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Serum Electrolytes, Creatinine (CRT) & Hematological (Hg) Indices of Rats Fed on Processed Atlantic Horse Mackerel

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Abstract
Trachurus trachurus (locally called *kote*) is a low cost table fish in Nigeria. Study determined effect of processed *kote* fillet; skin, head and bones (SHB), on serum electrolytes (calcium, potassium, chloride, sodium & creatinine) & full blood count in weaned male albino rat using standard methods. Chemical analyses were carried out on processed fillet; skin, head & bone (SHB) diets under standard conditions. 40 male rats (40.76 ± 2.42g) were fed with processed fillet or SHB diets for 14 days to assess. Individual blood samples were analyzed for serum electrolytes & full blood count. All data were subjected to analysis of variance by Duncan's multiple range test. Levels of calcium, potassium, chloride & sodium of rats fed with the test diets were insignificant ($p>0.05$) compared to the control groups. Levels of white blood cell & platelet in the wood smoked & poached fillet were elevated ($p<0.05$) than in the controls. Overall coal smoked fillet and SHB showed improved ($p<0.05$) levels of serum electrolytes, creatinine and hematological indices, at the expense of mild effects of high levels of processing on red blood count & haemoglobin. Processed *kote* SHB could be a good substitute for soy meal in animal feed.
Keywords: Wood & coal Smoking, Poaching, Full blood count, Serum electrolyte & *Trachurus trachurus*

1. INTRODUCTION

Fish has become increasingly important in the Nigerian diet since there is an increased awareness that regular red meat intake in adults above 40 years of age is not healthy (FOS, 1990; Olatunde, 1998; Omojowo *et al*., 2009). It is often imported into Nigeria and in resent time has gained good consumer acceptance because of its economic availability.

Fish is a very good animal source of protein, the muscle contains between 15 and 20 per cent protein, but values lower than 15 % or as high as 28 % are occasionally observed in some species (Wilcox, 2006; FIA, 2009). Fish protein provides a good combination of amino acids which is highly suited to man's nutritional requirements and compares favorably with that provided by meat, milk and eggs (Buchholz *et al*., 2000).

Amino acids have an important role in healthy and balanced nutrition. There are about twenty types of amino acid and certain of them are essential in the human diet for the maintenance of good health. If a diet is to be fully and economically utilized, amino acids must not only be present but must also occur in the correct proportions (Clark *et al*., 1999). These is because proteins contribute to key body functions, including blood clotting, fluid balance, production of hormones and enzymes, vision, and cell growth and repair (Wardlaw and Insel, 1996).

Nonetheless, fish processing methods, brings it in contact with water, smoke and high temperatures, which may interfere with the nutrients and are potential sources of RDCs and PAH generation. Furthermore the intensity of heat applied during processing greatly affects the fish protein concentration. Studies on some fish species have been fully elucidated considering their various processing methods, nutrient composition, keeping quality and biological value (Adeyemi *et al,* 2013), but to the knowledge of the researcher and scholars there still remains paucity of scientific information of how processing temperatures affect the serum electrolytes, creatinine & hematological indices in rats.

Hematological indices are indicative of the blood status of the animal and are used to assess protein quality and utilization (Oloyede *et al*., 2004). White blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hgb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and platelet count were analyzed in blood plasma of sacrificed experimental rats using the automatic analyzer techniques using the Beckman Coulter Synchron® Clinical Systems and UniCel® DxC 600/800 (2004) (Beckman Coulter Ireland Inc., 2004) systems.

Significant abnormalities in one or more of the blood cell populations can indicate the presence of one or more conditions (HCDAMBLM, 2007; Wintrobe's Clinical Hematology, 2009). The present study was therefore conducted to provide scientific data on the effect of processed *Trachurus trachurus* fillet; skin, head

and bones (SHB) on the serum electrolytes, creatinine & full blood count of weaned male wistar rats.

2. MATERIALS AND METHODS

2.1 Sample processing

The mean length and weight of *Trachurus trachurus* were; 30.52 ± 0.22 cm and 197.66 ± 3.67g respectively. Freshly purchased fish, packed in ice polystyrene boxes were transported to the laboratory within 30 min. The fish was thoroughly washed and drained, placed on wire gauze and cooked by poaching or smoking (firewood or charcoal). Poaching of the fish was done according to the method described by USDA (2006), modified by Larsen (2012). The procedure was followed without addition of any ingredient. *T. trachurus* weighing 7 kg was hot smoked using either firewood or charcoal in Altona smoke kiln as described by FAO/UN (2007). The smoking time, temperature and ambient conditions were monitored during the smoking operation. Smoking was terminated when fish was properly dried to an average moisture content of 10.41±0.02%, after 8 hours. The fish was turned at intervals and the smoked or poached fish samples kept in cane woven baskets, under laboratory conditions with no preservative, left to cool and subsequently packaged in low density and high-density polyethylene bag, sealed then stored at 8^{o}C until required for further use.

2.2 Rat diets formulation

Yellow maize (*Zea mays*) was purchased from Alice market, South Africa. The maize was soaked in warm water and changed daily for four days to soften the outer coat in preparation for milling. The corn was dried at 40^{o}C to constant weight using the Prolab Electrical Oven and milled to smooth powder using Polymix Dispersion and mixing Technology Kinemation Switzerland Blender. The animal diets were formulated following the protocol of Food and Agricultural Organization (FAO/WHO, 1991). The gross and chemical compositions of control and test diets formulated are shown in Table 1. A protein-free diet served as a negative control whereas the processed fish varieties (fillet and SHB) served as protein source in the experimental diets. All the diets for the experiment provided a minimum of 10% protein. Soy bean meal and groundnut cake were used as the protein source in the positive control. Both diets contained equal amounts of DL-methionine, sucrose, wheat meal, vitamin mix and mineral mix.

2.3 Proximate analysis of formulated diets

Raw and processed fish samples were oven dried to constant weight at 60^{o}C, fish fillet was separated from its skin, head and bones (SHB). Fish fillet or SHB was grounded to powder using a monillex kitchen blender for protein concentrate. The feed samples were analyzed for moisture and ash content (AOAC, 2002). Total crude fat was determined using the Soxhlet extraction method according to AOAC (2002) and Reinik et al. (2007). The crude fiber content was estimated by acid-base digestion method (AOAC, 2002). Crude protein content was determined by the Kjeldahl method (AOAC, 1984). Percentage nitrogen was calculated using the equation Y= 0.026x - 0.003 and R^2= 0.974 obtained from the calibration curve after nitrogen content determination (Okalebo et al., 2004). Crude protein was estimated by multiplying the nitrogen value by the converting factor of 6.25.

