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Cytogenetic and Heamatological Profile of *Sarotherodon melanotheron* Exposed to *Carica papaya* L. In Fish Feed as Replacement for Rice Bran

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Abstract: The aquatic environment makes up the major part of our environment and resources, its safety is directly related to the safety of our health and food security. This study investigated the cytogenetic effect of black jaw Tilapia, *Sarotherodon melanotheron* exposed to pawpaw leaf (*Carica papaya* L.) as replacement for rice bran for twelve weeks (90 days). The haematological effects of feeding *Carica papaya* leaf meal rations to *Sarotherodon melanotheron* Juvenile were also carried out. The study also investigated the haematological effects of feeding *Carica papaya* leaf meal rations to *Sarotherodon melanotheron* Juvenile. The treatments of the pawpaw leaves for the experiment were T_0 (0%), T_1 (25%), T_2 (50%), T_3 (75%) and T_4 (100%). The physico-chemical parameters showed a significant difference ($p<0.05$) and positive strong correlation coefficient of temperature with dissolved oxygen and pH (r = 0.998 and r = 0.999 respectively). The mean value for the physico chemical parameters measured thorough out the experiment are as given thus: Temperature (26.47 ± 0.89), Dissolved oxygen (7.80 ± 0.64) and pH (6.54 ± 0.43). The genotoxicity studies utilized micronucleus test of the exposed fish and there was no significant difference ($p > 0.05$) in the micronuclei present in 30 day in all the treatment while, day 60 and 90 showed a significant difference ($p < 0.05$) in all treatments. Analysis of Variance (ANOVA) showed a statistical significant difference ($p <0.05$) in the blood parameters: Mean Hb (g/dl),PCV (%), WBC (10^9/L), MCH (pg), Neutrophils (%) and Lymphocytes (%) values at 0 %, 25%, 50%, 75% and 100% level of inclusions. The trends observed in the haemoglobin, red blood cells and packed cell volumes' for the experimental fish were indications that the dietary inclusion levels of 50%, 75% and 100% favoured an increase in haematological parameters. *Carica papaya* leaf meal at 75% and 100% inclusion in *Clarias gariepinus* Juvenile diet showed significant weight gain and feed utilization and the ration was cost effective. It can serve as promising replacement for maize when Physico-chemical parameters of culture water were monitored within optimum range. It is evident from this finding that exposure of test fish to pawpaw (*Carica papaya* L.) replaced for rice bran in fish feed does not lead to cytogenetic damage and brings about little breakage of nucleus to form micronucleus cells.

Key words: Cytogenetic · Heamatology · *Sarotherodon melanotheron* · *Carica papaya* L · Rice Bran

INTRODUCTION

Feed is one of the major inputs in aquaculture production and fish feed Technology is one of the least development sectors of aquaculture particularly in Africa and other developing countries of the World [1]. High cost of fish feed was observed as one of the problems hampering aquaculture development [1].

Expensive feeds will marginalized or even nullify the profitability of fish farming thereby incapacitating the expansion of farms to increase production and consequently low yield in terms of quality and quantity, resulting in the scarcity of the commodity (fish) and eventually high cost of the few available ones to the disadvantage of the populace [2]. Fish feed account for at least 60% of the total cost of production [1].

Fish is an important source of both food and income to many people in developing countries.

Therefore, the need arises to search for alternative feedstuffs that are cheap, available and not in competitive demand for humans, livestock and industrial uses. In order to enhance the commercial production of

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fish, improve food security, reduce the level of poverty in developing countries and create employment, The feedstuff that are cheap, available and not in competitive demand for humans, livestock and industrial uses are referred to as un-conventional ingredients [3]. Nonconventional feed resources (NCFRs) are feeds that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production [4,5]. Non-conventional Feedstuffs are accredited for being non- competitive in terms of human consumption, very cheap to purchase, byproducts or waste products from agriculture, farm made feeds and processing industries and are able to serve as a form of waste management in enhancing good environmental impact.

The aquatic environment makes up the major part of our environment and resources; therefore, its safety is directly related to the safety of our health and food security. Biomarkers and bioindicators using fish micronucleus assay in eco-genotoxicology offers several types of unique information not available from other methods. These include: early warning on environmental damage, the integrated effect of a variety of environmental stresses on the health of an organism and the population, community and ecosystem, relationships between the individual responses of exposed organisms to pollution and the effects at the population level, early warning of potential harm to human health based on the responses of wildlife to population and the effectiveness of remediation efforts in decontaminating waterways [6].

