats to pet pigeons that are low cost to acquire, easy to maintain and are well appreciated by children (because they can fly) has brought the domestic pigeon to focus in recent times. There is also the increasing use of pigeons as laboratory models for experimental studies.

The evaluation of the haematological profile is of importance in animals and humans because the blood is the major transporter of substances in the body, and any deviations from normal caused by derangement of metabolic processes, invasion of the body by pathogens, deprivation, stress and other forms of injury/insult commonly translate to changes in the haematological parameters (Schalm *et al.*, 1975; Ihedioha, 2004; Ihedioha *et al.*, 2012). Specifically in birds, assessment of the haematology had been used for the evaluation of the state of health and nutrition, diagnosis of diseases, prognosis and the evaluation of the efficacy of therapeutic interventions (Campbell, 1994; 1998; Clark *et al.*, 2009; Ihedioha *et al.*, 2011).

Globally, there had been a focus of attention on zoonotic diseases and parasites that may be transmitted from street/feral pigeons to humans (Haag-Wackernagel and Moch, 2004; Haag-Wackernagel, 2005; Magnino *et al.*, 2008; Vasquez *et al.*, 2010; Geingenfeind *et al.*, 2012), to the detriment of appreciating the domestic pigeon as a preferred pet for some, laboratory animal model, and a commonly used ceremonial bird. Thus, apart from the numerous reports on diseases and parasites of street/feral pigeons cited above, there is a paucity of reports of studies on the domestic pigeon. In the area of haematology, there are some reports on the haematology of street/feral and racing pigeons (Pavlak *et al.*, 2005; Khan *et al.*, 2011; Opara *et al.*, 2012), and only few preliminary reports on the haematology of the domestic pigeon (Ritchie *et al.*, 1994; Lashev *et al.*, 2009), which are not comprehensive. The objective of this present study was to comprehensively evaluate the haematological profile of the domestic pigeon.

MATERIALS AND METHODS

A total of 75 domestic pigeons were acquired for the study from three major local breeders from whom pigeons are usually sourced in Nsukka agro-ecological zone, Enugu State, Nigeria. The breeders were located in Ibagwa (longitude 6°55.12' north, latitude 7°23.19' east), Orba (longitude 6°51.25' north, latitude

7°27.49' east) and Enugu-Ezike (longitude 6°58.69' north, latitude 7°24.74' east), Enugu State, Nigeria. Twenty five pigeons were sourced from each of the breeders/locations. Only adult pigeons were used for the study because the local breeders did not consider it humane and right to sell young ones and therefore refused to sell them out for the study. The pigeons were housed and acclimatized for two weeks in the Faculty of Veterinary Medicine Experimental Animal House, University of Nigeria, Nsukka. The university town of Nsukka is in Enugu State, Nigeria, and is situated within the derived savannah belt between latitudes 5° 50' and 7°00' north and longitudes 6°52' and 7°54' east, at an average elevation of approximately 500 m above sea level. It is an area of high temperature with yearly minimum and maximum temperature of 24.28° C and 32.19° C, with a mean of 28.24° C, and a relative humidity of about 70% during the rainy season that falls to about 20 % during the dry season.

During the period of acclimatization, the pigeons were examined individually and tagged. Those that showed any signs of abnormality of disorder were excluded from the study. The pigeons were fed *ad libitum* on pelletized growers mash (Vital Feed®, Grand Cereals and Oil Mills, PLC, Nigeria). Clean drinking water was also provided freely. At the end of acclimatization period, 11 of the pigeons were excluded from the study, and only 64 pigeons made up of 23 from Ibagwa, 21 from Orba and 20 from Enugu-Ezike were used for the study.

All through the study, the pigeons were humanely handled and all experimental procedures followed the University of Nigeria guidelines for handling of experimental animals. One millilitre of blood was collected from each pigeon by venipuncture of the jugular vein into a labeled sample bottle containing 1 mg of ethylene diamine tetra acetic acid (EDTA) anticoagulant. All haematological determinations followed standard procedures, and were done immediately upon collection of blood samples. Packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while haemoglobin concentration (HbC) was determined by the cyanomethaemoglobin method (Higgins *et al.*,

2008). Red blood cell (RBC) and total white blood cell (WBC) counts were done by the haemocytometer method using Natt and Herrick's solution as the diluting fluid (Campbell, 1994). The smears for differential leukocyte count were prepared and stained by the Leishman technique and enumerated by the battlement counting method (Thrall and Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Campbell, 1994). The body weights of the individual pigeons were measured and their sexes determined.

Statistical Analysis: Data generated from the study were subjected to descriptive statistics and presented as means \pm standard deviation (SD) with the minimum and maximum values. Differences between the sexes in all the parameters were analyzed for using students' t-test. Variations in the haematological parameters between the sources of pigeons were analyzed using one way analysis of variance, and the variant means were further separated using the least significant difference method post hoc. Significant differences were accepted at the probability level $p < 0.05$.

RESULTS

The overall means of the erythrocytic parameters of the domestic pigeons, with their minimum and maximum values were PCV (%) - 44.54 ± 4.73 [32.0 - 55.0], HbC (g/dl) - 12.89 ± 1.55 [7.76 - 16.00], and RBC counts ($10^6/\mu\text{l}$) - 3.34 ± 0.38 [2.12 - 3.95] (Table 1). There were no significant differences ($p > 0.05$) between the mean PCV, HbC and RBC counts of the males and females (Table 2). The mean PCV of pigeons sourced from Orba was significantly higher ($p < 0.05$) than that of pigeons obtained from Ibagwa, but the mean PCV of those obtained from Enugu-Ezike did not vary significantly from that of others (Table 3). There were however no significant variations ($p > 0.05$) in the HbC and RBC counts of pigeons sourced from Orba, Ibagwa and Enugu-Ezike (Tables 3).

For the mean red cell corpuscular values, the overalls, with their minimum and maximum values were MCV (fl) - 133.86 ± 19.37 [109.82 - 169.09], MCH (pg) - 38.67 ± 5.34 [26.86 - 50.41], and MCHC (g/dl) - 28.97 ± 2.59 [23.57 - 33.75] (Table 1). There were no significant differences ($p > 0.05$) in the MCV, MCH and MCHC between the male and female pigeons (Table 2), and no significant variations in these parameters between the pigeons sourced from the three different locations (Table 3).

The mean total WBC counts ($10^3/\mu\text{l}$) of the pigeons with the recorded minimum and maximum values were 23.36 ± 7.06 [12.50 - 35.50] (Table 4). There were no significant differences ($p < 0.05$) between the total WBC count of the male and female pigeons (Table 5), and no significant variations ($p > 0.05$) between that of pigeons obtained from the different locations (Table 6). For the percentage lymphocyte counts (%), the overall mean, and minimum and maximum values recorded for the pigeons were $45.76 \pm 4.77\%$ [32.00 - 58.00], while for the absolute lymphocyte counts ($10^3/\mu\text{l}$), the mean, and minimum and maximum values were 10.66 ± 3.49 [5.74 - 18.20] (Table 4). There were no significant differences ($p > 0.05$) between the percentage and absolute lymphocyte counts of the male and female pigeons (Table 5), but the percentage lymphocyte count of the pigeons obtained from Orba was significantly higher ($p < 0.05$) than that of pigeons obtained from other locations. There was however no significant variation ($p > 0.05$) in the means of the absolute lymphocyte counts of the domestic pigeons sourced from the three different locations (Table 6).

For the heterophil counts, the means of the percentage heterophil counts (%) of the pigeons with their minimum and maximum values were 33.37 ± 5.86 [18.00 - 50.00], while that of the absolute heterophil counts ($10^3/\mu\text{l}$) were 7.80 ± 2.89 [2.43 - 13.80] (Table 4). There was no significant difference ($p > 0.05$) in the percentage and absolute heterophil counts of the males and females (Table 5), and no significant variations ($p > 0.05$) in the percentage heterophil counts of the pigeons sourced from the three different locations (Table 6).

