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Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Activities in Selected Tissues of Rats Fed on Processed Atlantic Horse Mackerel (*Trachurus trachurus*)

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Abstract

Trachurus trachurus **(locally called kote) is a table fish that has become increasingly important in the Nigerian diet because of its low price. Therefore, this study investigated the effects of processed kote on the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in selected tissues of weaned male albino rats using standard methods. Chemical analyses were carried out on processed fillet, skin, head & bone (SHB) diets under standard conditions. 40 male rats (40.76 ± 2.42 g) were fed with processed fillet or SHB diets for 14 days to assay for the activities of ALP in harvested samples of brain, liver, kidney, heart, stomach, small intestine and spleen; ALT and AST in the liver and heart. All data were subjected to analysis of variance by Duncan's multiple range test and considered significant at a minimum of $p < 0.05$. Levels of ALP & AST in the liver, heart, kidney and brain were reduced ($p < 0.05$) in rats fed on test diets compared with the controls, but within accepted limit. The level of ALT in the kidney, stomach and small intestine were elevated ($p < 0.05$) compared to the controls, but within the accepted limit. In conclusion, coal smoked fillet and SHB greatly improved healthy growth of the rats, followed by the wood smoked and poached diets. Results suggested that processed kote SHB could**

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be a veritable source of valuable nutrients for human food and animal feed.

Keywords

Smoking, Poaching, Kote, ALP, ALT and AST

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 [1]-[3]. It is often imported into Nigeria and in resent time, has gained good consumer acceptance because of its economic availability. 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 Reactive Dicarbonyl Compounds (RDCs) and Polyaromatic Hydrocarbons (PAH) generation. Although these RDCs are responsible for the characteristic aromas of fish [4] [5], high RDC levels are caused by thermal processes [6] [7], moisture content and water activity [8] [9]. Studies on some fish species have been fully elucidated considering their various processing methods, nutrient composition, keeping quality and biological value [10], but to the knowledge of the researcher and scholars there still remains paucity of scientific information on the effect of processing on some hematological indices and the enzyme activities of selected rat tissues. Tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage [11]. It may be elevated if bile excretion is inhibited by liver damage. The tissue activities of the transaminase (ALT and AST) enzyme are markers for the functions and integrity of the heart and liver [12]. 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 full blood count, serum electrolytes, cholesterol, triglycerides, urea, creatinine and total proteins in the blood. As well as activities of alanine transaminase (ALT), aspatate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatinine (CRT), cholesterol and glucose levels in the brain, liver, kidney, heart, stomach, small intestine and spleen 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 ± 3.67 g 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 [15], modified by Larsen [14]. 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 [15]. 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˚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˚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 [16]. 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

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.

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˚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 [17]. Total crude fat was determined using the Soxhlet extraction method according to AOAC [17] and Reinik *et al*. [18]. The crude fiber content was estimated by acid-base digestion method [17]. Crude protein content was determined by the Kjeldahl method [19]. 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 [20]. 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˚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 ±2.00 g: 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. Activities of alanine transaminase (ALT), aspatate transaminase (AST), and alkaline phosphatase (ALP) were run in harvested samples of liver, kidney, heart, stomach, small intestine and spleen. Lactate dehydrogenase (LDH) and creatinine (CRT) in the heart and kidney, in addition to cholesterol and glucose levels of the brain.

2.6. Proximate Analysis of Formulated Diets

Raw and processed fish samples were oven dried to constant weight at 60˚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 [17].

2.7. 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˚C - 105˚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.

2.8. Determination of Crude Fat Content

Crude fat was determined using the Soxhlet extraction method described by the AOAC [17]. 2.0 g 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 250 mL of petroleum ether was introduced into the flask and sample was extracted for 6 h continuously as described by Reinik *et al*. [18]. The extract was concentrated in a rotary evaporator (RE-100, Stone Staffordshire, and England) at 60˚C to 2 mL. 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˚C for 45 min and weighed (W_2). The percent crude fat was calculated by the following formula:

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

where W_1 = weight of empty flask; W_2 = weight of flask and fat deposit.