Pawpaw (*Carica papaya*) is a common man's fruits, Eno *et al.* and Wilson *et al*. [7,8]. *C. papaya* is a soft-wooded perennial plant that has a life span of 5-10 years, although commercial plantations are usually replanted [6]. It normally grows as a single-stemmed tree with a crown of large palmate leaves emerging from the apex of the trunk, but plant stands may become multi-stemmed when damaged [9]. The fleshy part and of course the leaves of *Carica papaya* are available throughout the year in the Tropics thus it is believed that *Carica papaya* leaves can be a possible substitute for the expensive ingredient such as rice bran in tilapia fish ration.

Lohiya *et al.,* [10] suggested that ingestion of papaya seeds may adversely affect the fertility of human males or other male mammals. Ekanem and Okoronkwo [11] reported high success in using pawpaw seed powder in inducing sterility in male tilapia when administered through feed. Despite their widespread use, their toxicity and effectiveness of *C. papaya* to aquatic organisms, particularly fishes, have not been examined.*C. papaya* is a rare example of a species in which glucosinolate (benzylglucosinolate) and a cyanogenic glycoside (prunasin) co-occur [12]; generally plants that produce glucosinolates do not produce a cyanogenic glycoside and vice versa. Excessive consumption of plant parts that contain cyanogenic glycosides (leaves and roots, in the case of papaya) may induce adverse reactions, due to the formation of cyanide in the digestive system of the consumer, although none have recorded for papaya. Therefore, since there is a paucity of information on toxicity constituents of *C. papaya* L., this study is aimed at estimating the micronucleated cells of the Black Jaw Tilapia exposed to inclusion of *C. Papaya L* in formulated feed. The objectives of this study are to evaluate the potential of pawpaw as an alternative feed stuff in aquaculture and as dietary protein source for *S. melanotheron* fingerling and to examine the micronucleus present in the blood sample of Black jaw Tilapia and also investigate the effect of different concentration levels of Carica papaya leaf-meal on the heamatology of *S. melanotheron*.

MATERIALS AND METHODS

Experimental Animal: Juvenile *S. melanotheron* used for the bioassays (total length 8.5 ± 0.72cm and weight 9.76 ± 0.85g) were purchased from Department of Marine Sciences Aquaculture unit, University of Lagos, Akoka. The *S. melanotheron* was kept in a plastic tank (34 × 27 × 48.5 cm) which was half filled with dechlorinated water. During acclimatization, the juvenile were fed with commercial fish feed which contains 35% crude protein and they were fed 5ml/L of their body weight. The Juvenile *S. melanotheron* were fed thrice daily (morning, afternoon and evening) and the water was changed every day to prevent the accumulation of waste metabolite and food particles. The Juvenile *S. melanotheron* were maintained in the holding tank for a minimum of 14 days to allow them acclimatize to the laboratory conditions before commencement of the experiment.

Experiemental Feed: The feed fed to *S. melanotheron* in the course of the acclimatization was commercial feed (Coppens) of 2mm. At the start experiment, a total of fifteen plastic tanks each having ten fishes were used for the culturing of the fish. Each of the varying concentration of pawpaw leaf and rice bran were represented in triplicate with ten fishes in each tank.

The fishes were constantly fed with the formulated feed (using pearson's square method) with pawpaw leaf used to replace rice bran at 0%, 25%, 50%, 75% and 100%. The control had no inclusions of the pawpaw leaf (*Carica papaya* L.).

Ingredients: The ingredient pawpaw leaves (*Carica papaya*) were dried and grinded to powder form for the replacement with Rice bran. After acclimatization, 10 juvenile fishes were transferred to each contained treatment of various percentages of replaced feed. Three replicates were followed for each treatment. The feed was applied at the rate of 8% of body weight of the juvenile *S. melanotheron* throughout the experimental period of twelve weeks.

The diet was fed twice daily (morning and evening) in two equal portions. Dissolved oxygen and pH of water in each contained treatment was monitored by changing water daily. The range of temperature remained 22-26°C during study period. The morphometric characteristics i.e. body weight and total lengths were recorded to observe their growth performance. The ingredient used in formulating the fish feed were fish meal, soybean meal, groundnut cake, indomie, maize, mineral premix, vitamin premix, rice bran and pawpaw. The Pearson's square method was used in computing the feed with total crude protein (C.P) of 35% (Table 1).

Physico-Chemical Analysis: Physico-chemical parameters of the test media were collected and analyzed during the 3months of exposure. Physicochemical measurements of test media were made at the beginning of the experiment and at the end (that is, before change of test media). The parameters measured are dissolved oxygen, pH and Temperature using appropriate digital instruments (Horiber U-10) and were carried out at the Department of Marine Sciences laboratory, University of Lagos, Akoka.

