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Variations in the Levels of Total Protein, Urea and Ureate in Weaned Male Albino Rats Fed on Processed Atlantic Horse Mackerel

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Abstract
Trachurus trachurus (locally called *kote*) is a table fish sold for human consumption in Nigeria. Processing methods like poaching and smoking interfere with nutrients and are potential sources of reactive di-carbonyl compounds (RDCs) and polyaromatic hydrocarbons (PAHs). This study determined the variations in the levels of total protein, urea and ureate in weaned male albino rats fed on processed fillet; skin, head and bones (SHB), using standard methods. Chemical analyses were carried out on processed fillet & SHB diets under standard conditions. 40 male rats (40.76 ± 2.42g) were fed with processed fillet or SHB diets for 14 days to assay for on total protein, urea and ureate. All data were subjected to analysis of variance by Duncan's multiple range test and considered significant at a minimum of $p < 0.05$. Results indicated that levels of serum electrolyte, proteins and urea were insignificant ($p>0.05$). Study suggests that coal smoked *kote* SHB showed great promise as a possible protein substitute in animal feed, if gathered it could be utilized as a form of protein concentrate at little costs thus reducing costs of feeds due to highly priced casein, fish meal, soybean meal and groundnut cake (GNC) in south western Nigeria.
Keywords: Processed *kote*; Full blood count; total protein; Urea and Ureate

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. A few foods other than fish contain omega 3, which are also foods that can be included in a gout diet (Cleland et al, 1995). **F**ish oil in recent times has been reported to be effective in reduceing the presence of prostanglandins over longer periods of time, thus eliminating the need to take NSAIDs (non-steroidal anti-inflammatory drugs such as aspirin or ibuprofen), which often cause side effects (George Mateljan Foundation, 2015).

A substantial amount of research has also been accomplished regarding the strong anti-inflammatory properties of fish oil, or omega 3 fatty acids. By facilitating the production of prostanglandins, hormones responsible for reducing inflammation and regulating calcium dispersion, fish oil is a necessary part of a successful low purine diet intended to combat the pain of gout (Choi and Curhan, 2005). In addition, prostanglandins inhibit particular pro-inflammatory hormones that are part of the prostanglandin family (Word Press, 2015).

Uric acid forms during the metabolism of purines being during cellular degeneration and recycling of the cell's genetic material. While it is normal for the production of uric acid to result from purine break down, an inordinate amount eventually proves to generate harmful medical conditions. Certain amounts of uric acid is essential to promote healthy blood vessel linings that properly maintain blood flow throughout the body (Choi, 2004).

Purines, are natural compounds found in certain foods, are broken down into uric acid in the body. They're found in organ meats, beef, pork, lamb, sardines, anchovies, canned tuna, mackerel, lobster, scallops, shrimp, clams, asparagus, mushrooms, spinach, green peas, cauliflower, beans and lentils (Leslie, 2008). Purines are essential in our diets, because they help protect the blood vessels from damage and these purines are a common recycled product when body cells die (Brule et al, 1990).

Fish is rarely eaten raw and it is usually processed by various cooking methods, such as boiling, grilling, baking, and frying, before consumption (Adeyemi et al, 2015). Although cooking processes affect purine content, the nature of the changes are not clear. On the one hand, boiling high-purine foods in water caused break-down of the purine-containing components (nucleic acids), eventual freeing up of the purines for absorption. In some animal studies, rats were fed cooked versus non-cooked foods, the animals eating the cooked

version experienced greater absorption and excretion of purine-related compounds(George Mateljan Foundation, 2015).

In addition the cooking marine of fish at high temperatures, may interfere with the nutrients and are potential sources of RDCs and PAH generation. Although these RDCs are responsible for the characteristic aromas of fish (Milo & Grosh, 1996; Durnford & Shahidi, 1998; Adeyemi et al, 2015), high RDC levels are caused by thermal processes (Varlet, Prost & Serot, 2007; White, 2009), moisture content and water activity (Goldberg et al, 2004 and Golgi et al, 2006).

Total protein measurements reflect nutritional status and may be used to screen for and help diagnose kidney disease or liver disease. Protein in the plasma is made up of albumin and globulin (AACC, 2014). A low A/G ratio may reflect overproduction of globulins, such as seen in multiple myeloma or autoimmune diseases, or underproduction of albumin, such as may occur with cirrhosis, or selective loss of albumin from the circulation, as may occur with kidney disease (nephrotic syndrome). A high A/G ratio suggests underproduction of immunoglobulins as may be seen in some genetic deficiencies and in some leukemias (AACC, 2014).