2.9. Determination of Crude Fiber Content

Crude fiber was estimated by acid-base digestion following the method described by AOAC [17]. The residue obtained after lipid-extraction of 2 g from the sample was put in a 1 L beaker and 200 mL of boiling 2.5 M H_2SO_4 was added. The content was boiled for 30 min, cooled and filtered using a Buchner funnel followed by washing the residue three times with 50 mL boiling water. The washed residue was returned to the beaker for further digestion with 200 mL of 2.5 M NaOH for 30 min. The resulting solution was filtered, washed three times with 50 mL boiling water and then 25 mL ethanol. The washed residue was dried in an oven at 130˚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˚C for 2 h. It was cooled in a desiccator and re-weighed (W_2). Crude fiber was expressed as percentage loss in weight after ignition.

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

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

weight (g) of sample.

2.10. Determination of Crude Protein Content

Total nitrogen (crude protein) was determined by the method of micro-Kjeldahl [19]. A known weight (0.5 g) of dry and ground fish sample was weighed into a digestion tube. A volume of 12 mL (9 mL nitric acid + 3 mL 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 1 h, until the solution was clear. The mixture was allowed to cool after which it was made up to 50 mL with de-ionized water. The total nitrogen was determined colorimetrically using the method as described by [20]. 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.

2.11. 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 24 hr after respective 14 days of feed trial and water intake.

2.12. Preparation of Tissue Homogenates

Tissues were carefully removed from the sacrificed animals using a pair of gloves, dissecting set and collected in 15 mL 0.25 N sucrose and then homogenized. 1 g each of tissue (brain, liver, kidney, heart, stomach, small intestine and spleen) was weighed and homogenized in ice-cold 10 mL tris buffer (pH 7.8) using a triston homogenizer. The homogenates were centrifuged using the Beckman model TJ-6 refrigerated centrifuge (USA) at 2000 rpm for 10 minutes. The supernatant was carefully decanted into specimen bottles, kept frozen overnight to ensure maximum release of the enzymes in the tissue cells [21].

2.13. Determination of Tissue Enzyme Activities

Assay of Alkaline Phosphatase (ALP): The method described by Bassey *et al*. [22] as modified by Wright *et al*. [23] using Randox kits. In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm, and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation: ALP activity (IU/l) = 2742 × ΔA 405 nm/min; Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

Assay of alanine transaminase (ALT) activity: The method described by IFCC [24] using Randox kits was used. 50 µl of the sample and 500 µl of the ALT reagent were mixed in a test tube, and the initial absorbance at 340 nm was read after 1 minute. The timer was started simultaneously and further readings of the absorbance were taken after 1, 2, and 3 minutes. ALT activity (nm/min) = 1746 × ΔA 340 nm/min, ΔA 340 nm/min = change in absorbance per minute for the homogenate sample, 1746 = Extinction coefficient.

Assay of aspatate transaminase (AST) activity: The same assay method described for ALT was used with the exception that the ALT reagent was replaced with the AST reagent. AST activity (nm/min) = 1746 × ΔA 340 nm/min; ΔA 340 nm/min = change in absorbance per minute for the homogenate sample; 1746 = Extinction coefficient.

Determination of serum alkaline phosphatase (ALP): The substrate p-nitrophenyl phosphate is hydrolyzed by alkaline phosphatase from the sample in the presence of magnesium ions, to form nitrophenol which is yellow and can be read at 405 nm. The intensity of color produced is proportional to the activity of alkaline phosphatase.

$$\text{Para nitrophenolphosphate} + \text{HOH} \xrightarrow{\text{ALP}} \text{paranitrophenol} + \text{phosphate}$$

The procedure described by Bassey *et al*. [22] as modified by Wright *et al*. [23] using Randox kits was used for the assay. In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm, and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation: ALP activity (IU/l) = 2742 × ΔA 405 nm/min. Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

3. 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 [25]. Results were considered statistically significant at $p < 0.05$ with Duncan's multiple range test [26].

4. Result

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.

4.1. Organ/Body Weight

Organ/body weight ratio of rats fed with the test and control diets are represented in **Figure 1** & **Figure 2** respectively. **Figure 1** showed that the liver/body ratio of rats fed with charcoal smoked kote fillet meal based diet (CSKFBD), wood smoked kote fillet meal based diet (WSKFBD) and poached kote fillet meal based diet (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.*, charcoal smoked kote SHB meal based diet (CSHBBD) > wood smoked kote SHB meal based diet (WSHBBD) > poached kote SHB meal based diet (PSHBBD) > soy bean & groundnut cake meal based diet (Positive control) (S-GBD) > zero protein diet (Negative control) (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.