Determination of Temperature (°C): The temperature of the water was determined using a mercury-in-glass thermometer. The thermometer is calibrated in degree Celsius. It measures the maximum temperature of the day.

Determination of pH: The pH or Hydrogen ion concentrations were determined using a pH meter (Model 9405) with glass electrode. The electrodes were standardized using buffer solution and then washed with distilled water. It was thereafter, washed with the sample water to be tested, dipped into the sample water and the pH read on scale.

Determination of Dissolved Oxygen (DO) (Mg/l): The Dissolved oxygen (DO) in the water throughout the experiment was measured using appropriate digital instruments (Horiber U-10). The measurement is carried by inserting the probe into the water and the readings were taken.

Cytogenetic Analysis

Micronucleus Assay: Blood samples were collected from each treatment of the experiment. The peripheral blood smears were obtained through the blood by means of a serum imprint following dissection as described by

Table 1: Percentage composition of feed ingredient used

INGREDENTS	T_0	T_1	T_2	T_3	T_4
Fish meal	20.0	20.0	20.0	20.0	20.0
CPLM	-	7.1	14.2	21.2	28.3
Rice bran	28.3	21.2	14.2	7.1	-
SBM	25.0	25.0	25.0	25.0	25.0
GNC	20.0	20.0	20.0	20.0	20.0
Tapioca	5.0	5.0	5.0	5.0	5.0
Fish premix	0.5	0.5	0.5	0.5	0.5
Table Salt	0.4	0.4	0.4	0.4	0.4
DCP	0.1	0.1	0.1	0.1	0.1
TOTAL	100	100	100	100	100

Key

To: Treatment with 100% rice bran and 0% pawpaw leaf

T1: Treatment with 75% rice bran and 25% pawpaw leaf

T2: Treatment with 50% rice bran and 50% pawpaw leaf

T3: Treatment with 25% rice bran and 75% pawpaw leaf

T4: Treatment with 0% rice bran and 100% pawpaw leaf

Fagr *et al*. and Palhares, Grisolia, [13, 14]. The slides were then, air-dried for 24h, fixed in methanol for 30min, followed by 10ml/L Giemsa (v/v) staining. Erythrocytes of each fish were examined, from both peripheral blood. To determine micronuclei in erythrocytes, the slides were examined using oil-immersion (x 1000). For the scoring of micronuclei, the following criteria were adopted from [15]; the diameter of the micronucleus (MN) should be less than one- third of the main nucleus; MN should be separated from or marginally overlap with main nucleus as long as there is clears identification of the nuclear boundary; and MN should have similar staining as the main nucleus.

A drop of blood was placed on a fat-free slide. A cover slip was set on the slide at a sharp angle until it touched the blood drop. The blood spread along the side of the cover slip which was then pulled rapidly and evenly over the slide. Two slides per animal were prepared. Micronuclei have a round or ovoid shape and a maximum diameter of one third of that of the cell nucleus. These morphological characteristic was used in differentiating the meganucleus from the micronucleus present in the cytoplasm of the blood sample.

Haematological Examination of Fish: Some of the blood collected was used for heamatological analysis. Blood samples were emptied into 5ml heparinized blood sampling bottle treated with ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant.

Statistical Analysis: The statistical analysis for the micronucleus test utilized analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Values were considered significant when ($p < 0.05$). The data from the blood were also analyzed using graphical representatives. Analysis was performed using SPSS 18 for windows.

RESULTS

Water Quality Parameters: Water quality parameters in the bowls during the experimental period are presented in Table 2. The values observed were within the tolerant range of *S. melanotheron*. The pH was between 6.22-6.86, Dissolved oxygen 7.61-8.00 mg/l and temperature 25.00-27.80 °C (Table 2).

Micronucleus Analysis: The mean frequencies of micronucleus in *Sarotherodon melanotheron* exposed to different treatments of *Carica papaya* L. replaced with ricebran in fish feed are presented in table 3. The plates below show the photomicrograph of micronucleus taking with the aid of a microscope (x 1000) showing the presence of micronuclei in T_1, T_2, T_3 and T_4 with none in the control (T_0)(Tables 1-5). The absence of micronuclei in the control experiment was due to the absence of pawpaw leaf in the diet. The lowest number of micronucleus was noticed in the blood sample of *S. melanotheron* in treatment with 75% rice bran and 25% pawpaw leaf at day 30 while the highest number of micronucleus was seen in the treatment with 100% pawpaw leaf and 0% rice bran at day 90 of the experiment.