Table 1: The erythrocytic profile of domestic pigeons in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Mean	Minimum and maximum values
Packed cell volume (%)	44.54 ± 4.73	32.0 – 55.0
Haemoglobin concentration (g/dl)	12.89 ± 1.55	7.76 – 16.00
Red blood cell count (10 ⁶ /μl)	3.34 ± 0.38	2.12 – 3.95
Mean corpuscular volume (fl)	133.86 ± 19.37	109.82 – 169.09
Mean corpuscular haemoglobin (pg)	38.67 ± 5.34	26.86 – 50.41
Mean corpuscular haemoglobin conc. (g/dl)	28.97 ± 2.59	23.57 – 33.75

Table 2: Comparison of the erythrocytic profile of male and female domestic pigeons in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Sexes	
	Males (n = 30)	Females (n= 34)
Packed cell volume (%)	44.33 ± 4.85 [36.0 – 52.0]	44.66 ± 4.75 [32.0 – 55.0]
Haemoglobin concentration (g/dl)	13.15 ± 1.67 [9.43 – 15.84]	12.73 ± 1.47 [7.76 – 16.00]
Red blood cell count (10 ⁶ /μl)	3.43 ± 0.40 [2.31 – 3.95]	3.24 ± 0.74 [2.12 – 3.72]
Mean corpuscular volume (fl)	130.01 ± 19.99 [109.82 – 167.50]	138.02 ± 18.28 [113.43 – 169.09]
Mean corpuscular haemoglobin (pg)	38.35 ± 6.38 [26.86 – 50.04]	39.27 ± 4.69 [32.87 – 50.41]
Mean corpuscular haemoglobin concentration (g/dl)	29.72 ± 2.70 [23.57 – 33.75]	28.50 ± 2.46 [23.71 – 33.11]

Mean ± SD with minimum and maximum values in square brackets, No significant differences between the means of the males and females, $p > 0.05$

Table 3: Comparison of the erythrocytic profile of domestic pigeons sourced from different locations in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Locations		
	Ibagwa (n = 23)	Orba (n= 21)	Enugu-Ezike (n= 20)
Packed cell volume (%)	42.91 ± 4.32 ^a [32.0 – 49.0]	46.14 ± 5.81 ^b [35.0 – 55.0]	45.00 ± 3.12 ^{ab} [38.0 – 50.0]
Haemoglobin concentration (g/dl)	12.49 ± 2.06 [7.76 – 16.00]	13.35 ± 1.10 [10.88 – 15.56]	12.93 ± 0.97 [11.52 – 14.53]
Red blood cell count (10 ⁶ /μl)	3.24 ± 0.37 [2.12 – 3.75]	3.39 ± 0.39 [2.31 – 3.95]	3.36 ± 0.34 [2.46 – 3.85]
Mean corpuscular volume (fl)	133.18 ± 12.19 [113.43 – 158.62]	135.99 ± 19.53 [116.45 – 169.09]	134.02 ± 20.02 [109.82 – 166.23]
Mean corpuscular haemoglobin (pg)	38.58 ± 4.89 [26.86 – 47.27]	39.16 ± 4.52 [28.30 – 50.04]	38.48 ± 4.60 [34.71 – 50.41]
Mean corpuscular haemoglobin concentration (g/dl)	28.99 ± 0.62 [23.57 – 32.65]	28.93 ± 2.80 [23.71 – 33.75]	28.68 ± 1.94 [25.60 – 31.60]

Mean ± SD with minimum and maximum values in square brackets; ^{a b} Different superscripts in a row indicate significant differences between the means, $p < 0.05$

Table 4: The leukocytic profile of domestic pigeons in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Mean	Minimum and maximum values
Total leukocyte count ($10^3/\mu\text{l}$)	23.36 \pm 7.06	12.50 – 35.50
Percentage lymphocyte count (%)	45.76 \pm 4.77	32.00 – 58.00
Absolute lymphocyte count ($10^3/\mu\text{l}$)	10.66 \pm 3.49	5.74 – 18.20
Percentage heterophil count (%)	33.37 \pm 5.86	18.00 – 50.00
Absolute heterophil count ($10^3/\mu\text{l}$)	7.80 \pm 2.89	2.43 – 13.80
Percentage monocyte count (%)	10.04 \pm 3.02	5.00 – 19.00
Absolute monocyte count ($10^3/\mu\text{l}$)	2.32 \pm 0.93	0.63 – 4.09
Percentage eosinophil count (%)	9.83 \pm 2.77	4.00 – 17.00
Absolute eosinophil count ($10^3/\mu\text{l}$)	2.25 \pm 0.89	0.90 – 4.76
Percentage basophil count (%)	1.02 \pm 1.12	0.00 – 5.00
Absolute basophil count ($10^3/\mu\text{l}$)	0.24 \pm 0.30	0.00 – 1.70

Table 5: Comparison of the leukocytic profile of male and female domestic pigeons in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Sexes	
	Males (n = 30)	Females (n = 34)
Total leukocyte count ($10^3/\mu\text{l}$)	22.47 \pm 7.61 [12.50 – 35.50]	23.91 \pm 6.75 [13.50 – 34.00]
Percentage lymphocyte count (%)	45.52 \pm 4.82 [32.00 – 53.00]	45.91 \pm 4.80 [38.00 – 58.00]
Absolute lymphocyte count ($10^3/\mu\text{l}$)	10.17 \pm 3.42 [5.75 – 16.33]	10.97 \pm 3.57 [5.74 – 18.20]
Percentage heterophil count (%)	34.76 \pm 6.28 [20.00 – 50.00]	32.48 \pm 5.49 [18.00 – 41.00]
Absolute heterophil count ($10^3/\mu\text{l}$)	7.93 \pm 3.29 [3.20 – 13.80]	7.72 \pm 2.65 [2.43 – 13.26]
Percentage monocyte count (%)	9.42 \pm 2.69 [5.00 – 15.00]	10.42 \pm 3.19 [6.00 – 19.00]
Absolute monocyte count ($10^3/\mu\text{l}$)	2.10 \pm 0.85 [0.63 – 4.06]	2.46 \pm 0.96 [1.20 – 4.09]
Percentage eosinophil count (%)	9.38 \pm 2.82 [4.00 – 17.00]	10.12 \pm 2.74 [5.00 – 16.00]
Absolute eosinophil count ($10^3/\mu\text{l}$)	2.09 \pm 0.94 [0.90 – 4.49]	2.36 \pm 0.87 [1.08 – 4.76]
Percentage basophil count (%)	0.90 \pm 0.89 [0.00 – 3.00]	1.09 \pm 1.26 [0.00 – 5.00]
Absolute basophil count ($10^3/\mu\text{l}$)	0.19 \pm 0.21 [0.00 – 0.69]	0.27 \pm 0.35 [0.00 – 1.70]

Mean \pm SD, with minimum and maximum values in square brackets; No significant differences between the means of the males and females, $p > 0.05$

The absolute heterophil counts of pigeons obtained from Enugu-Ezike was however significantly higher ($p < 0.05$) than those of the pigeons obtained from the other locations (Table 6).

The mean of the percentage monocyte count (%) of the pigeons with their minimum and maximum values were 10.04 \pm 3.02 [5.00 – 19.00], while the mean absolute monocyte

count ($10^3/\mu\text{l}$) with minimum and maximum values were 2.32 \pm 0.93 [0.63 – 4.09] (Table 4). There were no significant differences ($p > 0.05$) in the percentage and absolute monocyte counts between the male and female pigeons (Table 5), and no significant variation ($p > 0.05$) in the percentage monocyte counts of pigeons obtained from the different locations (Table 6).