2.4 Experimental animals

A total of 40 weaned Wistar rats weighing between 30 and 40 g were obtained from the animal house of Central Analytical Laboratory, University of Fort Hare. The animals were kept in clean Plexiglas cages and maintained at a controlled temperature 24^{o}C with a 12 hour light-dark cycle and relative humidity of 45-50 %. They were fed with formulated diets or standard rat feed with water *ad-libitum* for 12 days. All animal experiments were conducted under NIH guidelines for care and use of laboratory animals after approval of animal ethics committee of the University of Fort Hare, South Africa.

2.5 Animal experimental design

Animals were randomly distributed into eight treatment groups with mean weight differing within ±2.00g: Group I: animal administered soya bean-groundnut cake meal (positive control). Group II: animals received basal diet (zero protein or negative control). Group III: animals received poached fillet diet. Group IV: animals treated with coal smoked fillet diet. Group V: animals fed with wood smoked fillet diet. Group VI: animals fed with poached SHB diet. Group VII: animals received coal smoked SHB diet. Group VIII: animals administered with wood smoked SHB diet for 12 days. Individual weights of the rats were taken prior to commencement of the experiment and afterwards on 4 day interval. Feed and water intake of rats were measured on a daily basis, while the cages were cleaned on 4th day, by which time the rat faces were collected. At the end of experiment the rats were sacrificed. Individual blood samples were analyzed for serum electrolytes, creatinine & full blood count.

2.6 Determination of Moisture Content

Moisture content was determined by oven drying method. A dry crucible was weighed (W_1) and 2.0 g of the

well-mixed sample was accurately weighed into the crucible and weighed ($W_{2)}$. The crucible and the content were dried in an oven at 100-105^{o}C for 12 h. Length of oven-drying time was based on bringing the samples to a constant weight. Then the crucible plus the dried content was placed in a desiccator for 30 min to cool. After cooling samples were weighed again (W_3); the percent moisture was calculated using the formula below:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100.$$

Where W_1 = weight of crucible; W_2 = weight of crucible and sample; W_3 = weight of crucible and sample after drying

2.7 Determination of Crude Fat Content: Crude fat was determined using the Soxhlet extraction method described by the AOAC (2002). 2.0g of moisture free sample was weighed into a fat free thimble, plugged with cotton wool and then introduced into the extraction tube. A clean dry boiling flask was weighed (W_1) and 250ml of petroleum ether was introduced into the flask and sample was extracted for 6 h continuously as described by Reinik et al. (2007). The extract was concentrated in a rotary evaporator (RE-100, Stone Staffordshire, and England) at 60^{o}C to 2ml. This was repeated for other samples. Then the remaining solvent removed from the extracted oil by placing the flask in the fume hood at 25^{o}C for 45min and weighed (W_2). The percent crude fat was calculated by the following formula:

$$\% \text{ Fat} = \frac{W_2 - W_1}{\text{Wt of sample}} \times 100$$

Where W_1 = weight of empty flask; W_2 = weight of flask and fat deposit

2.8 Determination of Crude Fiber Content: Crude fiber was estimated by acid-base digestion following the method described by AOAC (2002). The residue obtained after lipid- extraction of 2g from the sample was put in a 1L beaker and 200mL of boiling 2.5M H_2SO_4 was added. The content was boiled for 30min, cooled and filtered using a Buchner funnel followed by washing the residue three times with 50mL boiling water. The washed residue was returned to the beaker for further digestion with 200mL of 2.5 M NaOH for 30min. The resulting solution was filtered, washed three times with 50mL boiling water and then 25mL ethanol. The washed residue was dried in an oven at 130^{o}C to constant weight and cooled in a desiccator. The residue was carefully scraped into pre-weighed porcelain crucible, weighed (W_1) and ash at 550^{o}C for 2h. It was cooled in a desiccator and re-weighed (W_2). Crude fiber was expressed as percentage loss in weight after ignition.

$$\text{Crude Fiber (\%)} = 100 - \frac{[W_1 - W_2]}{W}$$

Where W_1= weight (g) of crucible and content before ashing W_2 = weight (g) of crucible containing ash; W= weight (g) of sample

2.9 Determination of Crude Protein Content

Total nitrogen (crude protein) was determined by the method of micro-Kjeldahl (AOAC, 1984). A known weight (0.5g) of dry and ground fish sample was weighed into a digestion tube. A volume of 12mL (9mL Nitric acid + 3mL HCL) digestion mixture was added to the tubes. The mixture was then digested using the Buchi 425 digester from Switzerland at number 4 setting for 1h, until the solution was clear. The mixture was allowed to cool after which it was made up to 50ml with de-ionized water. The total nitrogen was determined colorimetrically using the method as described by Okalebo et al. (2002). Percentage nitrogen was calculated using the equation Y= 0.026x - 0.003 and R^2= 0.974 obtained from the calibration curve using various concentration of the standards. The protein content was determined by multiplying the Nitrogen content value by 6.25.

2.10 Biochemical Evaluation of Diets on Rats Tissues

Rats were grouped and fed with compounded experimental feed and water *ad libitum*, but starved for 12 hours before the start of the experiment. All the animals from each group were sacrificed by chloroform anesthesia 24hr after respective 14 days of feed trial and water intake.

2.11 Determination of Serum electrolytes & Hematological Indices

Blood plasma was used prepared by collecting blood samples were in purple cap tubes coated with EDTAK3 in the interior of the tube wall.

Principle: EDTA binds the calcium ions and therefore blocks the coagulation cascade. Erythrocytes, leucocytes and thrombocytes in an EDTA anticoagulated blood sample are stable for up to 24 hours.

i. Blood Chemistry: The blood serum used to determine the serum electrolytes (Na^+, Cl^-, K^+ & Ca^{++}) was

prepared by collecting blood samples in green cap test tubes coated with lithium heparin on the interior of the tube wall to inhibit clotting. While serum creatinine was determined by collecting blood samples in the yellow cap test tubes coated with clot activator and inside the tube a barrier gel presented at the bottom, used for serum separation. The serum / plasma were then rapidly spun with a centrifuge in order to remove the blood cells or clot.