The mean frequencies of micronucleus in *S. melanotheron* exposed to different treatments of *Carica papaya* L. replaced with rice bran in fish feed showed that there is a significant difference ($p < 0.05$) observed at Day 90 and Day 60 while, Day 30 showed no significant difference ($p > 0.05$) in the micronuclei present (Figures 1, 2 &3). ANOVA (DMRT) showed that there was a significant different ($p < 0.05$) in the frequencies observed for micro nucleated cells in *S. melanotheron* exposed to all treatments at day 60 and day 90.

Table 2: Water quality parameters measured during the experiment

Parameters	Range	Mean ± S.E
Temperature (°C)	25.00 - 27.80	26.47 ± 0.89
Dissolved oxygen (mg/l)	7.61 - 8.00	7.80 ± 0.64
pH	6.22 - 6.86	6.54 ± 0.43

Table 3: Mean frequencies of micronucleus in blood of *s. melanotheron* exposed to different treatments of *carica papaya* l. replaced with rice bran in fish feed

Exposure period	T_0	T_1	T_2	T_3	T_4
DAY 30	1.78±0.36^a	2.22±0.49^a	2.00±0.37^a	2.22±1.49^a	2.11±0.42^a
DAY 60	1.89±0.35^{ab}	2.00±0.35^{ab}	2.78±0.49^b	2.67±0.33^b	1.11±0.11^a
DAY 90	1.78±0.36^a	1.89±0.26^a	2.11±0.42^a	1.67±0.24^a	2.11±0.35^a

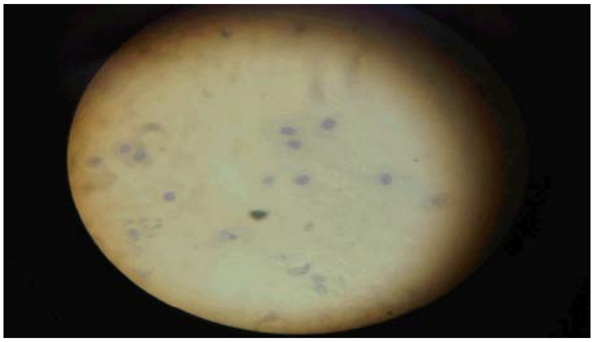

Plate 1: Micrograph of Rice Bran 100% with 0% Pawpaw Leaf (Control T_0)

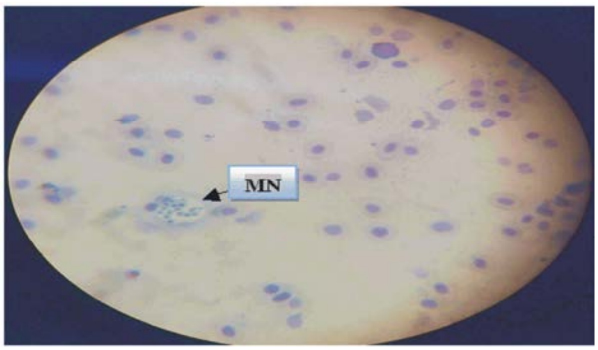

Plate 2: Micrograph of Rice Bran 75% with 25% Pawpaw Leaf (T_1)

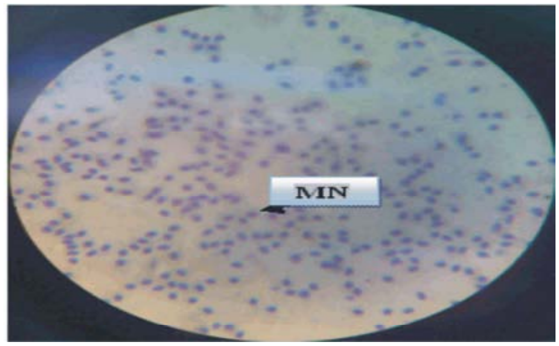

Plate 3: Micrograph of Ricebran 50% with

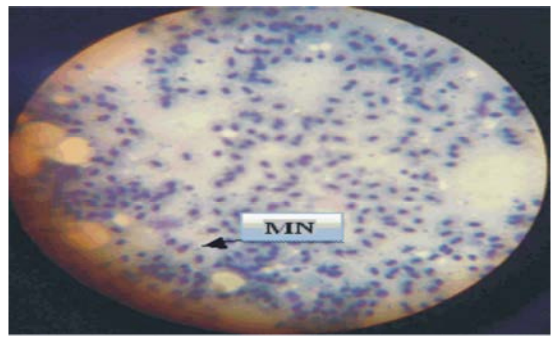

Plate 4: Micrograph of Ricebran 25% , Pawpaw Leaf 50% (T_2) with Pawpaw Leaf 75% (T_3)