Animal studies in this area have shown definite changes in purine content following the boiling and broiling of beef, beef liver, haddock, and mushrooms. Nonetheless research on cooking, total protein; Urea and Ureate content is very limited. Present study was therefore conducted to identify on the variations in the levels of total protein, urea and ureate in weaned male albino rats fed on processed fillet; skin, head and bones (SHB).

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 harvested fish from the wild sea were purchased from two major fish distributors in Oja Ipata, Ilorin, 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 (The Economic Research Service of the USDA, (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 bags respectively, 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.

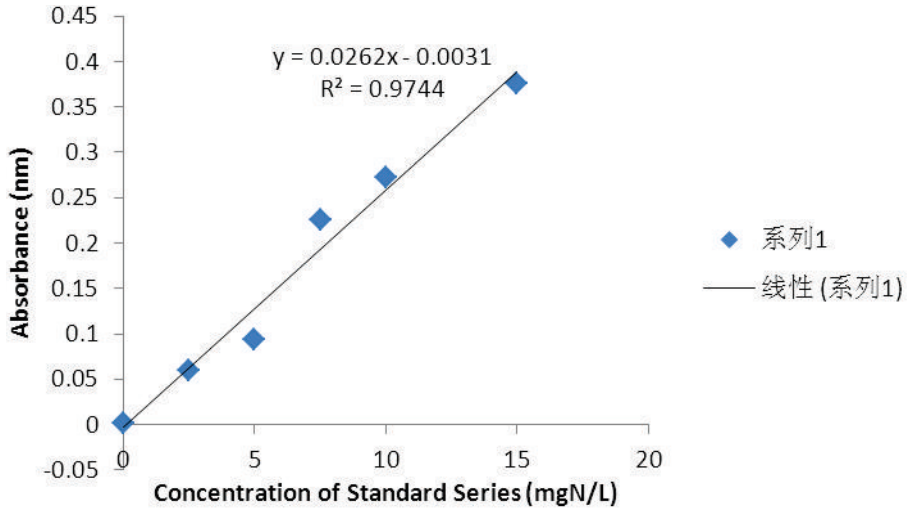

Figure 1: Graph Showing Calibration Curve Used for the Calculation of Nitrogen Content

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 total protein, urea and ureate.

2.6 Preparation of Corn Starch: Yellow maize (*Zea mays*) was purchased from Alice market, South Africa. The maize was soaked in warm water and changed daily for four days, as 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 like granules using the Polymix Dispersion and mixing Technology Kinemation Swizaland Blender.

2.7 Proximate Analysis of Formulated Diets: Raw and processed fish samples were oven dried to constant weight at 60^{o}C, and then fish fillet was separated from its skin, head and bones (SHB). Both fillet and SHB were grounded separately using a monillex kitchen blender, to produce protein concentrates. The feed samples were analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract according to the methods of AOAC (2002).

2.8 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

2.9 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

2.10 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

2.11 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.

2.12 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.

2.13 Collection / Preparation of Serum used to Determine Total Protein, Urea and Ureate

The blood serum used to determine the serum total protein, urea and ureate was prepared 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 was then rapidly spun with a centrifuge in order to remove the blood cells or clot.

2.14 Determination of Serum Total Protein (TP): The TP reagent was used in conjunction with the SYNCHRON® Systems, UniCel® DxC 600/ 800 and Synchron Systems Multi Calibrator systems for the quantitative determination of total protein in serum by a timed end point biuret method (Tietz, 1994). In the reaction, peptide bonds in the protein sample bind to cupric ions in an alkaline medium to form purple colored peptide - copper complexes.

$$\text{Protein} + Cu^{2+} \xrightarrow[\text{Alkali}]{} \text{Protein - Copper Complex}$$

The Synchron System automatically measure out 10µl of the sample and 500 µl of the TP reagent into the cuvette. The change in absorbance is monitored by the system at 560 nm. Change in absorbance was directly proportional to the concentration of urea in the sample and is used by the system to calculate and express the total protein concentration.