Principle: The anticoagulant heparin activates anti-thrombin, thus blocking the coagulation cascade and producing a whole blood/plasma sample instead of clotted blood plus serum.

ii. Hemoglobin Concentration (HgB) CN-Free Method and CN Method: The hemoglobin method used was a modification of the manual cyanmethemoglobin method developed by the International Committee for Standardization in Hematology (ICSH).

iii. RBC Indices: The red cell indices MCH and MCHC were derived from the mathematical calculation of the RBC count, total hemoglobin and the MCV determination. HCT was calculated from the RBC count and the MCV determination. RDW and HDW were calculated from cell by cell measurement of cell volume and hemoglobin concentration.

iv. White blood cell (WBC) Count: The whole blood sample was mixed with Advia®120 basso reagent that contained acid and surfactant, the red cells were hemolyzed and the white cells were then analyzed using 2 angle laser lights scatter signals.

v. WBC Differential Method - Peroxidase Method: The peroxidase method was developed by Cremin, Kim, Malin and Sclafani, based on the principles of differential cellular staining outlined by Ansley and Ornstein. According to these principles, leukocytes are classified by the characteristics properties exhibited by cell-specific constituents when the cells are treated with cytochemical stains. The enzyme peroxidase is present active in several leukocyte types. In the presence of hydrogen peroxide and an appropriate electron acceptor chromogen, peroxidase develops a darkly colored material which precipitates in the cells. Normal neutrophils and eosinophils possess significant levels of peroxidase activity, with enzyme activity corresponding to cell maturation. The monocytes contains lower amounts of peroxidase, which makes it possible to define them as a cell population with relatively large light-scatter signals and absorption signals that extend from the unstained cells up to, and partly overlapping, the most weakly-stained neutrophils. The lymphocytes population analyzed with the peroxidase method contained both lymphocytes and basophiles. The basophile count (obtained from the Basophiles / Lobularity method) is subtracted from the lymphocyte population to obtain the lymphocyte count. The peroxidase cytochemical reaction consists of 2 steps. In the first step, EDTA anti-coagulated whole blood sample was diluted with Advia 120 perox 1 reagent. Surfactants and thermal stress cause lysis of the red blood cells. Formaldehyde in Advia 120 perox 1 reagent fixes the white blood cells. During the second step, Advia 120 perox 2 reagents and Advia 120 perox 3 reagent were added to the peroxidase reaction chamber. The 4-chloro-1-napthol in Advia 120 perox 2 reagents and hydrogen peroxide in Advia 120 Perox 3 reagent stain the sites of peroxidase activities in the granules of neutrophils, eosinophils and monocytes. Lymphocytes, basophils and large unstained cells contain no granules with peroxidase enzyme activity. A constant volume of the cell suspension from the perox reaction chamber passes through the flow cell. The two fluids flowed as independent, concentric streams (no mixing), with the Advia 120 perox sheath stream encasing the sample stream. The absorbance and forward light-scattering signatures of each blood cell were measured. The optical signals were converted to electrical pulses by photodiodes. After processing, the information was displayed in two histograms. The perox Y histogram contained the forward scattering data (cell size). The Perox X histogram contained the absorption data (peroxidase scattering). The two histograms are combined to form the Perox cytogram from which the cells were identified and counted.

Determination of Serum Electrolytes (Sodium (N_a^+), Potassium (K^+), Calcium (Ca^{++}), Chloride (Cl^-)) & Creatinine in Rat Serum: The method as described in the manual for the Beckman Coulter Synchron® Clinical Systems and UniCel® DxC 600, 800 (2004) systems. 40 µl of the sample was mixed in the ratio1: 33 with the buffered solution. When the sample / buffer mixture made contact with the electrode, electrolytes ions in the sample underwent an ion exchange process with the corresponding ion in the hydrated portion of the electrode. Changes occur in the electrode potential as the ion exchange progresses, which are referenced to the reference electrode automatically by the system. The referenced potential follows the Nernst equation and is used to calculate the electrolyte ion concentration of the sample as follows: $E = \text{Constant} + (\text{Slope})(\log [\text{Ion}^+])$.

2.12. Statistical Analysis

Significant differences between means of experiments were determined by least significant difference. SPSS 14.0 statistical tool was used to analyze the data obtained. Results were considered statistically significant at $p < 0.05$ with Duncan's multiple range test (Duncan 1955).

3. RESULT

3.1. Proximate Analysis of Control and Test Diets: The data on the proximate analysis of formulated diets is presented in Table 1. The crude protein content was significantly high ($p < 0.001$) whereas crude fat content was highest ($p < 0.001$) in the WSCF as compared with the positive control diet. All formulated diets had sufficient nutrients required for growth and development of experimental animals.

3.2. Organ-Body Weight: Organ / body weight ratio of rats fed with the test and control diets are represented in Figures 1 & 2 respectively. Figure 1 showed that the liver / body ratio of rats fed with CSKFBD, WSKFBD and PKFBD were significantly higher ($p < 0.01$) compared to those in the control groups (positive and negative) respectively; no significant difference ($p > 0.05$) was observed in the brain / body, heart / body, stomach / body, small Intestine / body, kidney / body and spleen / body weight ratio of rats fed with the processed fillet diet compared to those fed with the control diets respectively. In Figure 2 rats fed with the SHBBD revealed that the liver/body and small Intestine/body weight ratios had the highest ($p < 0.001$) ratios in the following order of decreasing magnitude when compared to the control groups i.e., CSHBBD> WSHBBD > PSHBBD > S-GBD > ZPD. No difference ($p > 0.05$) was observed in the brain/body, heart/body, stomach / body and spleen / body weight ratio of rats fed with the SHBBD compared to those fed with the control diets respectively.

3.3 Serum Electrolytes & Creatine Contents of Rats Fed with Control and Test (Fillet and SHB) Diets

The serum calcium and potassium, chloride, sodium & creatinine levels of rats fed with the test and control diets are represented in Figures 3 and 4 respectively. Figure 3, showed insignificant difference ($p>0.05$) in the levels of calcium, Na^+ & Cl^- of rats fed with the processed fillet diets compared to those in the control groups respectively; while serum potassium was significantly reduced ($p<0.05$) in rats fed with processed fillet diets than in the positive control group.

Figure 4 shows result of the serum calcium and potassium, chloride, sodium & creatinine levels of animals fed with the test (SHB) and control diets. Levels of serum calcium and potassium were significantly lesser ($p < 0.05$) in rats that fed with the SHB meal based diets compared to the positive control diets. While the levels of serum Na^+ & Cl^- showed no significant difference ($p>0.05$) compared to those fed with the control diets. Furthermore, values obtained were within the serum clinical range (2.10- 8.50mmol/l) for Cl^- and (135 – 145 mmol/L) for Na^+.