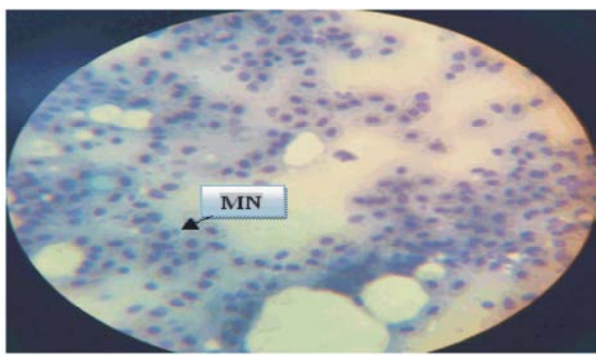

Plate 5: Micrograph of Ricebran 0% with Pawpaw, Leaf 100%.(T4)

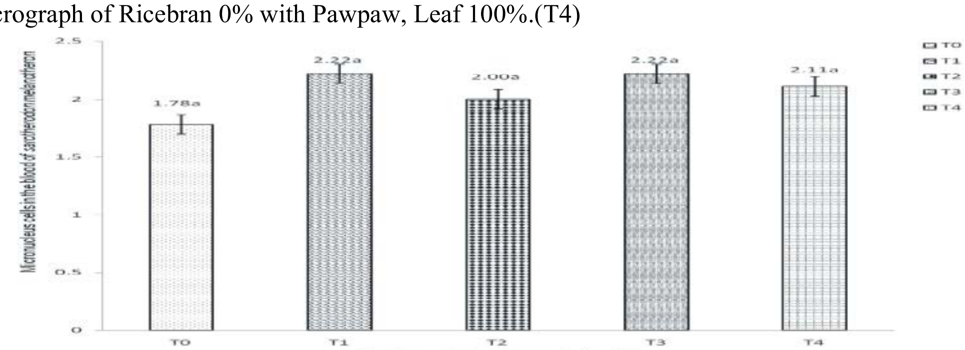

Fig 1: Micronucleus frequencies in the blood erythrocytes of *Sarotherodon melanotheron* exposed to different treatments of *Carica papaya* L. replaced with Rice bran in fish feed at DAY 30 [Means ± SE bars with the same alphabet in each exposure period are not significantly different (p >0.05, DMRT)].

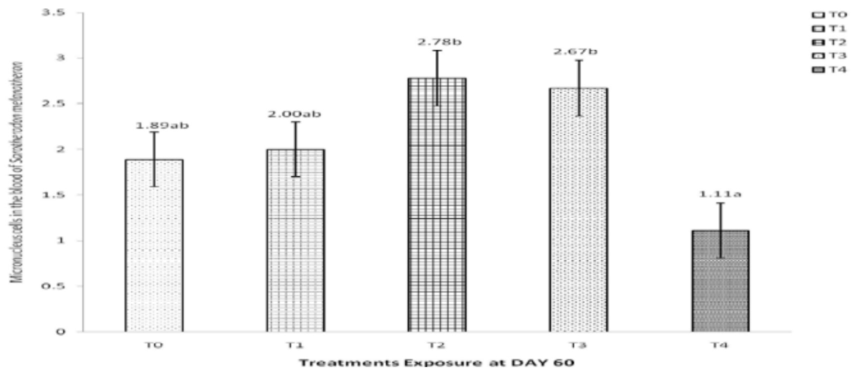

Fig 2: Micronucleus frequencies in the blood erythrocytes of *Sarotherodon melanotheron* exposed to different treatments of *Carica papaya* L. replaced with Rice bran in fish feed at DAY 60[Means ± SE bars with the same alphabet in each exposure period are not significantly different (p >0.05, DMRT)].