4.2. Serum ALP

ALP activities of rats fed with the test and control diets are represented in **Figure 3**. Results indicated that levels

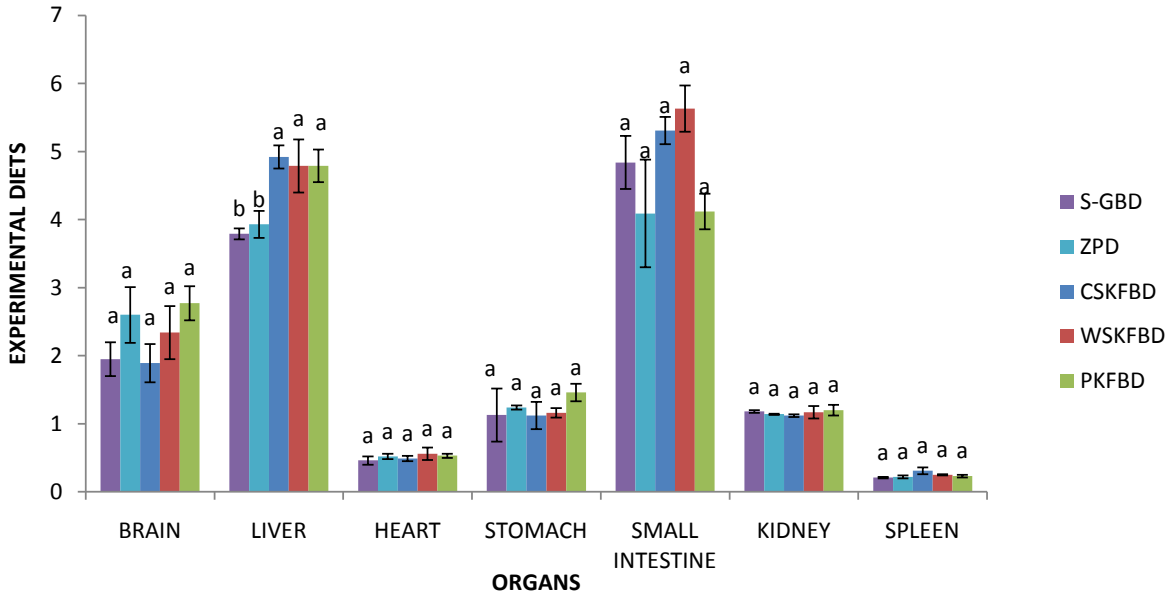


Figure 1. Organ/body weight of rats fed on control and test (fillet) diet. *Values are means of 3 determinations ± SEM. n = 3. Bars with the same colour but different letters are significantly different ($p < 0.05$K). CSFBD: coal smoked kote fillet meal based diet; WSKFBD: wood smoked kote fillet meal 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).