3.4. Hematological Indices of Rats Fed with Test and Control Diets

Hematological indices of animal fed with the test and control diets are shown in Figures 5 and 6 gives hematological indices of animal fed with test and control diets. Present investigation showed that the RBC, Hgb, HCT, and LYMP were significantly reduced ($p < 0.05$) in rats fed with test diets compared to the controls respectively. Variations observed in the values of haematocrit (HMCT), monocytes (MONO), mean corpuscular volume (MCV), mean corpuscular haematocrit concentration (MCHC), and red blood cells width (RDW) were not significant ($p>0.05$). Levels of White blood cell (WBC) and platelet (PLT) in the wood smoked and poached fillet were significantly elevated ($p<0.05$) compared to the controls.

Table 1: Gross and proximate composition (%) of experimental (fillet and SHB) based diets

Parameters	Protein	Fat	Ash	Crude fibre	Moisture	CHO
γS-GBD	13.76±4.91^{b}	2.15±0.01^{b}	3.17±0.17^{c}	8.43±0.01^{b}	46.67±0.17^{a}	28.19±0.96^{b}
†S-GBD	13.76±4.91^{a}	2.15±0.01^{c}	3.17±0.17^{c}	8.43±0.01^{c}	46.67±0.17^{a}	28.19±0.96^{a}
γZPD	11.52±4.35^{d}	2.86±0.03^{b}	3.17±0.17^{c}	8.30±0.10^{b}	47.50±0.50^{a}	24.28±1.12^{c}
†ZPD	11.52±4.35^{b}	2.86±0.03^{c}	3.17±0.17^{c}	8.30±0.10^{c}	47.50±0.50^{a}	24.28±1.12^{b}
CSKFBD	14.62±2.23^{a}	3.24±0.08^{a}	5.5±0.29^{a}	6.36±0.01^{d}	38.17±1.42^{b}	32.11±0.81^{a}
CSHBBD	10.44±3.32^{c}	5.03±0.08^{a}	7.67±0.44^{a}	19.06±0.03^{b}	40.17±1.09^{b}	17.63±1.92^{d}
WSKFBD	12.43±3.24^{c}	3.45±0.09^{a}	6.17±0.17^{a}	13.18±0.02^{a}	47.00±1.00^{a}	17.77±0.97^{d}
WSHBBD	12.68±3.28^{b}	5.62±0.26^{a}	4.5±0.00^{b}	18.90±0.03^{b}	46.17±0.33^{a}	12.13±0.78^{e}
PKFBD	14.66±1.01^{a}	3.05±0.01^{a}	3.5±0.00^{b}	7.85±0.09^{c}	46.50±0.00^{a}	24.44±0.21^{c}
PSHBBD	14.25±2.45^{a}	3.69±0.02^{b}	8.17±0.33^{a}	24.82±0.01^{a}	29.00±3.00^{c}	20.07±0.82^{c}

*Data= Mean ± SEM, n=3. Values with different superscripts along a row are significantly different ($p < 0.05$). CSKFBD: charcoal smoked *kote* fillet meal based diet; WSKFBD: wood smoked *kote* fillet meal based diet; PKFBD: poached *kote* fillet meal based diet; CSHBBD: charcoal smoked *kote* SHB meal based diet; WSHBBD: wood smoked *kote* SHB meal based diet; PSHBBD: poached *kote* SHB meal based diet S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control).† stands for SHB group and γ stands for fillet fed group; CHO stands for carbohydrate.

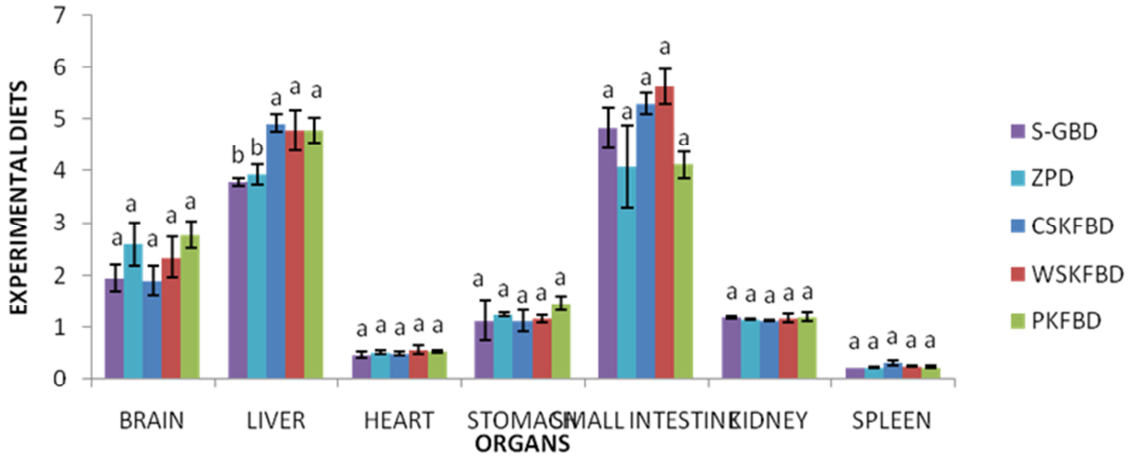

Figure 1: Organ/Body Weight o Rats Fed on Control and Test (Test) Diet*

*Values are means of 3 determinations ± SEM. n=3.Bars with the same colour but different letters are significantly different (P < 0.05K). CSFBD = coal smoked *kote* fillet meal based diet, WSKFBD= wood smoked *kote* fillet meal based diet, PKFBD= poached *kote* fillet meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control)