Table 6: Comparison of the leukocytic profile of domestic pigeons sourced from different locations in Nsukka agro-ecological zone, Enugu State, Nigeria

Parameters	Locations		
	Ibagwa (n = 23)	Orba (n= 21)	Enugu-Ezike (n= 20)
Total leukocyte count ($10^3/\mu\text{l}$)	22.64 ± 7.31 [12.50 – 34.50]	21.78 ± 7.68 [12.50 – 35.50]	26.33 ± 5.20 [16.00 – 34.00]
Percentage lymphocyte count (%)	44.91 ± 5.52 ^a [32.00 – 56.00]	48.17 ± 3.73 ^b [43.00 – 58.00]	44.27 ± 3.73 ^a [38.00 – 51.00]
Absolute lymphocyte count ($10^3/\mu\text{l}$)	10.22 ± 3.80 [5.74 – 18.20]	10.31 ± 3.76 [5.75 – 16.70]	11.67 ± 2.62 [7.20 – 17.34]
Percentage heterophil count (%)	32.23 ± 7.46 [20.00 – 50.00]	33.29 ± 5.50 [18.00 – 39.00]	35.13 ± 2.56 [32.00 – 41.00]
Absolute heterophil count ($10^3/\mu\text{l}$)	7.29 ± 2.99 ^a [3.20 – 13.80]	7.13 ± 2.95 ^a [2.43 – 13.49]	9.29 ± 2.18 ^b [5.28 – 12.71]
Percentage monocyte count (%)	10.41 ± 3.46 [5.00 – 19.00]	9.41 ± 2.72 [6.00 – 15.00]	10.20 ± 2.73 [6.00 – 15.00]
Absolute monocyte count ($10^3/\mu\text{l}$)	2.36 ± 1.04 ^{a,b} [0.63 – 4.09]	1.97 ± 0.78 ^a [1.05 – 4.08]	2.67 ± 0.81 ^b [1.28 – 3.92]
Percentage eosinophil count (%)	10.77 ± 3.38 ^a [4.00 – 17.00]	8.82 ± 1.74 ^b [6.00 – 12.00]	9.60 ± 2.38 ^{a,b} [5.00 – 13.00]
Absolute eosinophil count ($10^3/\mu\text{l}$)	2.37 ± 1.01 [1.26 – 4.76]	1.89 ± 0.89 [0.90 – 3.74]	2.48 ± 0.62 [1.05 – 3.36]
Percentage basophil count (%)	1.68 ± 1.32 ^a [0.00 – 5.00]	0.35 ± 0.12 ^b [0.00 – 1.00]	0.80 ± 0.77 ^b [0.00 – 2.00]
Absolute basophil count ($10^3/\mu\text{l}$)	0.39 ± 0.39 ^a [0.00 – 1.70]	0.08 ± 0.11 ^b [0.00 – 0.36]	0.21 ± 0.20 ^{a,b} [0.00 – 0.65]

Mean ± SD, with minimum and maximum values in square brackets; ^{a,b} Different superscripts in a row indicate significant differences between the means, $p < 0.05$

The absolute monocyte count of pigeons obtained from Enugu-Ezike was significantly higher ($p < 0.05$) than that of pigeons obtained from Orba (Table 6).

The overall mean percentage eosinophil count (%) for the pigeons with their minimum and maximum values was 9.83 ± 2.77 [4.00 - 17.00], while the mean absolute eosinophil count ($10^3/\mu\text{l}$) with minimum and maximum values was 2.25 ± 0.89 [0.90 - 4.76] (Table 4). There was no significant difference ($p > 0.05$) in the percentage and absolute eosinophil counts between the males and females (Table 5).

Furthermore, there was no significant variations ($p > 0.05$) in the absolute eosinophil counts of pigeons from the different locations (Table 6). The percentage eosinophil counts of the pigeons obtained from Ibagwa was significantly higher ($p < 0.05$) than that of pigeons obtained from Orba (Table 6). For the basophil counts, the overall mean percentage basophil count (%) with their minimum and

maximum values was 1.02 ± 1.12 [0.00 - 5.00], while the mean absolute basophil count ($10^3/\mu\text{l}$) with minimum and maximum values was 0.24 ± 0.30 [0.00 - 1.70] (Table 4). There was no significant difference ($p > 0.05$) in the percentage and absolute basophil counts between the male and female pigeons (Table 5). The percentage basophil count of the pigeons obtained from Ibagwa was significantly higher ($p < 0.05$) than those of pigeons obtained from Orba and Enugu-Ezike, and the absolute basophil count of the pigeons from Ibagwa was significantly higher ($p < 0.05$) than that of pigeons from Orba (Table 6).

The mean body weight (g) of the pigeons was 211.46 ± 20.74 , with minimum and maximum values of 153.20 and 229.02 (Figure 1). There was no significant difference ($p > 0.05$) between the body weights of the males and females (Figure 1), and no significant variations between the body weights of the pigeons obtained from the different locations (Figure 2).

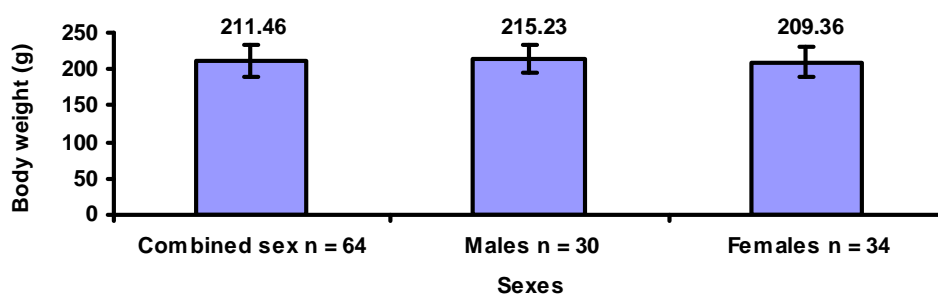


Figure 1: Sexual dimorphism in the body weights of the domestic pigeons in Nsukka agro-ecological zone, Enugu State, Nigeria

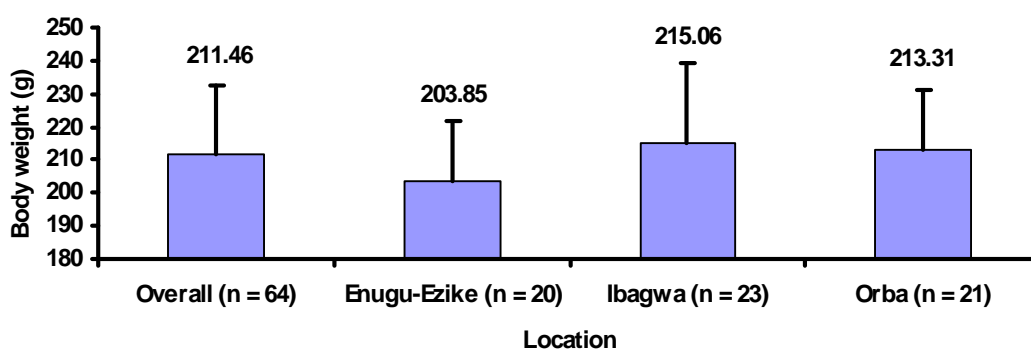


Figure 2: Spatial variations in the body weights of the domestic pigeons sourced from different locations in Nsukka agro-ecological zone, Enugu State, Nigeria