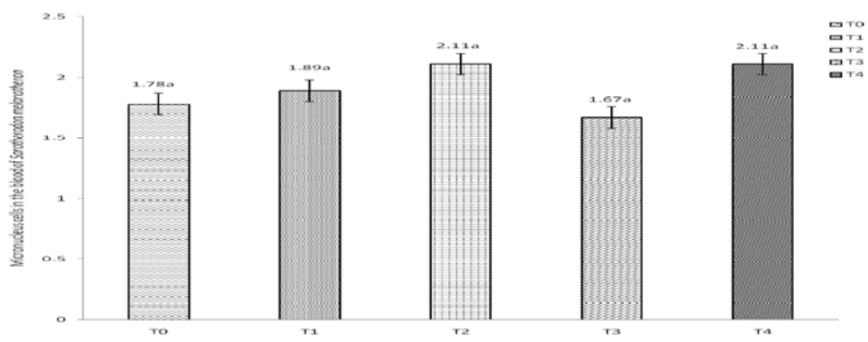

Fig 3: Micronucleus frequencies in the blood erythrocytes of *Sarotherodon melanotheron* exposed to different treatments of *Carica papaya* L. replaced with Rice bran in fish feed at DAY 90 [Means ± SE bars with the same alphabet in each exposure period are not significantly different (p >0.05, DMRT)].

Haematological Parameters: Results of Mean Haematological parameters of experiment fish samples were represented in Table 4. The mean haemoglobin values of 5.99±0.01 g/dl,6.94±0.06g/dl and 8.33±0.01g/dl for 50%, 75% and 100% respectively were significantly (p <0.05) higher than the control (5.33±0.00).

Result of Mean red blood cell value slightly increased from 2.00±0.00 to 2.90±0.10(10^{12}/l) in the (50%, 75%, 100%) levels of inclusions. There was a significant drop in the RBC count in the 25% level of inclusion. Packed cell volume values at 50%,75% and 100% were significantly higher than the values in the control and 25% level of inclusions.

The trends observed in the haemoglobin, red blood cells and packed cell volumes' for the experimental fish were indications that the dietary inclusion levels of 50%,75% and 100% favoured an increase in haematological parameters. The white blood cell count in the 50% (4.18±0.02 10^9/l) significantly higher than the control 25%(5.44±0.10 10^9/l) and 25% (1.60±0.10). The mean values of packed cell *volume* (PCV) in *Sarotherodon melanotheron* obtained from this study were within acceptable range for different inclusion of *Carica papaya* leaf meal at 50%, 75%, 100% dietary levels. The range of Mean Corpuscular volume MCV (86.36±0.64, 86.25±0.36, 90.33± 0.67, 91.30±0.00 and 86.21±0.09 fl.) in (25%, 50%, 75% and 100%) levels inclusions respectively were high. Result of the Mean Corpuscular Haemoglobin ranges (28.88±0.02-30.43±0.00 pg) in this study. There was no significant difference in the mean values of MCHC (33.33±0.00) between inclusion levels.

Analysis of Variance (ANOVA) showed a statistical significant difference (p <0.05) in the mean Hb (g/dl), PCV (%), WBC (10^9/L), MCH (pg), Neutrophils (%) and Lymphocytes (%) values at 0 %, 25%, 50%, 75% and 100% level of inclusions. No statistical significant difference(p >0.05) was recorded between mean MCHC (g/dl) values at 0%, 25%, 50%, 75% and 100% level of inclusions. There was a statistical significance (p <0.05) between mean RBC (10^{12}/L) counts between (0% and 25%), (0% and 75%) and (0% and 100%) levels of

Table 4: Mean Haematological Parameters of *Clarias gariepinus* Fed with Different Levels of *Carica papaya* Leaves

Haematological Parameters	Mean±SD				
	Control (0%)	25%	50%	75%	100%
Hb (g/dl)	*5.33±0.00*	*4.33±0.01*	*5.99±0.01*	*6.94±0.06*	*8.33±0.01*
PCV (%)	*16.33±0.58*	*13.33±0.58*	*18.00±0.00*	*20.67±0.58*	*25.00±0.00*
RBC (10^{12}/ L)	*2.00±0.00*	*1.53±0.58*	*2.00±0.10*	*2.33±0.58*	*2.90±0.10*
WBC (10^9/L)	*5.44±0.10*	*1.60±0.10*	*4.18±0.02*	*6.08±0.04*	*3.40±0.02*
MCV (fl)	*80.36±0.64*	*86.25±0.36*	*90.33±0.67*	*91.30±0.00*	*86.21±0.09*
MCH (pg)	*26.66±0.01*	*28.88±0.02*	*30.00±0.02*	*30.43±0.00*	*28.73±0.05*
MCHC (g/dl)	*33.22±0.19*	*33.33±0.00*	*33.33±0.00*	*33.33±0.00*	*33.33±0.00*
Neutrophils (%)	*12.00±0.00*	*18.00±1.00*	*21.00±0.00*	*28.00±1.00*	*35.00±0.00*
Lymphocytes(%)	*87.67±0.58*	*82.00±0.00*	*78.67±0.58*	*72.00±0.00*	*67.00±0.00*
Monocytes (%)	-	-	-	-	-
Eiosinophils (%)	-	-	-	-	-
Basophils (%)	-	-	-	-	-