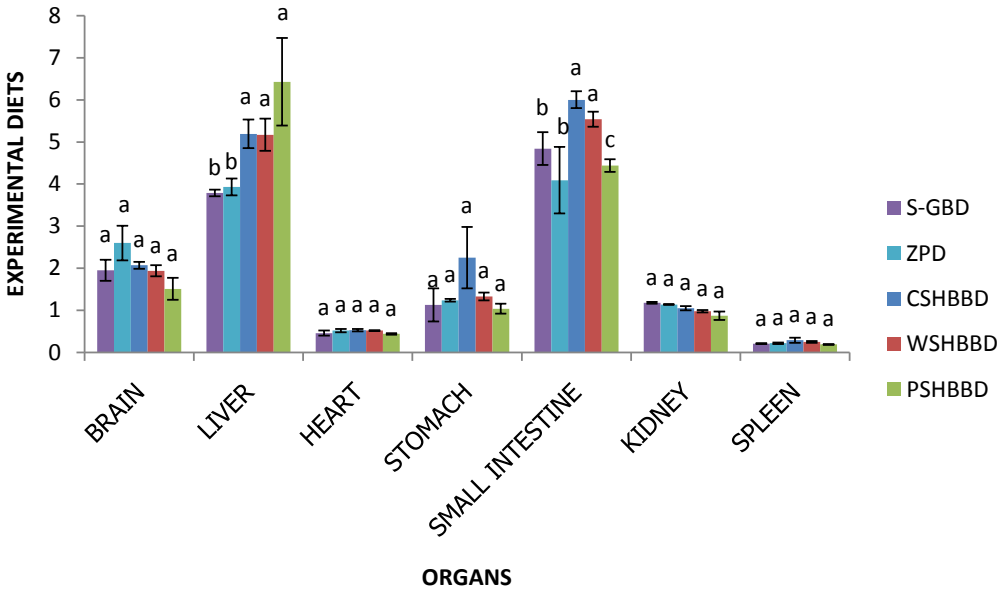


Figure 2. Organ/body weight of rats fed on control and test (SHB) diet. *Values are means of 3 determinations ± SEM. n = 3. Bars with the same colour but different letters are significantly different ($p < 0.05$). CSSHBBD: charcoal smoked kote SHB meal based diet; WSSHBBD: wood smoked kote SHB meal based 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).

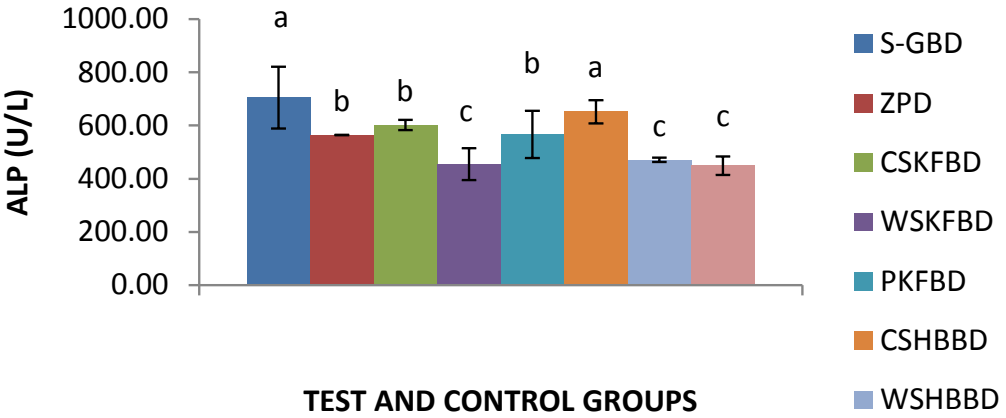
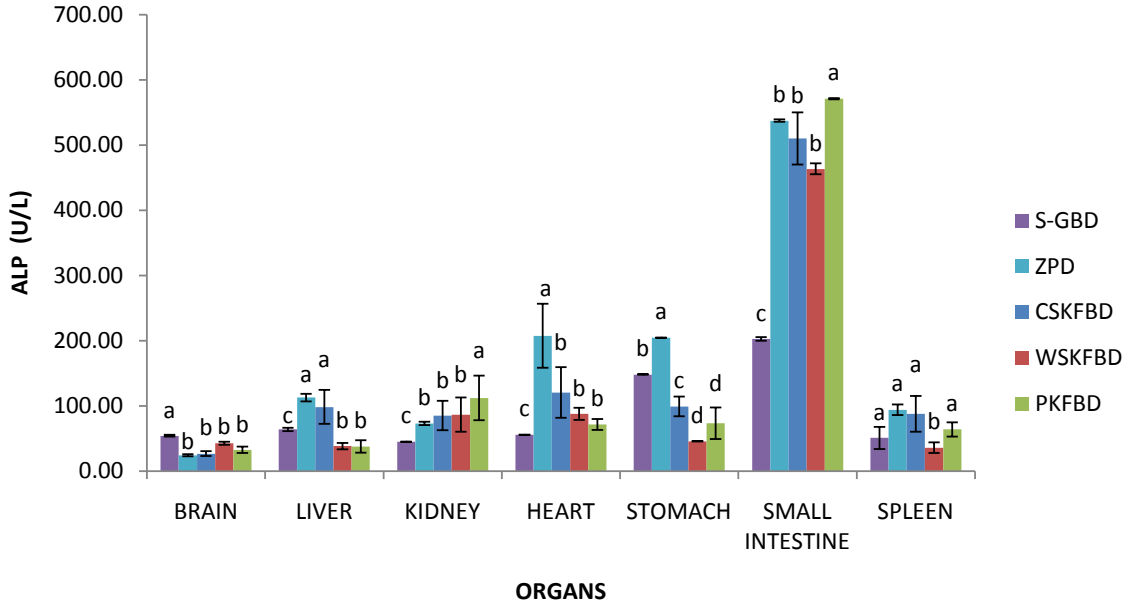


Figure 3. Serum ALP activity in rats fed with control and test (fillet) meal based diets. *Values are means of 3 determinations ± SEM. n = 3. Bars with the same colour but different letters are significantly different ($p < 0.05$). CSKFBD: coal smoked kote fillet meal based diet, WSKFBD: wood smoked kote fillet meal based diet, PKFBD: poached kote fillet meal based diet; 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); ALP = alkaline phosphatse.