DISCUSSION

The overall mean PCV recorded for the pigeons in this present study (44.54 ± 4.73 %) was comparable to and slightly higher than that reported by Ritchie *et al.* (1994) for domestic pigeons (42.5 %). It was however relatively lower than the 49.36 ± 6.40 % reported for street rock pigeons by Khan *et al.* (2011). The relatively higher PCV reported for street pigeons may be part of adjustment for greater demands for constant flight of street pigeons (Viscor *et al.*, 1985). The HbC recorded for the pigeons in this study (12.89 ± 1.55 g/dl) were comparable to, but relatively lower than the mean value (14.46 ± 0.19 g/dl) reported by Lashev *et al.* (2009) for domestic pigeons. The minimum and maximum values for HbC reported by Ritchie *et al.* (1994) (8.1 – 9.9 g/dl) lied within the minimum and maximum recorded in this study (7.76 – 16.00), though the range in this study was wider. The mean RBC counts of the pigeons in this study (3.34 ± 0.38) were comparable to,

but slightly lower than RBC counts (3.96 ± 0.05) reported by Lashev *et al.* (2009) for domestic pigeons and the minimum and maximum values recorded in this study (2.12 – 3.95) were relatively lower than values reported by Ritchie *et al.* (1994) for domestic pigeons (3.1 – 4.5). The relatively lower HbC and RBC counts recorded in this study when compared to that reported by Ritchie *et al.* (1994) and Lashev *et al.* (2009) may be due to difference between the temperate environment (with its relatively lower environmental temperatures) in which the earlier reported studies were conducted compared to the tropical environment (higher environmental temperature) under which the present study was done. Olsen (1973) had earlier reported higher values of erythrocyte parameters in cattle exposed to controlled cold environmental temperatures. This difference between the erythrocytic profile of the same species at temperate and tropical environments also concurred with the earlier reports of such differences in albino rats (Ihedioha *et al.*, 2004).

There were no reported values for the erythrocyte MCV, MCH and MCHC of domestic pigeons in available literature to compare with the values obtained in this study. However, the MCV, MCH and MCHC recorded in this study were relatively lower than that reported for street pigeons by Khan *et al.* (2011) and Opara *et al.* (2012). These higher values reported for the erythrocyte corpuscular values of street pigeons relative to domestic pigeons may be due to physiological adjustments for the greater demand for constant flight by street pigeons (Viscor *et al.*, 1985).

The mean total WBC count recorded in this study (23.36 ± 7.06) was comparable to, and in agreement with the mean of 23.80 ± 1.27 reported by Lashev *et al.* (2009), and the minimum and maximum values reported by Ritchie *et al.* (1994) for domestic pigeons (13.0 – 22.3) lied within the minimum and maximum values recorded in this study (12.5 – 35.50), though the upper limit of the values obtained in this study were higher. The minimum and maximum values of the absolute lymphocyte, heterophil, monocyte and basophil counts recorded in this study were comparable to that recorded by Ritchie *et al.* (1994) but were of a wider range. The wider range of the absolute values recorded in this study in comparison with that reported by Ritchie *et al.* (1994) may be because domestic pigeons used in this present study were sourced from three different breeders/locations within the same geographical zone. The minimum and maximum values for the absolute eosinophil counts obtained in this study (0.90 – 4.76) were however higher than the 0.1 – 0.3 reported by Ritchie *et al.* (1994). It was also of a wider range when compared to that reported by Ritchie *et al.* (1994). Lashev *et al.* (2009) reported the differential WBC counts in mean percentages; and the mean percentage lymphocyte and heterophil counts recorded in this study were slightly lower than that reported by Lashev *et al.* (2009), while the mean percentage monocyte, eosinophil and basophil counts recorded in this study were higher than that recorded by Lashev *et al.* (2009). These differences in the differential WBC counts may be attributed to differences in environmental and geographical factors.

The absence of sex (male and female) related differences in all the haematological parameters recorded in this study may not be unrelated to the documented lack of obvious gender related differences in the outward secondary sexual characteristics, body size, morphology and specific behavior between male and female pigeons (Vogel *et al.*, 1994; Kigir *et al.*, 2010). The findings of no significant sex related differences in the haematological parameters of pigeons in this study were in agreement with the reports of Lashev *et al.* (2009) who also reported no differences in the haematology of male and female domestic pigeons. Our findings however is slightly at variance with the reports of Pavlak *et al.* (2005) who reported sex related differences only in the MCV values and in the percentages of lymphocytes and neutrophils.

The significantly higher mean PCV and slightly higher RBC count and HbC recorded for the pigeons sourced from Orba relative to those sourced from Ibagwa and Enugu-Ezike may be attributable to the differences in the altitudes of the locations; Orba is located at 452 metres above sea level, while Ibagwa and Enugu-Ezike are located 334 m and 391 m above sea level respectively. Higher altitudes with their relatively lower oxygen tension had been reported to be associated with higher hematocrit, haemoglobin and red blood cell values (Frisancho, 1975; Ihedioha, 2004; Nepal *et al.*, 2012). The significant differences between the pigeons sourced from the three locations in their absolute heterophil, monocyte, basophil counts and percentage lymphocyte, eosinophil and basophil counts may be attributed to possible differences in the condition of keeping and management of the pigeons at the sourced locations. These differences were however negligible even when they are statistically significant, as the minimum and maximum values lied within the same range.

The minimum and maximum values recorded for the body weights of the pigeons in this study (150.20 – 229.02) lied within the range reported for pigeons by Kigir *et al.* (2010) who studied pigeons in northern Nigeria. The lack of significant differences between the body weights of pigeons in this study concurred with

earlier reports that male and female pigeons may not be easily differentiated based on body size (Vogel *et al.*, 1994). The findings in this study of slightly higher body weight of males relative to females which was not statistically different was in agreement with the findings of Kigir *et al.* (2010).

Conclusion: The haematological profile of domestic pigeons in Nsukka, Nigeria were in some respects comparable to that already reported in literature, but also varied in some respects. However, there were no sex-related significant differences in all the haematological parameters evaluated and the body weight.

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PRELIMINARY ASSESSMENT OF THE IMPACT OF IVERMECTIN IN THE TREATMENT OF ONCHOCERCIASIS IN EZZA NKWUBOR AND UMUHU COMMUNITIES IN ENUGU STATE, NIGERIA

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ABSTRACT

Blackflies have been implicated over the years as the vector of onchocerciasis (River blindness). Ivermectin on the other hand has been the drug of choice for the treatment of onchocerciasis and to a great extent indirectly serving as systemic insecticide thereby breaking the transmission cycle of the disease. This study tried to evaluate the impact of ivermectin in Ezza Nkwubor and Umuhu communities of Enugu East and Isi Uzor LGAs of Enugu State, respectively. Epidemiological survey was carried out to check for the presence of microfilariae in skin snip samples collected from both left and right iliac crests of subjects living in the community. A total of 147 subjects were evaluated in the two communities. There was a significant difference $p = 0.266$ in the age distribution and also in the gender distribution $p = 0.316$. It was observed that the knowledge of ivermectin in the study areas was significantly different $p = 0.012$ and for those that have been skin snipped before there was also a statistically significant difference $p = 0.000$. More so, all samples subjected to microscopic analysis tested negative. It will be hasty to conclude that onchocerciasis has been eliminated from these communities. Further studies on the presence of the vector and their vectorial capacity could also assist in determining the actual status of onchocerciasis in the study areas.