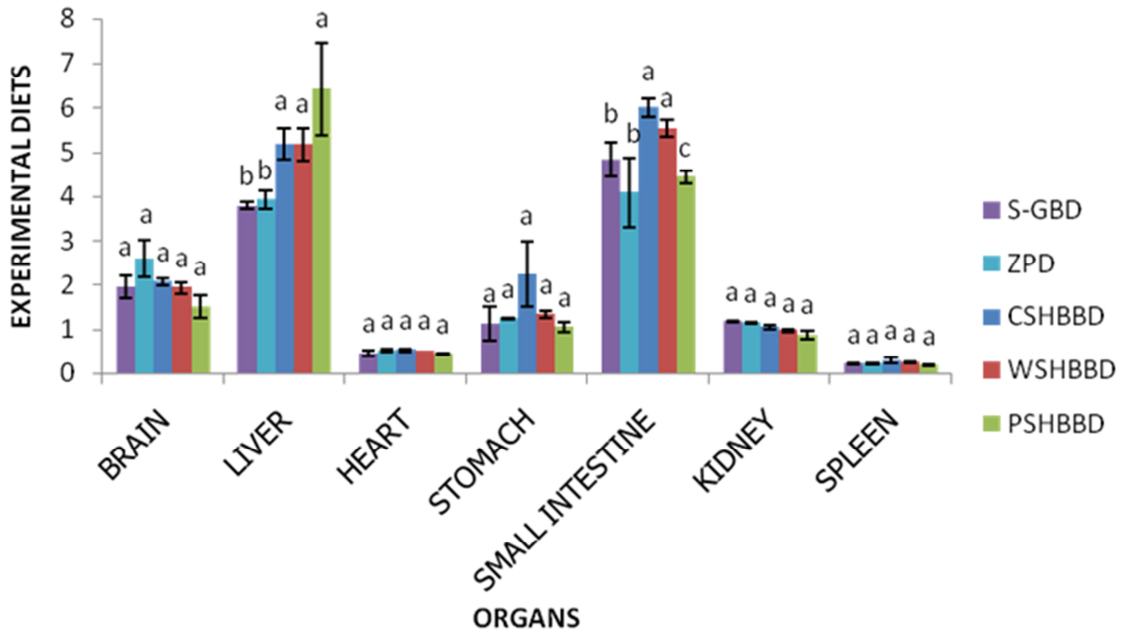

Figure 2: Organ / Body Weight of Rats Fed on Control and Test (SHB) Diet*

*Values are means of 3 determinations ± SEM. N= 3. Bars with the same colour but different letters are significantly different (P < 0.05). CSSHBBD: charcoal smoked *kote* SHB meal based diet; WSSHBBD: wood smoked *kote* SHB meal based diet; PSHBBD: poached *kote* SHB meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control)

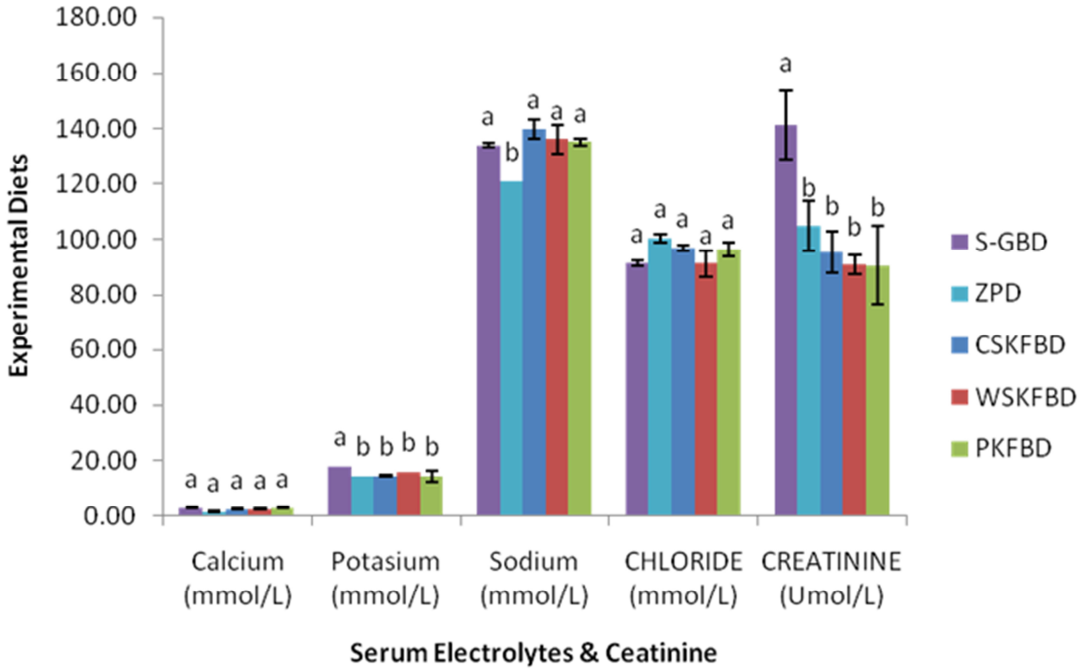

***Figure 3: Serum Electrolytes & Ceatinine Contents of Rats Fed with Control and Test (Fillet) Diets.**
*Values are means of 3 determinations ± SEM. n= 3. Bars with the same colour but different letters are significantly different (P < 0.05). CSKFBD = coal smoked *kote* fillet meal based diet, WSKFBD= wood smoked *kote* fillet meal based diet, PKFBD= poached *kote* fillet meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control).

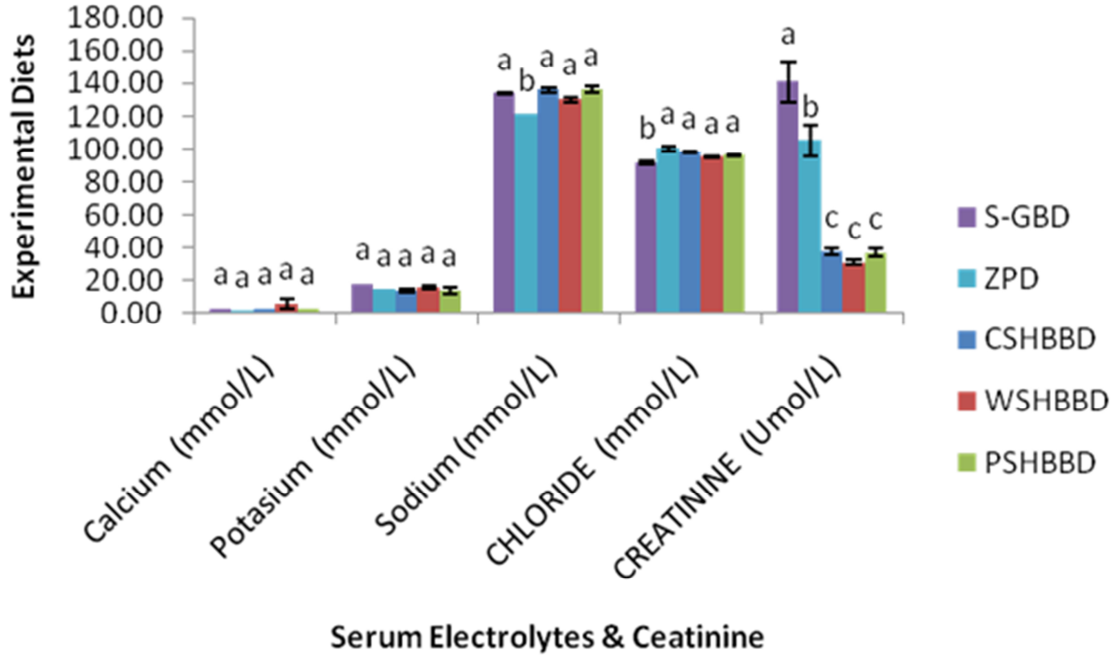

***Figure 4: Serum Electrolytes & Ceatinine Contents of Rats Fed with Control and Test (SHB) Diets.**
*Values are means of 3 determinations ± SEM. n= 3. Bars with the same colour but different letters are significantly different (P<0.05). CSSHBBD: charcoal smoked *kote* SHB meal based diet; WSSHBBD: wood smoked *kote* SHB meal based diet; PSHBBD: poached *kote* SHB meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control).