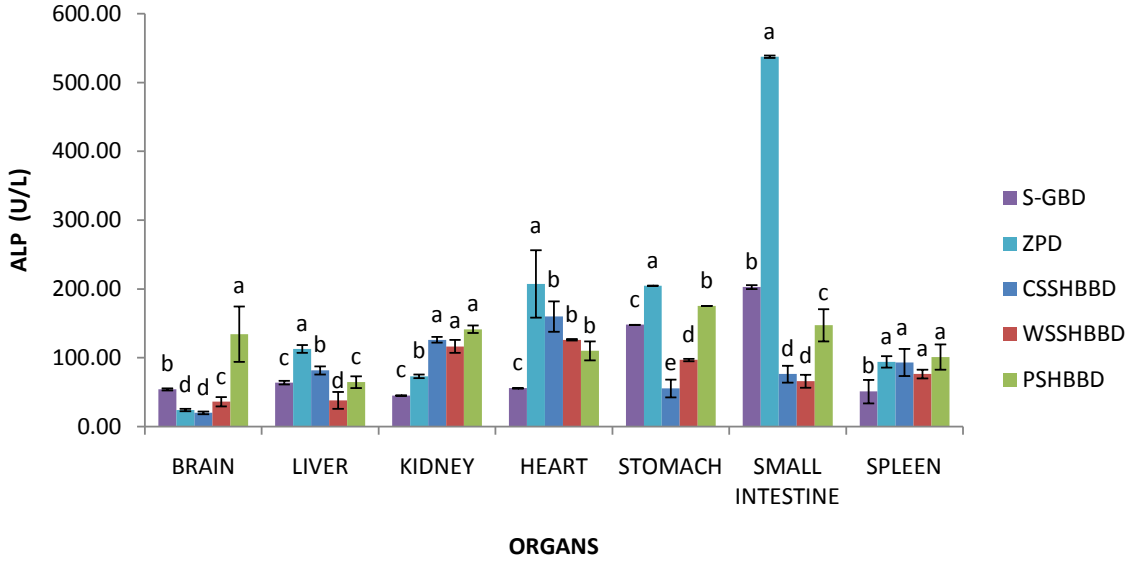
of ALP were significantly ($p < 0.05$) lower in rats fed on the test diets compared with those in the control.

4.3. Tissue Enzyme Activities of Rats Fed with Control and Test Diets

ALP, AST & ALT activities: Tissue enzyme activities for ALP, AST & ALT of rats fed with the test and control diets are represented in **Figures 4-6** respectively. In **Figures 4(a)-(b)**, which represents result of ALP activity for rats fed with fillet and control diets; levels of ALP were slightly higher in the kidney, heart, small intestine & spleen of rats fed with the fillet diets compared positive control. While ALP elevations ($p < 0.05$) in rats



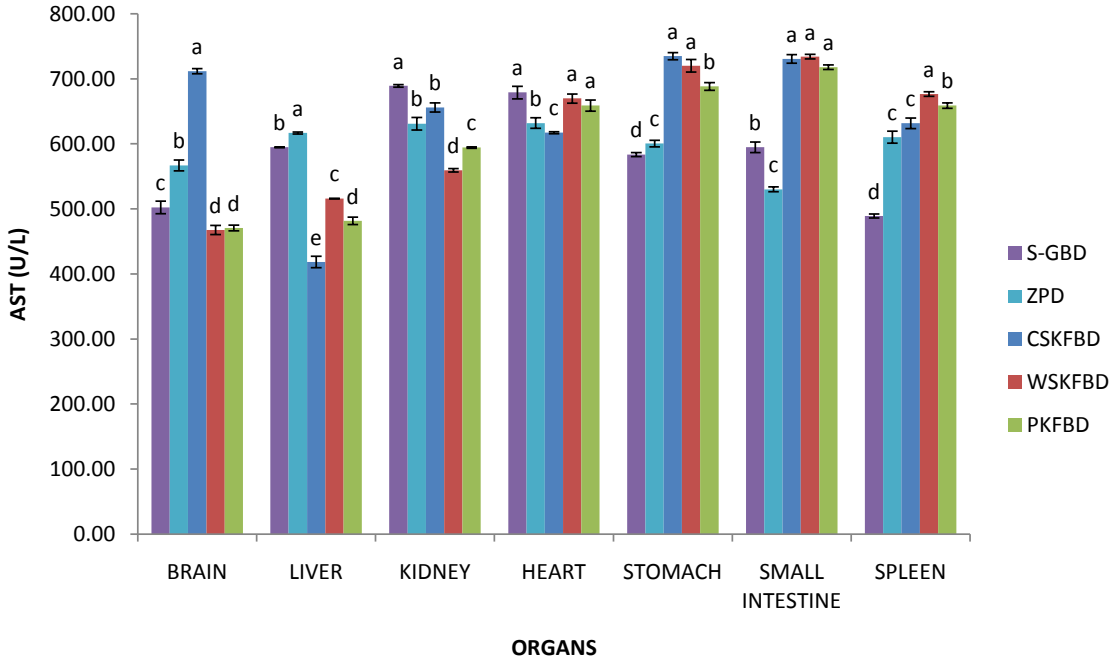
(a)