2.15 Determination of Serum Albumin (ALB): The ALB reagent was used for the quantitative estimation of Albumin in the rat serum by a timed end point method (Wang and Zakowski, 1986; Tietz, 1994). In the reaction, albumin combines with bromocresol purple (BCP) to form a colored product. (i.e. Albumin + BCP ----------→ Albumin- BCP complex). The Synchron System automatically measures out 10 µl of the sample and 1000µl of the ALB reagent into the cuvette. The change in absorbance is monitored by the system at 600nm. Change in absorbance is directly proportional to the concentration of albumin in the sample and is used by the system to calculate and express ALB concentration.

2.16 Determination of Serum Urea: Urea nitrogen measurements are used in the diagnosis and treatment of certain renal and metabolic diseases. UREA reagent was used in conjunction with the SYNCHRON® Systems, for the quantitative determination of Urea in the serum by an enzymatic method (Talke and Schubert, 1965; Tiffany *et al*., 1972). In the reaction, urea is hydrolyzed by urease to ammonia and carbon dioxide. The condensation of ammonia and α-ketoglutarate to glutamate and the subsequent oxidation of the product (NADH), to NAD^+ is catalyzed by glutamate dehydrogenase.

$$\text{Urea} + H_2O \xrightarrow[\text{Urease}]{} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-ketoglutarate} + NADH + H^+ \xrightarrow[\text{Glutamate dehydrogenase}]{} \text{Glutamate} + NAD^+ + H_2O$$

The Synchron System automatically measured out 10 µl of the sample and 1000 µl of the UREA reagent into the cuvette. The change in absorbance is monitored by the system at 340 nm. Change in absorbance was directly proportional to the concentration of urea in the sample and is used by the system to calculate and express urea concentration.

2.17 Uric acid: The Roche/Hitachi analyzer (Synchron System) automatically measured out 10µl of the sample and 1000µl of the URIC ACID reagent into the cuvette. The change in absorbance is monitored by the system at 340 nm. Change in absorbance was directly proportional to the concentration of uric acid in the sample and is used by the system to calculate and express uric acid concentration.

2.18 Statistical analysis

The data from all the analyses were collected and statistically analyzed and expressed as the mean ± standard error (s.e.) (n=3), the significant differences between means were compared for each group of rats using the least significant difference test after ANOVA for one-way classified data. SPSS 14.0 (SPSS, 2005) statistical tool was used to analyze data obtained. Results were considered statistically significant at a level of $p < 0.05$, chosen as the minimum for significance 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 2 & 3 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 3 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 Total Protein, Urea and Ureate Contents of Rats Fed with Control and Test (Fillet and SHB) Diets

The serum total protein, urea and ureate levels of rats fed with the test and control diets are represented in Figures 4 and 5 respectively. Figure 4, showed insignificant difference ($p>0.05$) in the levels of albumin, globulin, AG (albumin/globulin ratio), as well as total proteins in rats fed on the fillet meal based diet compared to those in the control meal based diets. The level of serum urea was significantly reduced ($p < 0.05$) in rats fed with fillet diet compared to those in the positive control group. In addition variations observed in the levels of ureate in rats fed with fillet diet was not difference ($p > 0.05$) compared to the positive control.

In figure 5, the level of serum urea was significantly reduced ($p < 0.05$) in rats fed with SHB diet compared to those in the positive control group. In addition variations observed in the levels of ureate in rats fed with fillet diet was not difference ($p > 0.05$) compared to the positive control. While insignificant differences ($p>0.05$) were observed in the levels of albumin, globulin, AG (albumin/globulin ratio) & total proteins in rats fed on the SHB meal based diet compared to those in the control meal based diets.

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.

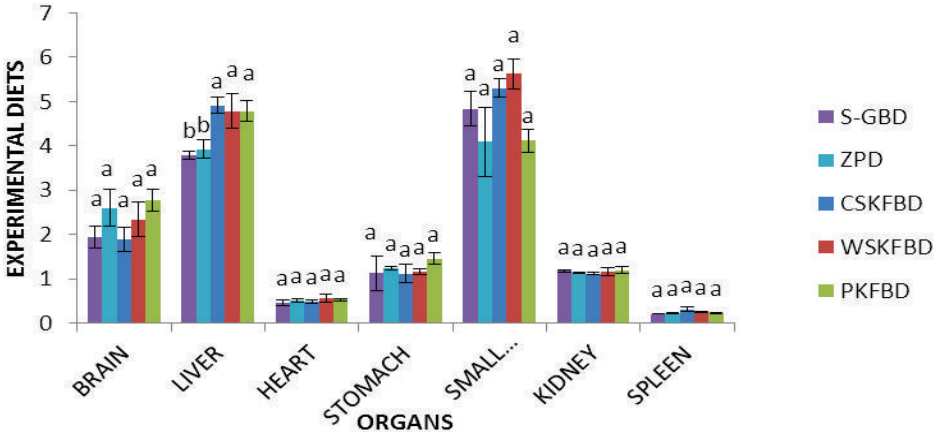

Figure 2: Organ/Body Weight o Rats Fed on Test (Fillet) and Control Diet*

*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)