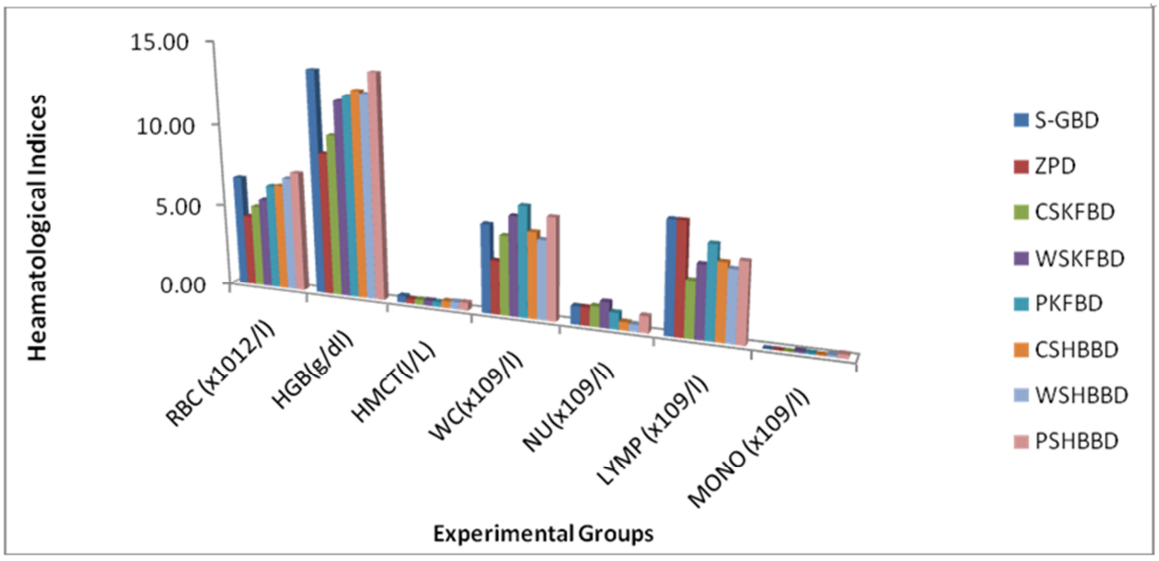

Figure 5: Heamatological Indices of Rats Fed with Control and Test Diets (I)

*Values are means of 3 determinations ± SEM. n= 3. Bars with the same colour but different letters are significantly different (P<0.05). CSSHBBD: charcoal smoked *kote* SHB meal based diet; WSSHBBD: wood smoked *kote* SHB meal based diet; PSHBBD: poached *kote* SHB meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control); RBC= red blood count; , Hgb= hemoglobin, HMCT= Haematocrit, WBC= white blood cells, NU= Neutrophiles, LYMP= lymphocytes, MONO= Monocytes.

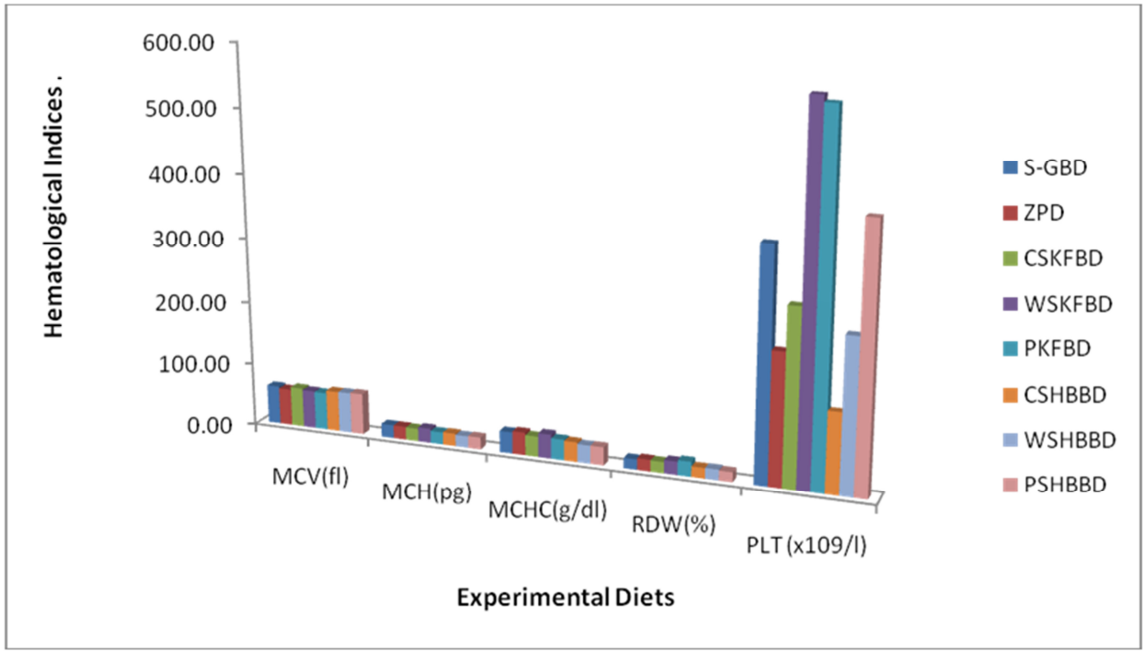

Figure 6: Heamatological Indices of Rats Fed with Control and Test Diets (II)

*Values are means of 3 determinations ± SEM. n= 3. Bars with the same colour but different letters are significantly different (P<0.05). CSSHBBD: charcoal smoked *kote* SHB meal based diet; WSSHBBD: wood smoked *kote* SHB meal based diet; PSHBBD: poached *kote* SHB meal based diet; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control); MCV= mean corpuscular volume, PLT= Platelet count, MCH= mean corpuscular haematocrit, MCHC= mean corpuscular haematocrit concentration, RDW= red blood cells width.

4. DISCUSSION

The role of test and control diets on protein digestibility and bioavailability, in weaned male rats showed that the test and control diets conformed to the recommended feeding protocol (Food and Agricultural Organization Protocol, 1991; Aduku, 2005) and were adequate to meet growth requirements of the weaned rats. Although the

crude fat content was highest ($p < 0.001$) in the WSCF compared with the positive control, this was not significant ($p > 0.05$) because the observed value was less than 30% reported by Delorme and Gordon, (1983) and Benevenga et al, (1995) that cause a decrease in the growth of rats. Furthermore, the analyzed nutrients components in test and controls diets fell within the acceptable recommendation range of the nutrient required for laboratory animal (Benevenga et al, 1995). Thus values for all the nutrients in test compared well with the positive and were similar to those reported by Benevenga et al, (1995).