Keywords: Epidemiological survey, Onchocerciasis, Ivermectin, Microfilariae, Ezza Nkwubor and Umuhu communities, Enugu State, Nigeria

INTRODUCTION

Onchocerciasis is a vector-borne parasitic disease caused by a filarial nematode worm *Onchocerca volvulus*. The adult worms (macrofilariae) lodge in palpable nodules under the skin of infected humans, although they can also be found free in subcutaneous tissue (Nnochiri, 1964; Samba, 1994). The microfilariae are found in the intercellular fluid, including that of the eye, and their death and subsequent disintegration result in inflammatory reactions. If microfilarial load is high following a prolonged period of exposure to massive

infection, this may lead to serious visual impairment including blindness. In addition, the microfilariae give rise to intensely itching rashes, to wrinkling, thickening and depigmentation of the skin, to lymphadenitis resulting in hanging groins and elephantiasis of the genitals and to general debilitation, including loss of weight (Samba, 1994). The disease affects rural communities and is a major cause of blindness and skin disease in endemic areas usually accompanied with serious morbidity, psychosocial problems and reduced work, especially reduced agricultural productivity in populations affected by the disease. Estimates

shows that approximately 500,000 people are blind due to onchocerciasis (Boussinesq *et al.*, 2001; TDR, 2005; WHO, 2011). Globally it is estimated that 37 million people are infected with onchocerciasis and more than 99 percent of cases occur in 27 countries in sub-Saharan Africa with 120 – 122.9 million at risk of the disease (WHO, 1995; Gemade *et al.*, 1998). In Nigeria, onchocerciasis is widespread and the cause of blindness in most rural communities. Of all the countries of the world, Nigeria is worst hit and has the greatest number of persons with onchocerciasis (WHO, 1995). Visual impairment due to onchocercal eye disease can be demonstrated in about 30% of children aged 5 years who live in hyper-endemic communities in Nigeria; 35% of males and 27% of females in such communities are visually impaired at the age of 30 years (Edungbola *et al.*, 1993; Gemade *et al.*, 1998). The disease is endemic in much of tropical Africa and parts of Central and South America and Yemen (Gemade *et al.*, 1998). In the warm tropical environment, the disease thrives under conditions favorable for their development all year round (Gemade and Utsalo, 1990). Duke (1972) described the disease in Africa, as a disease of the future because as the development of the hinterlands proceed, particularly as dams and water projects increase, it will cease to be a disease affecting only small, isolated, poverty stricken and primitive communities in the bush and will become more and more a threat to sophisticated development personnel and other workers. It is thus expected that the battle for control of onchocerciasis will require more effort. There have been reports on different epidemiological patterns of infection between savanna and forest regions. Blindness and impaired vision are the most dangerous disabilities associated with the disease and are seen more among endemic communities living around the foci of transmission (Murdoch *et al.*, 2002). In West African savanna areas, ocular onchocerciasis is common; it particularly affects the anterior segment of the eye, though the posterior eye segment can also be affected. The risks of visual impairment increase, in part, as the prevalence and intensity of infection in a community rises (Crosskey, 1990; Nwoke and

Ikonne, 1993). Onchocercal blindness is more common in the savanna bio-climatic zone than in the rain forest zone with sclerosing keratitis standing out as the ocular lesion with the highest prevalence (Murdoch *et al.*, 2002). In African forest areas with a comparable intensity of onchocerciasis as savanna areas, onchocercal skin disease predominates, with much less blindness. Furthermore, ocular lesions, when present, usually involve the posterior eye segment (Murdoch *et al.*, 2002).

A multi-country study in highly endemic forest communities found that itching affected 42 percent of the population aged ≥ 20 years, and onchocercal skin lesions affected 28 percent of the population aged ≥ 5 years. Strong associations were found between the prevalence of skin lesions and troublesome itching and onchocercal endemicity (Remme *et al.*, 1989).

The drug of choice for onchocerciasis is ivermectin, which has been shown to reduce the occurrence of blindness and severity of skin symptoms. Ivermectin kills the microfilariae (larvae), but not the adult worms. Treatment has to be continued annually for at least 15 years in order to cover the life span of the adult worms (WHO, 2011). Contraindications for administration of ivermectin include being younger than 5 years or 90 cm in height, being pregnant or and lactation of infant less than one week of age, having serious health problem, e.g. asthma, renal or hepatic disease (WHO, 2011).

MATERIALS AND METHODS

Study Area: The study was undertaken in Umuhu and Ezza Nkwubor communities of Isi Uzor and Enugu East Local Government Area of Enugu State, respectively. Isi Uzor is a Local Government Area of Enugu State, Nigeria bordering Benue State and Ebonyi State. Its headquarter is in Ikem town. It has an area of 877 km² and a population of 148,415 at the 2006 census. It is located around 6°47'N 7°43'E. Umuhu is a rural community located in Eha-Amufu, Isi-Uzor, Enugu State, located at 5.8° N 7.2° E. The soil is of lateritic type and the vegetation is mostly composed of lowland forest trees, mainly *Piptadeniastrum africanum*,

Uapaca spp., *Pycanthu* spp., *Lophiraalata* and *Khaya ivorensis*.

The area rises up to 300 m above sea level and is dominated by a tropical interland climate with an average temperature of over 27° C. The total annual precipitation is 2000 – 3000 mm (Wikipedia, 2014). Umuhu is a village where residents are predominantly farmers. There is a common river called 'Ebonyi' where all the community gets their source of water for drinking, bathing and washing. The highest educational institution in the village is secondary school both private and public. Enugu East is a Local Government Area in Enugu State, Nigeria. Its headquarter is in the town of Nkwo Nike. It has an area of 383km² and a population of 279,089 at the 2006 census. Ezza Nkwubor is a village in Emene located on latitude 6.30115°, longitude 7.37001° (Maplandia, 2014). It is basically a farming community with secondary school being the highest educational institution. University. The rivers commonly used by some of the community dwellers are River Oboja and River Owo which are between 0.6 – 1 km from the village settlement.

Study Design: A descriptive epidemiological study was used to carry out the research. A cross sectional type of descriptive study was employed.

Limitation of Study: Wider population coverage would have been better, but unfortunately, only a small proportion of people in the community presented themselves for skin snipping. More so, entomological observations were to be done simultaneously but it was not possible.

Ethical Review: Ethical clearance was obtained from the University of Nigeria Teaching Hospital Ethics Committee. Clearance from the State Ministry of Health and the State Onchocerciasis unit was also obtained before visiting the communities. Informed consent of all eligible subjects was obtained verbally before they were sniped.

Data Collection: Skin-snip surveys were done in Umuhu and Ezza Nkwubor communities and

were carried out between September 2013 and February 2014.

In each village, all subjects above the age of 10 year who agreed to participate. The questions on the validated and pre-tested questionnaire sought information on the age, sex, occupation, knowledge of the fly, disease caused by the black fly, duration of stay in study area and knowledge of ivermectin.

Epidemiological Evaluation: The age of the cohort range from 10 – 75 years. Subjects were selected at convenience for the skin snip. Although majority of the subjects that presented themselves declined to be registered for the skin snip. All members of a family present at the venue of registration were all registered in one form bearing the "Family name" for easy access should there is any positive case. The surveys used established skin-snip examination methods in which the National Onchocerciasis Control Program (NOCP) had previously been trained by the WHO African Programme for Onchocerciasis Control (APOC). Two skin biopsies were obtained from the right and left iliac crests of all individuals who presented themselves for the survey. A 2 mm Holth Corneoscleral Punch (MDP^{CE} IW811) was used to obtain the skin biopsies. After each series of two bloodless skin-snip obtained from a subject, the scleral punch was sterilized sequentially in sodium hypochlorite solution, distilled water and then autoclaved by pressure for 15 minute. The entire process was to ensure that blood-borne infections are not transferred to individuals in the community. The samples were microscopically examined after 24 hours incubation in normal saline to allow the microfilariae (larvae) to emerge.

Data Analysis: The analytical tool employed in the study includes descriptive statistics and chi-square test of independence. It was used to obtain the frequencies and percentages of the research variables. Chi-square symbolically represented as χ^2 was used to compare actual observed distribution with the hypothesis or expected distribution. The test was done at 95% confidence level.