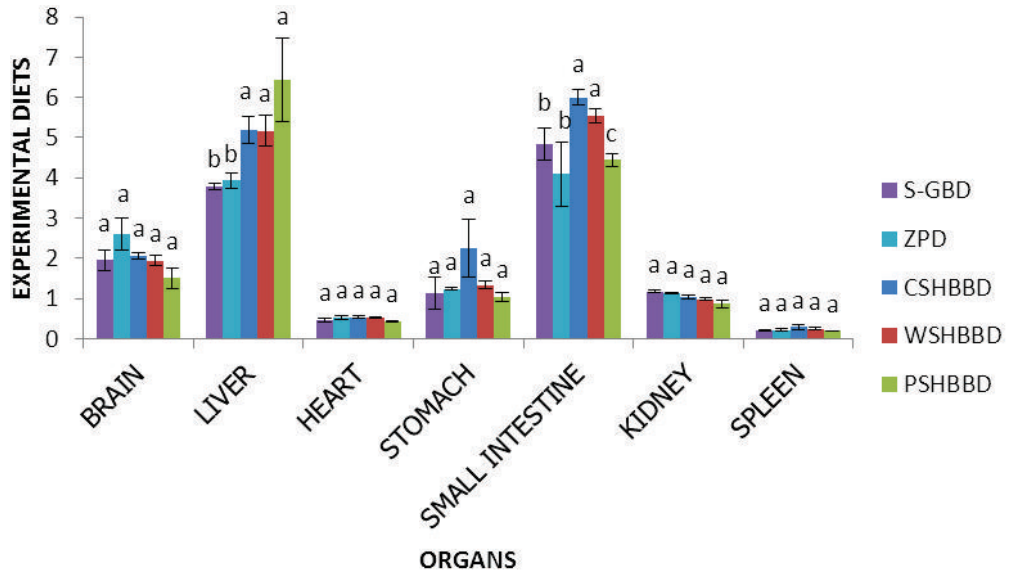

Figure 3: Organ / Body Weight of Rats Fed on Test (SHB) and Control Diet*

*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)

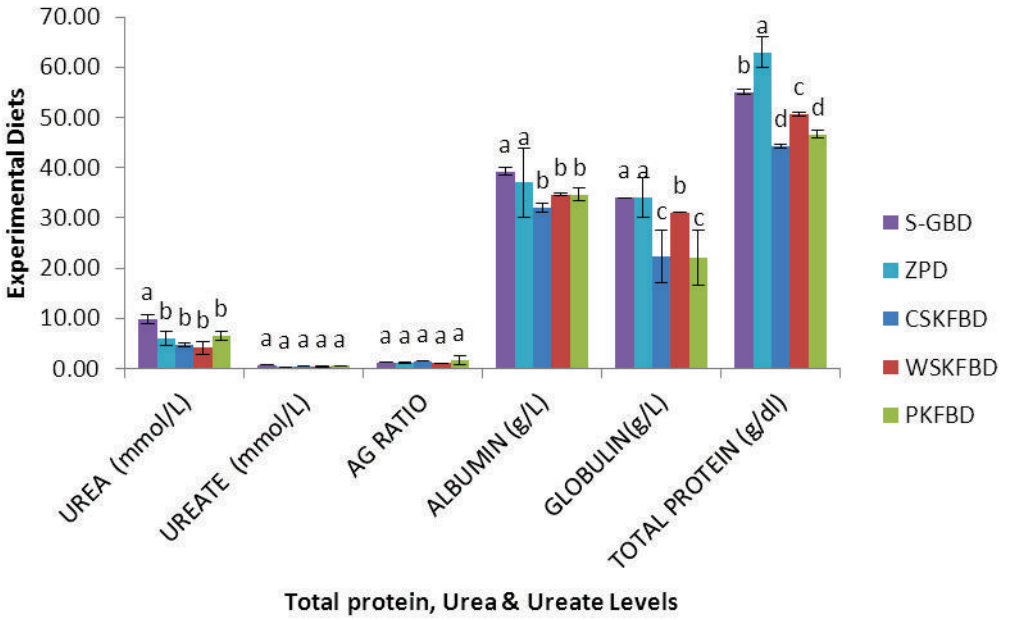

***Figure 4: Serum Electrolytes, Total Protein, Urea and Ureate Contents of Rats Fed with the Control and Fillet Meal Based Diets.**