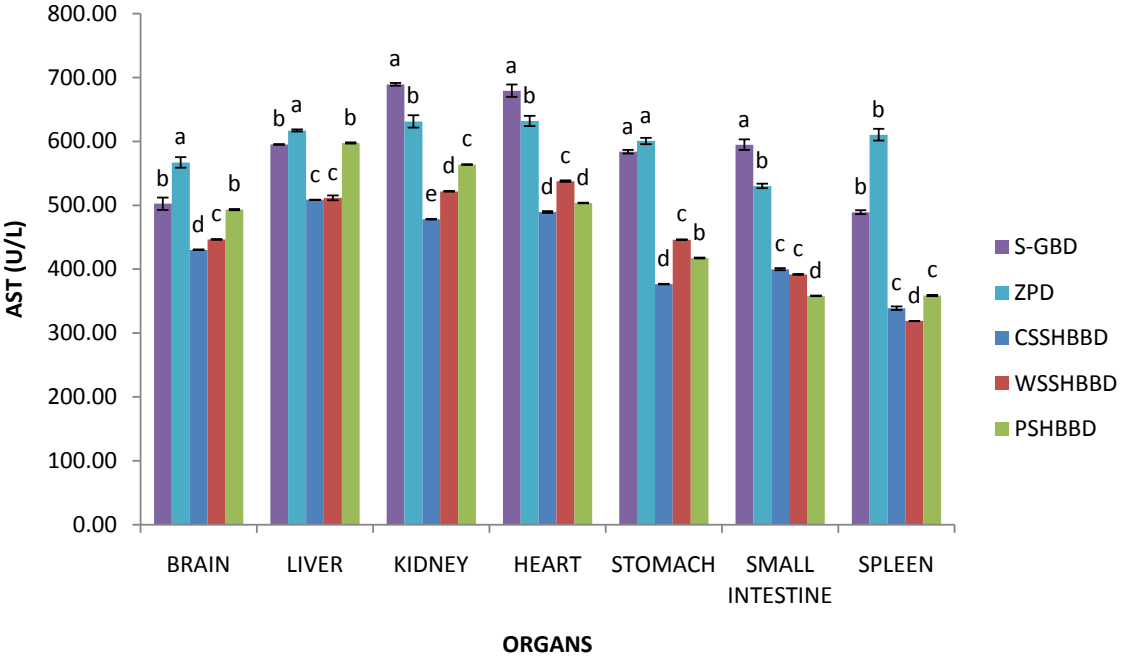
(b)

Figure 4. (a) Levels of tissue ALP of rats fed with control and fillet 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); ALP = alkaline phosphatse. (b) Levels of tissue ALP of rats fed with control and SHB 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); ALP = alkaline phosphatse.

fed with the SHB diets were observed in the brain (PSHBBD only), heart, stomach, liver & spleen compared positive control. In **Figures 5(a)-(b)** the AST activity was reduced ($p < 0.05$) in rats fed on the test diets in the liver, kidney and heart compared to the controls. While in **Figures 6(a)-(b)**, variations observed in the liver ALT level in rats fed on fillet diet were not significant ($p > 0.05$), but levels of ALT were higher ($p < 0.01$) in the liver of rats fed, with the SHB diets compared with the controls.