RESULTS

Only 75 subjects agreed to be registered in Umuhu community and these were the number that was snipped. The turnout was similar in Ezza Nkwubor with only 68 persons agreeing to be registered but only 65 of them yielded to be snipped. In total, 143 subjects registered but only 140 were snipped in both communities yielding to 97.9% compliance, and 2.1% refusal (Table 1).

Age and Sex Distribution: Out of the 65 subjects that were snipped in Ezza Nkwubor, 23(35.4%) were below the age range <35 years and 45(69.2%) were >35 years with the $P = 0.266$ showing that there is significant difference in the age of subjects that were snipped in both communities. In Umuhu community, 19(25.3%) were in the age range <35 years while a greater proportion 56(74.7%) were in the age group >35. There was a significant difference in the gender of the subjects in both rural and urban $P = 0.316$, although in Ezza Nkwubor community 27(41.5%) were males 38(58.5%) were females likewise Umuhu community, 25(33.3%) were males and 50(66.7%) were females (Table 1).

Knowledge of Ivermectin and Skin Snip: Among those that were registered in the Ezza Nkwubor, 45(66.2 %) were aware of ivermectin and have been given ivermectin by the CDDs and 23(33.8%) had no knowledge on ivermectin distribution nor have taken it before. While in Umuhu community 34(45.3 %) were also aware of ivermectin distribution and have been taking the annual rounds. There was a significant difference $P = 0.012$ between the two community on the knowledge of ivermectin distribution. Moreso, none of the subjects had any knowledge of being snipped before. Whereas in the rural area, 39(55.7%) remembered they have been snipped before in the community while 31(44.3 %) have never been skin snipped before. Knowledge on previous skin snip information was statistically significant at $p = 0.000$ (Table 2).

Microfilariae: All samples subjected to microscopic analysis after 24 hours incubation were confirmed negative for microfilariae.

DISCUSSIONS

Several studies have indicated that elimination of onchocerciasis with long term mass drug administration with ivermectin is achievable. Such studies have been carried out in Mali and Senegal (Diawara, 2009) where treatment had started as early as 1988/1989 and also in Kaduna State, Nigeria where more evidence have shown elimination of onchocerciasis with long term mass ivermectin administration (Tekle *et al.*, 2012).

This study tried to evaluate the impact of ivermectin within the study area through the skin snip method to check for the presence of microfilariae in the skin of the subjects. The statistically significance that was observed in the age distribution of subjects could be that most of the subjects that fall on this range <35 are students and very youthful and feel they are not sick. Many of them most have gone to school or may have travel to neighboring town in search of greener pastures. Oyekanmi (2010) in a study in Kwara State, Nigeria found out that males were more prone to migration. This could be a reason why we had more female subjects compared to the males. The age range >35 was considered aged because majority of them were far above 40 years of age. They might have presented themselves to be snipped because of the feeling of ill health usually attributed to age and will seek and respond to any health intervention program. There was a significant difference on previous skin snip information on both communities. It was clear from the study that there was a more active CDTI and CDDs in Umuhu community compared to Ezza Nkwubor community. Umuhu community dwellers perceived onchocerciasis to be a burden in their community compared with the community dwellers in Ezza Nkwubor. This perceived risk might have changed the attitude of subjects towards the demand for Ivermectin.

Table 1: Socio-demographic parameters of Ezza Nkwubor and Umuhu communities in Enugu State, Nigeria preliminary assessed on the impact of ivermectin in the treatment of onchocerciasis

Variables	Umuhu community n=75 Frequency (%)	Ezza Nkwubor community n=68 Frequency (%)	Total n=143 Frequency (%)	X ² value	P-value
Age in years				1.239	0.266
< 35	19 (21.0)	23(33.8)	42 (29.4)		
> 35	56 (74.7)	45 (66.2)	101 (70.6)		
Sex				1.004	0.316
Male	25 (39.7)	27 (33.3)	52 (38.5)		
Female	50 (66.7)	38 (66.3)	88 (61.5)		

Statistically significant at p<0.05

Table 2: Knowledge of the use of ivermectin and skin snip among members of Ezza Nkwubor and Umuhu communities in Enugu State, Nigeria preliminary assessed on the impact of ivermectin in the treatment of onchocerciasis

Variables	Umuhu community n=75 Frequency (%)	Ezza Nkwubor community n=68 Frequency (%)	Total n=143 Frequency (%)	X ² value	P-value
Knowledge of ivermectin				6.267	0.012
Yes	34 (45.3)	45(66.2)	79(55.2)		
No	41 (54.7)	23(33.8)	64(44.8)		
Skin snip information				52.810	0.000*
Snipped before	39(55.7)	0(0.0)	39(28.3)		
Not been snipped	31(44.3)	68 (100.0)	99 (71.7)		

**Statistically significant at p<0.05*

Microscopic analysis revealed that all 140 samples of skin snips examined were negative. The fact that no skin microfilariae were found indicated that there might not be adult worms in the subjects that were evaluated or even if there are still adult worms in the subjects, they may not longer be productive or may be producing too few microfilariae to be detected by microscopic analysis of the skin snip. It was also observed that drug distribution was not carried out routinely. There were irregularities with drug administration. It might be that subjects took in ivermectin just a few days or week prior to evaluation of the skin biopsy. It has been proven that ivermectin can clear 85% of the microfilariae within the first 24 hours of intake and 98 – 99% after 1 – 2 months (Basanez *et al.*, 2008).

Recommendation: The study focused only on the epidemiological aspects of the community dwellers using skin snip diagnosis, it is therefore important to undertake an entomological survey

to ensure the actual and present situation of the vectorial capacity of the flies through dissection to detect microfilaria. It is evident that mass drug distribution is effective. It is therefore important to encourage the CDDs in their task of ivermectin distribution. Moreso, there is the need for more awareness, sanitization and epidemiological survey prior to drug administration.

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PREVALENCE OF MALARIA INFECTION IN CHILDREN IN ANAMBRA STATE, NIGERIA AFTER CHANGE OF POLICY FROM PRESUMPTIVE/CLINICAL TO CONFIRMED DIAGNOSIS

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ABSTRACT

In 2011, WHO change malaria case-management policy from presumptive treatment of fevers to parasitological diagnosis and targeted treatment with artemisinin combination therapy (ACTs). Between 2010 and 2012, a series of activities were undertaken to support the implementation of the new policy. Regular monitoring of the quality of malaria case-management was carried out to inform policy makers, implementers and donors agencies on the implementation progress. This study was carried out to estimate the effects of this new WHO policy on the prevalence of malaria parasite infection in children from selected communities in Anambra State, Nigeria. This study was conducted in thirteen communities purposively selected from thirteen local government areas in Anambra State using children aged 0 – 14.90 years. Venous blood samples were collected from 82 and 166 children from the communities and hospitals, respectively for thick films blood smears for microscopy. Chi-square (χ^2) and Fisher least significance difference test were used to analyse the data collected. The overall prevalence of malaria based on the community survey in Anambra State was 46.30 %, while the prevalence of malaria based on hospital survey was 94.60 %. The result of this study showed that there was no significant difference in infection rate in relation to age in both community and hospital survey. There should be proper management of childhood malaria in the homes and hospitals. This could be achieved by training and retraining of health care workers and mothers/care givers in the formal health care delivery systems to ensure quick and accurate diagnosis of malaria parasite infection of children in Anambra State, Nigeria.

Keywords: Prevalence of malaria, Children, Anambra State, Nigeria, Presumptive policy, Confirmed diagnosis, Hospital malaria vs. Community malaria

INTRODUCTION

Malaria is one of the most important causes of morbidity in the world. It is a vector borne infectious disease caused by a eukaryotic protista of the genus *Plasmodium*. The disease is transmitted by female *Anopheles* mosquitoes which carry infective sporozoite stage of *Plasmodium* parasite in their salivary glands (Akinleye, 2009). It is transmitted from person to person through the bite of a female

Anopheles mosquito that is infected with one of the four species of *Plasmodium*: *Plasmodium ovale*, *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae*.