Table 5: Comparative costs of different CPLM rations

INGREDIENTS	T_0	T_1	T_2	T_3	T_4
Fish meal	20.0	20.0	20.0	20.0	20.0
CPLM	-	7.1	14.2	21.2	28.3
Rice bran	28.3	21.2	14.2	7.1	-
SBM	25.0	25.0	25.0	25.0	25.0
GNC	20.0	20.0	20.0	20.0	20.0
Tapioca	5.0	5.0	5.0	5.0	5.0
Fish premix	0.5	0.5	0.5	0.5	0.5
Table Salt	0.4	0.4	0.4	0.4	0.4
DCP	0.1	0.1	0.1	0.1	0.1
TOTAL	100	100	100	100	100
COSTOF100Kg	17,255.00	16,187.00	16,127.00	15,551.00	14,991.00
COST/TONNE	172,255.00	161,870.00	161,270.00	155,510.00	149,910.00

inclusion. No significant difference (p >0.05) between (0% and 50%) levels of inclusions. There was a significant difference (p <0.05) in the mean MCV (fl) values at 0%, 25%, 50%, 75% and 100% levels of inclusions. There was no difference (p >0.05) between the mean value of MCV at 25% and 100% levels of inclusions. A significant (p <0.05) positive correlation (r=0.527) occurred between mean neurophils (%) and MCV (pg). There was a significant (p <0.05) correlation between Neutrophils (%) and MCH (pg).A significant negative (p <0.05) correlation (r=-0.527) occurred between lymphocytes (%) and MCV (fl). A significant negative (p <0.05) correlation (r=-0.537) occurred between lymphocytes and MCH (pg). A positive correlation between mean values of neutrophils (18.00±1.00%-35.00±0.00%) and mean MCV values (86.25±0.36%-91.30±0.00%) in juvenile fed with *Carica papaya* leaf meal rations.

Cost Benefit of *Carica Papaya* Leaf Meal: Table 5 showed the comparative cost benefit of different inclusion levels of *Carica papaya* in practical diets of *Sarotherodon melanotheron.* The cost of compounding each kg of experimental ration (0%, 25%, 50%, 75% and 100%) were (N17.3/kg, N16.2/kg, N16.1/kg, N15.6/kg, N14.9/kg) respectively.

DISCUSSION

The cytogenetic effect of *S. melanotheron* exposed to different treatments of Pawpaw (*Carica papaya* L.) replaced for rice bran in fish feed was evaluated for 90 days. Throughout the experiment variations was observed in terms of growth rate of the fishes, micronucleus present in the smeared blood sample and the physico-chemical parameters of the water. The presence of micronuclei in the blood sample of the fish was due to the inclusion of pawpaw leaf in the diet which triggered the rapid cell division of the nucleus. These serves as biomarkers in response to environmental chemicals at the individual level or below demonstrating departure from normal status [16]. The relationship between the physico-chemical parameters also attribute to the growth of the fishes. The physico-chemical parameter for pH has a mean value of 6.5 which falls within the FAO's limit range while the mean value for water temperature was 26.5C which also falls below limit range. Dissolved oxygen has a mean value of 7.80mg/L.

The physico-chemical parameter showed a significant difference ($p< 0.05$) between Temperature, Dissolved oxygen and pH. Generally, there was a strongly positive correlation amongst the parameters (Temperature and DO, r = 0.998*; Temperature and pH, r = 0.999*) which is an indication that there is an inter-relationship that the physico-chemical parameter of the water has a major influence on the genetic material of the *S. melanotheron*

According to Odeigah *et al.* [17], the impact of genotoxic waste water on the environment and the significance to human health are difficult to predict, because waste water are complex mixtures of chemical substances. Complete interpretation of their effect requires chemical analysis of the constituents that may indicate the components of the waste water that can persist and accumulate in exposed biota and thus potentially pose a hazard to human health.Fish are often used as sentinel organism because they play a number of roles in the trophic web, accumulate toxic substances and respond to low concentrations of mutagens [18]. Therefore, the use of ?sh biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems [19].