*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);

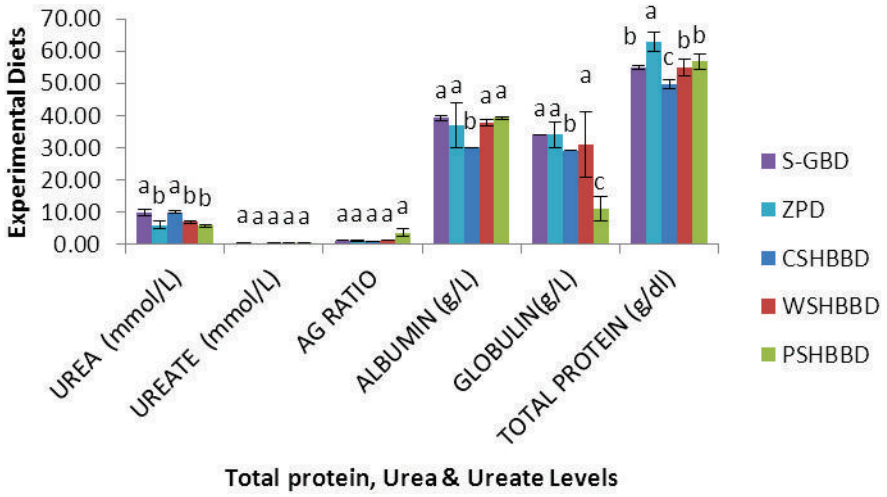

***Figure 5: Serum Electrolytes, Total Protein, Urea and Ureate Contents of Rats Fed with the Control and SHB Meal Based Diets.**

*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)

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.

Serum urea is an important test for knowing the conditions of the kidneys. They are major catabolic products of muscles and protein respectively. Normal clinical range for serum urea is 2.86 to 8.57mmol/l; an increase in urea level may indicate impairment of renal functions, dehydration, shock, burns, fever or high protein diet (Cameron and Greger, 1998). This was not the case in present study because the serum urea 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.

Total serum protein consists of albumin and the globulins and gives information regarding the nutritional status and malnutrition of animals (Allison *et al*., 1995, Adeyemi, 2013). An increase in globulin is indicative of chronic liver diseases due to reduced clearance by the hepatocytes. However, current study showed no difference ($p > 0.05$) in serum total protein and albumin of rats fed with the test diets compared to those in the control group; also values were within the clinical range (60-82g/l).

Serum albumin is important for maintaining oncotic pressure, and transportation of many substances in the blood. When there is inadequate protein intake, the body begins to breakdown to obtain enough amino acids for the synthesis of serum albumin (Green, 1980). A low serum albumin therefore indicates poor liver function (Naganna, 1989).

While the albumin / globulin (A/G) ratio, describes the relationship between albumin and globulins. A/G ratio of the animals fed with test and control diets were within the clinical range of >1.00, thus implying that the test diet, though contained some anti-nutrients (RDCs and PAH) in low ($p < 0.05$) amount (Adeyemi *et al.*, in press), contained adequate protein and nutrients, which aided and sustained healthy growth and development in the weaned male rats.

5. Conclusion

In this study the effect of processed atlantic horse mackerel (fillet & SHB) on serum electrolytes, total protein, urea and ureate in weaned male albino rats was detected for the first time in South western Nigeria. The change in cooking losses tended to have a linear relationship with the time and temperature of cooking, this concurs with the reports of Garsia-Segovia *et al*., (2007). 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. Knowledge about quality and composition is a necessity; *Trachurus trachurus* SHB is rich in potentially valuable nutrients, which aided and sustained healthy growth and development in the weaned male rats. Currently, the major wastes (heads, skin, and skeleton) are underutilized and often create disposal problems and environmental concerns in many developed countries. These waste may also have many alternative uses in pharmaceutical, agricultural, aquaculture and industrial applications such as being used as a good substitute for fish meal in live stock feed.

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