In Nigeria, malaria is holoendemic in the rural areas and mesoendemic in the urban areas. In the southern part of the country the transmission rate is approximately uniform throughout the year (Nwaorgu and Orajaka, 2011). Farming activities which takes place mostly during the rainy season period of the

year favours the breeding of mosquitoes and this makes the effects of malaria apparently noticeable in rural areas due their proximity to farmlands (Kalu *et al.*, 2012). The degree of malaria infestation varies from region to region in Nigeria (Onwuemele, 2014). Globally, the disease caused an estimated 453 000 under-five deaths in 2013. Between 2000 and 2013, an expansion of malaria interventions helped to reduce malaria incidence by 30 % globally, and by 34% in Africa. During the same period, malaria mortality rates decreased by an estimated 47% worldwide and by 54 % in Africa. In the under-five age group, mortality rates have declined by 53 % globally, and by 58 % in Africa (WHO, 2014). WHO noted that progress in adopting and rolling out preventive therapies for children has been even slower than ever. In 2013, only six of the 16 countries where WHO recommend preventive therapies for children under five have adopted the treatment as national policy. Only one country has adopted the recommended preventive therapy for infants (WHO, 2014).

The decline in malaria incidence and mortality may be attributed to the volume of RDT sales to the public and private sectors of endemic countries that increased from 46 million in 2008 to 319 million in 2013. Also the number of patients tested by microscopic examination increased to 197 million in 2013, with India accounting for over 120 million slide examinations and globally, 392 million courses of ACTs were procured by endemic countries in 2013, up from 11 million in 2005. In 2013 the total number of diagnostic tests (RDTs and microscopy combined) provided in the public sector in Africa exceeded the total number of ACTs distributed. This indicates a prominent shift away from presumptive treatment and is an encouraging sign (WHO, 2014). Therefore, study was carried out to estimate the effects of this new WHO policy on the prevalence of malaria parasite infection in children from communities in Anambra State.

MATERIALS AND METHODS

Study Area: The study was conducted in Anambra State. Anambra State is located in

south-eastern Nigeria with a population of over 4 million people made up of 2,117,984 males and 2,059,844 females (NPC, 2006; MLS, 2010). Anambra State is made up of twenty one local government areas. This study was conducted in thirteen communities purposively selected from thirteen local government areas in Anambra State. The communities are; Umueri in Anambra East, Atani in Ogbaru, Awka in Awka South, Amanuke in Awka North, Enugu-Ukwu in Njikoka, Ifite-Dunu in Dunukaofia, Ekwulobia in Aguata, Agulu in Aniocha, Nnobi in Idemili, Umunya in Oyi, Osomala in Ogbaru, Onitsha in Onitsha South and Nnewi in Nnewi South Local Government Areas. The prevailing climatic conditions are high rainfall ranging from 1,400 mm in the north to 2,500 mm in the south with four months of dryness (November – February), constant high temperature and a mean of 30 % atmospheric humidity. The vegetation types are mangrove and freshwater swamps communities, rainforest, forest, savannah mosaic and derived savannah zone. The mainstay of the communities is subsistence agriculture and trading.

Study Population: Children aged 0 – 14.9 years from the sampled households in the communities and their counter parts that attended the outpatient clinics of the selected general hospitals in Anambra State were recruited into the study. The sampled communities and hospitals were visited twice monthly.

Sampling Technique: Stratified random sampling was used. All hospitalized or non-hospitalized patients (children 0 – 14.9 years) with acute febrile illness were randomly sampled two times in a month from April 2012 to March 2013. Fifty homes were randomly sampled by balloting in each of the 13 communities for 12 months.

Ethical Clearance and Exclusion Criteria: Ethical clearance was obtained from the University of Nigeria Teaching Hospital Ituku-Ozalla in Enugu State, with which permission was obtained from the management of the selected General Hospitals for the study.

Informed consent was obtained from the mothers/caregivers before the collection of blood samples and the administration of questionnaires.

Sample Collection: The research was conducted between the months of April 2012 to March 2013. With the help of the medical team, blood samples were collected from children whose mother consented according to Sood (2006). One (1) ml of venous blood was obtained from each sampled child after cleaning the site with spirit and put in labelled ethylenediamine tetra-acetic disodium acid (EDTA) vacutainers to avoid clotting and ensure preservation of the samples. The samples were kept in ice chips and taken to the laboratory for parasitological analyses. Venous blood samples were collected from 82 and 166 children from the communities and hospitals, respectively.

Preparation of Blood Smears for Microscopy: Thick film blood smears were prepared from the blood samples according to Sood (2006). Large drop of blood samples were deposited at one end of the slide and were spread out evenly with the corner of another slide to a diameter of about 20mm. They were put in distilled water for 10 minutes for dehaemoglobinisation, dried in a flat position to ensure even distribution of blood and stained with Gynea's stain for 20 minutes. The stain was washed out with buffered water of pH 6.8 and stood upright to dry in the air, and viewed under x 100 objective (oil immersion) lens. The thick smears were used to confirm the presence or absence of malaria parasite. The asexual forms of the parasite were counted in 200 leucocytes. The degree of parasitaemia were graded according to the number of parasite per micro litre thus, 1-999 (+), 1000-9999 (++) and >100000 (+++) (Cheesbrough, 2006). Negative blood samples served as control.

Statistical Analysis: The presence or absence of *Plasmodium* infection (prevalence) was calculated and the significant difference in prevalence across age groups for both community and hospital survey was determined using Chi-square (χ^2). The significant difference

in the prevalence of infection for sex (community and hospital) was done using Fisher least significance for 2 x 2 tables. For all determination, the significant difference was set at $p < 0.05$.

RESULTS

Characteristics of Studied Population:

Children (asymptomatic and symptomatic) between the ages of 0 – 14.90 (4.12 ± 0.27) years were sampled from thirteen communities and eleven general hospitals in Anambra State. A total of 82 children were sampled in the communities which included 39(47 %) males and 43(53 %) females. One hundred and sixty six (166) children who attended the outpatient clinic of general hospitals/comprehensive health centres in Anambra State were sampled, comprising of 95(57.2 %) males and 71(42.8 %) females. The overall prevalence of malaria based on the communities surveyed in Anambra State was 46.3 %, while the prevalence of malaria based on hospitals surveyed was 94.6 %. The infection prevalence among the communities (Table 1) showed that Awka community had the highest prevalence (75 %) followed by Amanuke and Osomala (70 %), Umunya (66.7 %), Onitsha (50 %), Umueri and Ekwulobia (40 %), Ifitedunu (37.5 %), Atani and Agulu (33.3 %), Enugu-Ukwu and Nnewi (25 %) and Nnobi with the lowest prevalence of 20 %.

Table 1: Community prevalence of malaria in children in Anambra State, Nigeria

Variables	Number examined (n)	Number positive (%)
Communities		
Amanuke	10	07(70.0)
Awka	04	03(75.0)
Osomala	10	07(70.0)
Atani	06	02(33.3)
Onitsha	04	02(50.0)
Umueri	10	04(40.0)
Umunya	03	02(66.7)
Ifite-Dunu	08	03(37.5)
Enugu-Ukwu	04	01(25.0)
Nnewi	04	01(25.0)
Ekwulobia	05	02(40.0)
Nnobi	05	01(20.0)
Agulu	09	03(33.3)
χ^2	-	104.000
p-value	-	0.03*

* = significant

The differences were statistically significant ($p < 0.05$) using Chi-square (χ^2). The significant

difference in the prevalence of infection by sex (community and hospital) done using Chi-square test of significance difference (Table 2) indicated that female 22(55.0 %) were more infected than males 16(41.0 %).