Fish serve as useful genetic models for the evaluation of pollution in aquatic ecosystems [20]. The erythrocyte micronucleus test has been used with different fish species to monitor aquatic pollutants displaying mutagenic features [21]. Therefore, it is suggested that micronuclei tests in fish erythrocytes be carried out at various times following treatments, thus making it possible to follow-up the changing micronuclei frequencies. Studies of the micronuclei rates of various fish species showed that they generally peaked after day forty of the treatment [22].The frequencies of micronucleus in *S. melanotheron* exposed to different treatments of Pawpaw (*Carica papaya* L.) replaced with Rice bran in fish feed showed that there is a significant difference ($p < 0.05$) observed at Day 60 and 90 while Day 30 showed no significant difference ($p > 0.05$).

The haematological response of the experimental fish fed varied dietary levels of *Carica papaya* leaf meal as summarized on the table showed significant differences in the various haematological indices for treatments (0%, 25%, 50%, 75% and 100%). However, it was observed that at 25% inclusion of *Carica papaya* there was a steep drop in the mean Haemoglobin, packed cell volume, red blood cell, white blood cell and lymphocytes values with an afterward progressive increase in mean Haemoglobin, packed cell volume, Red blood cell, white blood cell and neurophils values. The mean haemoglobin values of 5.99±0.01 g/dl,6.94±0.06g/dl and 8.33±0.01g/dl for 50%, 75% and 100% respectively were significantly ($p <0.05$) higher than the control (5.33±0.00).This is in consonance with the work of [23] for the Haematological response of *S. melanotheron* fed dietary levels of *Carica papaya* leaf meal. The red blood cell value slightly increased from 2.00±0.00 to 2.90±0.10 (10^{12}/l) in the (50%, 75%, 100%) levels of inclusions. There was a significant drop in the RBC count in the 25% level of inclusion. Packed cell volume values at 50%, 75% and 100% were significantly higher than the values in the control and 25% level of inclusions. The trends observed in the haemoglobin, red blood cells and packed cell volumes' for the experimental fish were indications that the dietary inclusion levels of 50%,75% and 100% favoured an increase in haematological parameters. The white blood cell count in the 50% (4.18±0.02 10^9/l) significantly higher than the control.The variability in WBC response of the fish might be due to the dietary inclusion levels of the leaf meal in the diets. The mean values of packed cell *volume* (PCV) in *S. melanotheron* obtained from this study were within acceptable range. It is higher than values obtained by Gabriel *et al.* [1].This increase may be associated with the inclusion of *Carica papaya* leaf meal at 50%, 75%, 100% dietary levels. The range of Mean Corpuscular haemoglobin MCV (86.36±0.64, 86.25±0.36, 90.33± 0.67, 91.30±0.00 and 86.21±0.09) in (25%, 50%, 75% and 100%) levels inclusions respectively were higher but within the range reported by Gabriel *et al*. [1]. The Mean Corpuscular Haemoglobin ranges (28.88±0.02-30.43±0.00 pg) of this study were similar to findings of Nilza *et al*. [24].There were no significant difference in the mean values of MCHC (33.33±0.00) between inclusion levels. This implies that different levels of inclusions of *Carica papaya* had no effect on this parameter. A positive correlation between mean values of neurophils (18.00±1.00%-35.00±0.00%) and mean MCV values (86.25±0.36%-91.30±0.00%) is suggestive that increase in these parameters was due to feeding fish with *Carica papaya* leaf meal rations. A negative correlation between mean MCH (pg) values (28.88±0.02 pg-30.43±0.00 pg) and lymphocytes values (82.00±0.00%-67.00±0.00) perhaps could be an indication that the *Carica papaya* leaf meal inclusion is immune-suppressive. Similar reasons may be suggested for the reduction in mean lymphocyte counts from 87.67±0.58 to 67.00±0.00%.A significant increase in mean weight of experimental fish was observed. There was however a progressive increase in mean weight of experimental fish at 25%-75% inclusion from (10.13±7.39g-16.43±0.55).A drop in weight was thereafter observed at 100% level of inclusion.

CONCLUSION

In conclusion, it is evident from this finding that exposure of test fish to Pawpaw (*Carica papaya* L.) replaced with Rice bran in fish feed does not lead to cytogenetic damage but brings about little breakage of nucleus to form micronucleus cells. The buildup of the formation regarding micronucleus indicates that home waste and agricultural pollution can increase the clastogenic effects on peripheral erythrocytes of aquatic organisms and could have similar effects on human population. The inclusion of *Carica papaya* in the feed of the experimental diets of (*S. melanotheron*) increased the growth and haematological parameters of *S. melanotheron* at 50%, 75% and 100% levels of inclusion to replace rice bran in conventional feed formulations.

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