The organ to body weight ratio gives a proportional size of the organ to body weight. It has been suggested that the use of organ - body weight ratios may be valuable in evaluating the relationship between certain experimental situations and the biological response of a test organism (Charles and Richard, 1965). The liver being the major organ carrying out metabolic and detoxification processes is unique among the body's vital organs in that it can be regenerated. WSKFBD had the highest organ/body weight in the small intestine / body of animals in the fillet, while PSHBBD was highest in the liver / body and CSHBBD in Stomach/body and small intestine/body ratios of animals fed on the SHB based diets compared with the control. Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences ($p < 0.05$) in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Steven et al, 2004). Thus the organ-body weight ratio showed better ($p<0.05$) performance in animals placed on the SHB meal based diets in the brain, liver, stomach and small intestine compared to those placed with the fillet and control diet respectively.

Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of an organism (Kakade *et al.*, 1972; Luiz, *et al.*, 1998*)*. Both muscle tissue and neurons are considered electric tissues of the body. Muscles and neurons are activated by electrolyte activity between the extracellular fluid and intracellular fluid and the balance of these electrolytes in the body is essential for normal function of the cells and organs (Coso *et al.*, 2008). Electrolytes occur in large quantities in both extracellular and intracellular fluids, with respect to serum sodium and chloride levels. Muscle contraction is dependent on the presence electrolytes like; calcium (Ca^{2+}), sodium (Na^{+}), and potassium (K^{+}), without sufficient levels of these key electrolytes, muscle weakness or severe muscle contractions may occur (Kamil *et al.*, 2011). However, no significant ($p > 0.05$) difference was observed in rats fed with the fillet and SHB diets of serum sodium and chloride compared to those in the control groups. Sodium regulates the total amount of water in the body and the transmission of sodium in and out of individual cells plays a critical role in the body functions (Syzdek *et al*., 2010).

Many processes in the body, especially in the brain, nervous system, and muscles, require electrical signals for communication (Syzdek *et al*., 2007). The movement of sodium is critical in generation of these electrical signals. Too much or too little sodium therefore can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal (Syzdek *et al*., 2009). Reduction in serum Na & Cl below the clinical accepted range is indicative of dehydration and shock, while an increase in serum albumin is indicative of stress on the liver organ and a reduction of the same is indicative of chronic liver diseases (Coso *et al*., 2008), this was however not the case in the present study.

Calcium is a major structural element in bones and teeth. It is necessary to stabilize or allow for optimal activity of a number of proteins and enzymes. The binding of calcium to the protein, troponin-c, initiates a series of steps that lead to muscle contraction. The binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen, which provides energy for muscle contraction (Heaney, 2000). Also the binding of calcium ions is required for the activation of the seven "vitamin K-dependent" clotting factors in the coagulation cascade. The normal range for blood calcium is 2-10 to 8.50mmol/l. Hence, with the exception of ZPD (1.54mmol/l), rats fed on control and experimental diets fell within the normal clinical range for serum calcium. A low blood calcium level usually implies abnormal parathyroid function, and is rarely due to low dietary calcium intake since the skeleton provides a large reserve of calcium for maintaining normal blood levels (Davies *et al*., 2000).

Conversely, serum calcium levels in the rats fed with the PKFBD and WSKFBD were high ($p < 0.05$) compared to the controls, indicating that *kote* may be a rich source of calcium, especially the skin, head and bones (SHB). Furthermore tests that measure the concentration of electrolytes are needed for both the diagnosis and management of renal, endocrine, acid-base, water balance and many other conditions. Their importance lies in part with the serious consequences that follow from the relatively small changes that diseases or abnormal conditions may cause (Henry, 2001). Potassium is often a STAT (needed immediately) test because values below 3.0 mmol / l are associated with arrhythmia (irregular heartbeat), tachycardia (rapid heartbeat), and cardiac arrest. Abnormal potassium cannot be treated without reference to bicarbonate, which is a measure of the buffering capacity of the plasma (Tierney *et al.*, 2001).

Sodium measurements are very useful in differentiating the cause of an abnormal potassium result. Thus serum potassium levels in present study was clinically significant; because high levels of serum potassium is indicative of cardiac arrhythmia (irregular heart beat) (Ruothalo, 2008). Conditions such as the overuse of

diuretics (drugs that promote lower blood pressure) often result in low levels of both sodium and potassium. On the other hand, Cushing's (adrenocortical over-activity) and Addison's disease (adrenocortical under-activity) drive the sodium and potassium in opposing directions (Wallach, 2000). Thus the levels of potassium, sodium, calcium and chloride for rats fed with the test and positive control diets showed no significant difference ($p > 0.05$) in the serum electrolyte, confirming that the nutrients in these diets were adequate to sustain growth and maintenance in the rats. This result is in agreement with the reports of Lin *et al*., (2000) that there is an interaction of calcium and energy intake in predicting changes in body weight.

Serum creatinine is an important test for knowing the condition of the kidneys. It is a major catabolic product of the muscles and protein respectively. An increase in CRT is clinically indicative of muscular dystrophy or wasting disease. This was not the case in present study because the creatinine levels of rats fed on the fish meal based (fillet and SHB) diets were lower ($p<0.05$) in rats fed with the test diets compared to those fed with the control diet.

In addition, serum creatinine (CRT) levels were also lower ($p < 0.05$) in rats fed with the fillets diet compared with the control. These results are positive because values obtained were within the stipulated ranges; more so an increase in serum creatinine is indicative of diseases affecting the cardiac muscle (Rao, 2006), while an increase in serum albumin is indicative of stress on the liver, but a decrease indicates chronic liver diseases (Rao, 2006).

In the SHB groups however, the levels of serum creatinine was also lower ($p < 0.05$) in the rats fed with the SHBBD compared to those fed on control diets. According to Ravel (1995), secretion of creatinine occurs through a combination of glomerular filtration and tubular secretions. Increases in serum creatinine may therefore be as a result of these two factors and could indicate renal damage (Keller, 1986).

Hematological indices are indicative of the blood status of the animal and are used to assess protein quality and utilization (Oloyede *et al*., 2004). A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. CBC also helps in checking for weakness, fatigue, or bruising, that may be present in an organism (Web Medical Reference, 2010). Abnormally high or low counts may indicate the presence of many forms of disease conditions, such as anemia, infection, and many other disorders (Web Medical Reference, 2010).