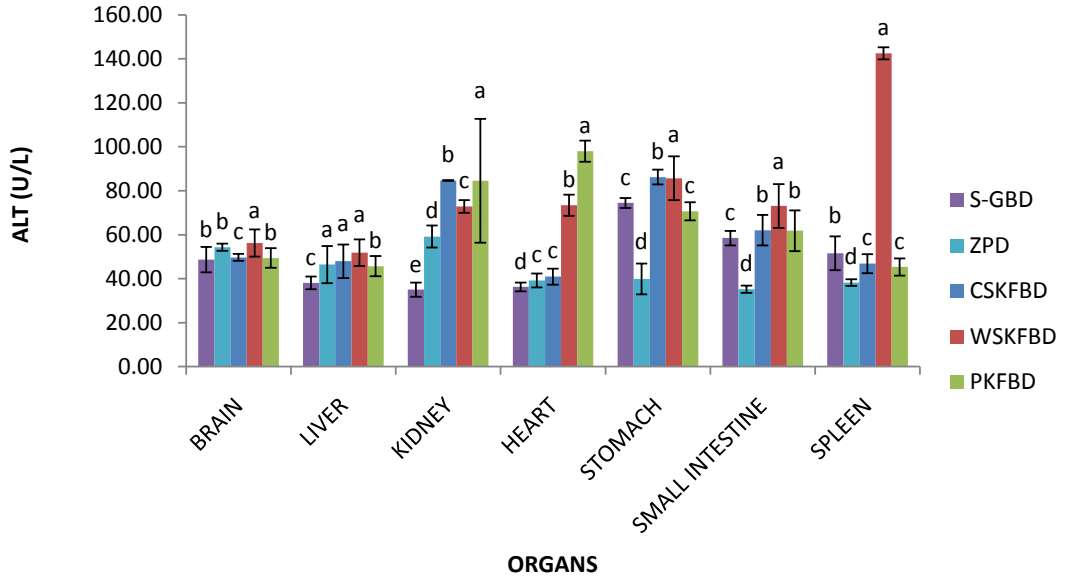


(a)

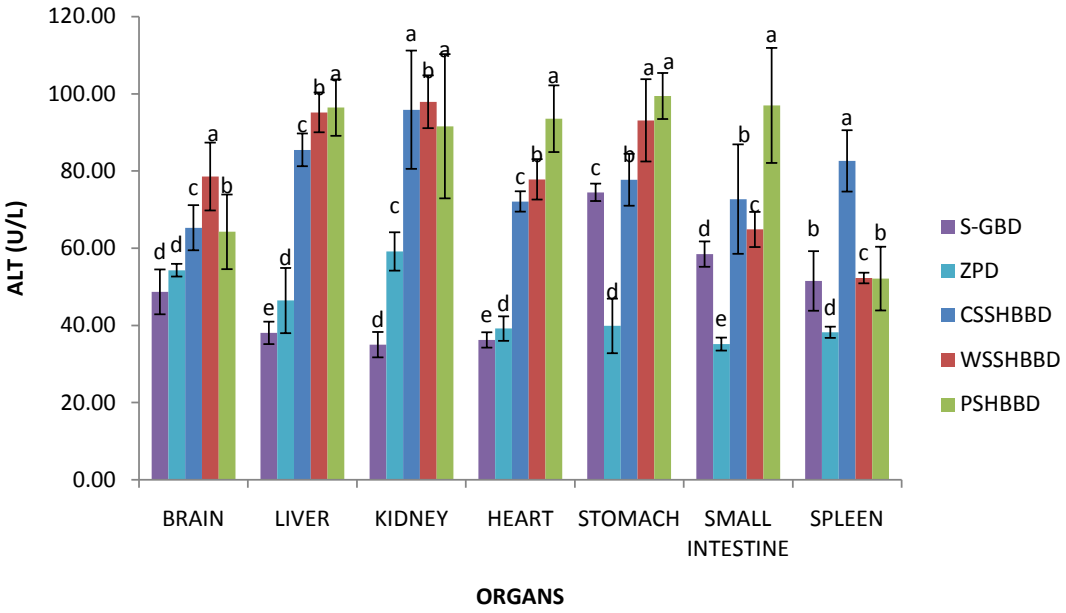


(b)

Figure 5. (a) Levels of tissue AST level of rats fed with control and fillet 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); AST= aspatate transaminase. (b) Levels of tissue AST level of rats fed with control and shb 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); AST = aspatate transaminase.