Table 2: Sex prevalence of malaria in children in Anambra State, Nigeria

Variables (sex)	Number examined (n)	Number positive (%)
Communities		
Male	39	16(41.0)
Female	43	22(55.0)
χ^2		0.845
P- value		0.38 ^{ns}
Hospitals		
Male	95	90(94.7)
Female	71	67(94.4)
χ^2		0.651
P- value		0.92 ^{ns}

ns = not significant

Table 3 showed the age group prevalence of malaria infection in children in Anambra State, Nigeria. In the communities, children between 5 – 9.9 years (48.9 %) were more infected followed by children between 10 – 14.9 years (44.9 %) and 0 – 4.9 years (42.3%) while in the hospitals, children aged 0 – 4.9 years and 5 – 9.9 years had 93 %, while 10 – 14.9 years had 100 % infection.

Table 3: Age related prevalence of malaria in children in Anambra State, Nigeria

Variables (Age – years)	Number examined (n)	Number positive (%)
Communities		
0 – 4.9	26	11(42.3)
5 – 9.9	47	23(48.9)
10 – 14.9	09	04(44.4)
Total	82	38(46.3)
χ^2	-	0.310
P – value	-	0.856 ^{ns}
Hospitals		
0 – 4.9	98	92(93.9)
5 – 9.9	44	41(93.2)
10 – 14.9	24	24(100)
Total	166	156(94.6)
χ^2	-	1.637
P – value	-	0.651 ^{ns}

ns = not significant

The monthly prevalence of malaria in Anambra State, Nigeria is summarized Table 4. The months of March, April, June, July, August, September, October and November had 100 %

infection respectively followed by December (93 %), May (92 %), February (83 %) and January (73 %). The differences in the monthly infection were highly significant ($p < 0.05$).

Table 4: Monthly and seasonality prevalence of malaria in children in Anambra State, Nigeria

Variables	Number examined (n)	Number positive (%)
Months		
April	10	100 (100)
May	14	3 (92.9)
June	13	13 (100)
July	13	13 (100)
August	16	13 (100)
September	19	19 (100)
October	15	15(100)
November	12	12 (100)
December	15	14 (93.3)
January	15	11 (73.3)
February	12	10 (83.3)
March	11	11 (100)
χ^2	-	21.699
P- value	-	0.027*
Seasonality		
Rainy	88	81 (92.0)
Dry	78	76 (97.6)
χ^2	-	2.34
P- value	-	0.13

* = significant difference ($p < 0.05$)

There was more infection in the rainy season (97.4 %) than in the dry season (92.0 %). Comparison of the prevalence of malaria infection in the communities and hospitals showed that hospital infection was significantly ($p < 0.05$) higher (94.6 to 46.3 %) than community malaria infection (Table 5).

DISCUSSION

The high prevalence of malaria in the hospitals in Anambra State may attribute to increase awareness of mothers/caregivers of the dangers of malaria infection on children especially the under fives. This must have resulted in their increase visit of government hospitals for the treatment of their children's malaria infection. This result is in agreement with the report that in Nigeria, over 50 % of out patients' attendance, 40 % of hospital admissions and 30% of child mortality are due to malaria infection (Okafor and Amzat, 2007; Olasehinde *et al.* 2010).

Table 5: Comparison of the prevalence of community and hospital managed childhood malaria infection in Anambra State, Nigeria

Variables	Infection status		
	Uninfected (%)	Infected (%)	Total (%)
Hospital	9(5.4)	157(94.6)	166(100)
Community	44 (53.7)	38(46.3)	82(100)
Total	53(21.4)	195(78.6)	248(100)
P-value	-	-	0.00*

* = significant difference ($p < 0.05$)

In this study the overall prevalence of malaria based on hospital attendance of febrile children is 94.0 %, while in the communities it is 46.3 % which is very high showing the endemic nature of malaria in Anambra State, Nigeria. The high prevalence rate in the study area could result in childhood anaemia and other malaria related conditions such as cerebral malaria (Chessed *et al.*, 2013). This may be attributed to the climate which influences the development of the mosquitoes as well as the human behaviour. The high prevalence of asymptomatic infection in the communities suggests the development of some degree of immunity to malaria infection by the children (Greenwood, 1984; Nwaorgu and Orajaka, 2011). This could be attributed to immunity derived from persistent malaria attacks.

The prevalence of malaria infection in the communities varied and the differences were significant. Community prevalence of malaria infection showed that Awka had the highest infection. This may be as a result of the fact that the hospital serves as a Teaching hospital to Anambra State University and also the location of the hospital in the State capital. Also differences in the community prevalence may be attributed to lack of use of preventive measures like ITNs and the geographical location of the community. The community prevalence also showed that the river line towns had high prevalence of malaria infection. This is as a result of their closeness to the river Niger that has tributaries that serve as a breeding site for the mosquito vector of *Plasmodium* parasite. The percentage prevalence of malaria infection in the different communities was relatively low compared to 93.4% prevalence of malaria

infection reported by Ilozumba and Uzozie (2009).

Gender related prevalence of malaria infection in the communities in Anambra State, Nigeria showed that female had a higher prevalence than the males but the difference was not significant. This result is in line with Nwaorgu and Orajaka (2011) who reported that malaria infection in Awka North Local Government Area of Anambra State was not gender biased. Furthermore, hospital childhood malaria was not selective for sex. This is in line with Sarki (2012) who reported no significant difference in the prevalence based on sex. Nigeria is an endemic country for malaria with all the year round transmission and the children in the hospitals actually came from the communities, hence the no significant differences in sex prevalence in both communities and hospitals.

The result of this study also showed that there was no significant difference in infection rate in relation to age in both community and hospital survey. This result contradicts the significant difference in prevalence of *Plasmodium* infection among the age groups and sex in Igbo-Eze South Local Government of Enugu State and Umuchieze / Uturu in Abia State, all in Nigeria (Ekpenyong and Eyo, 2008; Kalu *et al.*, 2012). The children in the communities were equally disposed to *Plasmodium* infection, hence the insignificant differences in the prevalence, even though the children between the ages 0 – 4.9 years had the lowest prevalence which may be attributed to higher attention given to children under five years of age malaria infection such as adequate protection against mosquito bites through the use of insecticide treated bed nets.

The monthly prevalence of malaria infection is influenced by conditions suitable for malaria parasite transmission. The conditions that are suitable for both the development of *Plasmodium* and mosquitoes were defined as the coincidence of precipitation accumulation greater than 80 mm, mean temperature between 18^o C and 32^o C and relative humidity greater than 60 % (Ayanlade *et al.*, 2010). These climatic conditions were prevalent in Anambra State, thus the all year round high

prevalence of malaria with significant variations in percentage prevalence. The months of April to November are suitable for malaria transmission in Anambra State, hence the presence of malaria infection in both wet and dry season (Ayanlade *et al.*, 2010; Iwuora, 2014).

People seek treatment for malaria from a wide range of providers ranging from patent drug sellers to hospitals. Childhood malaria infection treated at home is based on presumptive diagnosis by mothers/care givers. The comparison of malaria infection prevalence in the homes and hospitals in this study showed that the prevalence was significantly higher in the hospitals. This is in line with a study in Northern Nigeria where children of illiterates in sub-urban villages had the highest mean parasite density of 950 with 17.1 % prevalence rate for malaria infection (Chessed *et al.*, 2013). The encouragement given to mothers/caregivers to report any febrile childhood illness to the nearest health centre must have contributed to wide difference between the hospital prevalence and community.

Conclusions: The result of this study calls for the proper management of childhood malaria in the homes and hospitals by training and retraining of health care workers and the mothers/care givers in the formal health care systems to ensure quick and accurate diagnosis of childhood malaria in Anambra State, Nigeria.

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