There was no significant difference ($p>0.05$) observed in the MCV, MCH, MCHC and RDW levels of rats fed on test diets compared with those in the control groups. MCV is an important trait which determines the cell size of erythrocytes and is therefore an important factor in determining the ability of rats to withstand prolonged oxygen starvation (Miruka and Rawnsley, 1997; Mmereole, 2008). Hemoglobin (Hgb) and haematocrit are basic values revealing the degree of anemia, while MCHC is a useful index of the average hemoglobin concentration of the red cells (Edem, 2009). Low values of RDW indicate uniformity in size of RBCs. Thus, RDW result in present investigation confirms uniformity in size of RBCs of rats fed with the test and control diets respectively.

Nevertheless, significantly lower ($p<0.05$) levels were observed in the RBC and hemoglobin (Hgb) of rats fed with the test diet compared to those in the control groups. Low RBC is indicative of anemia caused by nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency); While an increased ($p<0.05$) RBC and Hgb level indicates polycythemia, usually caused by; dehydration, smoking or genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release) (Pagana and Pagana, 2006). Polycythemia is triggered as a result of the presence of cyto-toxins (like RDCs and PAHs). Nonetheless this increase was insignificant because the RBC and Hgb levels were still within the given clinical range.

An increase in RBC is indicative of thallasaemia and decrease points to anaemia (Rao, 2006; CBC, 2012); while an increase in WBC is indicative of inflammation / hemorrhage and a decrease is implicative of viral infection or bone marrow failure. Also an increase in Hgb is implicative of lung diseases and a decrease may result in anemia, while an increase MCHC may result in prolonged dehydration and a decrease Iron deficiency (Ruothalo, 2008; CBC, 2012). In the Nu, an increase is implicative of inflammation / hemorrhage and a decrease viral infection or bone marrow failure. Increase in Lymph may imply viral infection / leukaemia and a decrease indicative of bone marrow infiltration or lupus. Increase in Mono is indicative of Protozoan diseases, leukemia or malignant diseases (Ruothalo, 2008; CBC, 2012).

Alterations in the qualitative and quantitative composition as well as the biochemistry of the blood cells are warnings and indications of an impaired function (Stroev, 1989; Oloyede *et al.*, 2004). Therefore the significant increase ($p<0.05$) in the platelet count of rats fed on the WSKFBD and PKFBD over those in the control groups could be due to the significantly high ($p < 0.05$) levels of RDC and PAH earlier observed in the WSKF and PKF (Adeyemi *et al.*, in press). Nonetheless, platelet count of rats fed with CSKFBD was insignificant ($p \geq 0.05$) relative to the control, because animals that do not have enough platelets ($<190 \times 10^{-9}$) may be at an increased risk of excessive bleeding and bruising (Harmening, 2009), this was not the case in the present study.

According to Kwan, (1992), increase in platelet values above the control value (800×10^{-9}) may serve as

a marker of vascular diseases such as micro-angiopathy. In rats fed with the SHB diets however, levels of RBC, Hgb, Nu, and platelets were significantly increased ($p < 0.05$) in those fed with the PSHBD compared with those in the control groups. This may have been due to the presence of RDC and PAH levels earlier observed in the SHB, which was significantly highest ($p < 0.001$) in the poached SHB. While the significant decrease ($p < 0.05$) recorded in WBC and lymph of rats fed with the PSHBBD, CSHBD and WSHBD compared to those on control diets, could predispose the rats to reduced immunological responses to infections. Low levels of NU are indicative of neutropenia and high levels are indicative of neutrophilia (Kasper *et al.*, 2005).

Furthermore, several studies have reported that a significant decrease ($p < 0.05$) in WBC of blood indicates a decline in the proportion of the defensive mechanism to combat infections, a situation which would naturally make the animal more susceptible to various physiological stresses resulting in diseases, greater mortality and poor growth (Mohammed, 2007). What may be responsible for the destruction of these cells are toxic components (like the RDCs and PAHs in the boiled, coal and wood smoked SHB the animals fed on.

This corroborates the earlier observation that thermal processes favours RDC and PAH volatile formations in fish muscle. Thus hematological parameters of the blood of weaned male albino rats fed on the processed SHB meal-based diet, though not the same with the control in WBC and Lymphocytes; showed an improvement in the HCT, MCH and RDW levels which compared significantly well ($p < 0.05$) with rats fed with the test and control diets. This invariably suggests that feeding of the test diet for a longer period may be detrimental to the rats since these two factors are involved in the formation of immunoglobins responsible for the development of antibodies, thus resulting in health hazards to such animals when challenged with infections.

The levels of lymphocyte in animals fed on the processed fillet and SHB meal based diets were also significantly lower ($p < 0.05$), WBC of the same fluctuated compared with the control groups animals. The decrease observed in the lymphocytes of rats fed on experimental diet is of a positive impact, because an increased number of lymphocytes are indicative of a viral infection. In certain disease states, such as leukemia, abnormal (immature) white cells rapidly multiply, increasing the WBC count, this might have been the reason for the significantly high ($p < 0.05$) levels in WBC and Monocytes of rats fed on WSKBD and PSKBD compared with the rats fed on the control diets respectively.

The relatively high values of the WBC and Monocytes can be attributed to the immune system of the rats attempting to detoxify the toxic chemicals (RDC and PAHs) present in the feed (Calhon and Brown, 1975; Liener, 1989). The significantly high ($p<0.05$) levels of blood platelet in the test diets compared to the positive control further confirms the presence of these toxins, because high ($p<0.05$) levels of platelet in the test group compared to the control is indicative of the presence of toxic chemicals within the diet. This lends credence to the reports of Iglesias, (2010) that RDCs and PAH in raw and processed food samples could be cyto-toxic.

5. CONCLUSION

This study concludes that the wide variability of nutrient profile and content between the processing methods observed in the present study strengthens the importance of producing data derived from a selection of effect of cooking methods. Nonetheless, slight aberrations observed in hematological parameters like RBC and Hgb of the rats fed with the test diets compared to those in the control group might have been as a result of the presence of effects RDC and PAH in raw and processed samples. RBC (i.e. glyoxal) and PAH (Naphthalene) cause the break down and dysfunction on red blood cell in addition to suppressing of the immune system, indicating that prolonged feeding of the rats on these diets could be harmful to the red blood cells, haemoglobin and platelets. *Trachurus trachurus* SHB could be a veritable source of valuable ingredients for human food and animal feeds. Especially if milled into flour and used to fortify food / feed stuff, it may be a valuable alternative food in the human diet and animal feed.

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