(a)



(b)

Figure 6. (a) Levels of tissue ALT level of rats fed with control and fillet 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; ALT = Alanine transaminase. (b) Levels of tissue ALT level of rats fed with control and SHB 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); ALT = alanine transaminase.

5. 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 [27] [28] 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 [29] and Benevenga *et al*. [30] 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 [30]. Thus values for all the nutrients in test compared well with the positive and were similar to those reported by Benevenga *et al*. [30].

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 [31]. 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 [32]. 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 [33] [34]. Serum ALP in rats fed on the test diets were lower ($p < 0.05$) compared to the controls. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum [35] [36], it is therefore an ectoenzyme of the plasma membrane [37]. It is often used to assess the integrity of the plasma membrane [38], such that in the tissue and serum would indicate likely damage to the external cell boundaries (plasma membrane). An increase in ALP level may quarry the possibility of membrane damage, because ALP is a membrane bound enzyme [39] [40]. High levels of serum ALP activity is usually noticed in liver damage, cancer and heart infections [41]. Consequently significant decrease ($p < 0.05$) in serum ALP observed in the rats fed on test diets may be an indication of the healthier state of the plasma membranes of these rats compared to those in the control groups.

Alkaline phosphatase is involved in bone growth and excreted in the bile. It may be elevated if bile excretion is inhibited by liver damage [11]. ALP activity in selected tissues were significantly high ($p < 0.05$) in the kidney, liver, heart, stomach, small intestine and spleen but least in the brain of rats fed on test and control diets. This is in conformity with previous reports that the mammalian organs having very high ALP activities are those involved in active transport mechanism [11].

However the significantly lower ($p < 0.05$) levels of ALP in rats fed with the positive control diet may be due to enzyme induction rather than leakage since the ALP activity was also higher ($p < 0.01$) in the serum of rats fed on the control diets compared to those in the test groups. Such reduction in ALP activity from the tissues of animals fed on the positive control diet could be attributed to the disruption in the ordered lipid-bilayer of the membrane structure leading to escape of detectable quantity of ALP out of the cell into the extra cellular fluid. This could hinder adequate transport of required ions or molecules across their cell membrane that may lead to starvation of the cells [42]. In addition, the observed reduction in the ALP activity for animals in the positive control groups might also have adversely affected other metabolic processes where the enzyme was involved, such as synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters.

The tissue activities of the transaminase (AST and ALT) enzyme are markers for the functions and integrity of the heart and liver [12] [13]. They rearrange the building blocks of proteins. It is released from damaged liver cells [43] [44]. Elevation of these enzymes in the serum have been reported to indicate cellular damage, tissue necrosis, as well as a calculated risk for cardiovascular diseases, with higher risk of cardiovascular disease and elevated myocardial infarction being attributed to elevation of ALT and AST respectively [45]. Although ALT activities of liver and heart were elevated in rats fed on the test diets compared with the control, this elevation was not confirmed in the AST activity which was significantly ($p < 0.05$) reduced in rats fed on test diets than in the controls. Obtained values were within the acceptable range, thus implying that the earlier observed elevations in the ALT of rats fed on the test diet was insignificant ($p > 0.05$). Result therefore suggests that the test diets protected and prevented damage to the plasma membranes of not only the liver and heart, but also the brain, kidney, stomach, small intestine and spleen in the rats. Further lending credence to earlier observations in serum

and tissue ALP of rats fed with test diets compared to the controls.

6. Conclusion

In conclusion, coal smoked fillet and SHB had markedly best effect in terms of the organ/body weight ratio, serum ALP as well as tissue ALP & ALT activities, followed by the wood smoked kote. Especially, the processed SHB diets showed better ($p < 0.05$) performance in animals in the brain, liver, stomach and small intestine compared with those placed on the fillet and control diet respectively. Thus, processed kote SHB could be a veritable source of valuable nutrients for human food and animal feeds. However, it is necessary to further improve the processing methods of the fillet and SHB in such a way that all traces of anti-nutritional and hemolytic factors (RDC and PAHs) which are capable of distorting the hematological parameters are eliminated. This done, the SHB can be gathered and utilized at little costs, thus reducing costs of feeds due to highly priced casein, soybean meal and GNC.

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