Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol 30, 2015

Influence of Processing Methods on Protein Quality of Atlantic Horse Mackerel (Trachurus trachurus)

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The research is financed by National Research Foundation (NRF) of South Africa for their financial support.

Abstract
Protein quality is a long-accepted paradigm for human protein needs however the quality is affected with processing methods. The protein and amino acids content of poached or charcoal or wood smoked horse mackerel fish (HMF) were determined using standard methods. Chemical analyses were carried out on the processed fillet and skin, head and bone (SHB) diets under standard conditions. A total of 40 weaned male rats were fed with processed fillet or SHB diets for 12 days to assess protein quality and amino acids digestibility. Fish samples were also analyzed for protein efficiency ratio (PER), net protein ratio (NPR), Protein Digestibility-Corrected Amino Acid Scores (PDCAAS), true and apparent protein digestibility (TPD and APD). Results showed that the wood smoked fillet had the highest PDCAAS regarding to the controls whereas PER, NPR and TPD was highest in SHB. The best results were obtained from animals fed on coal or wood smoked fillet/SHB on protein quality and amino digestibility parameters selective. The SHB could be used as a valuable source of ingredients for animal feeds and human consumption instead of being discarded as agricultural waste.
Keywords: Processing methods**,** protein quality, growth performance; amino acids, Atlantic horse mackerel, agricultural waste/discard.

1. INTRODUCTION

Fish consumption is related to health reimbursement, because of a rich content in protein (Adeyemi et al., 2013) high dietary value, vitamins and distinguishing lipids (like the ω3 and ω fatty acids) as well as micro and macro minerals such as calcium, phosphorus and manganese (Nnaji et al 2010; Akinwumi, 2011; Lordan et al, 2011). Although fish are nutritious and rich in protein but are highly perishable and get spoilt very easily, even in temperate climates; except disposed off quickly after captured or preserved in certain ways (Olafsdottir et al., 1997; Okeyo et al., 2009). For this reason, a number of fish preservation methods and processing techniques are used to increase its availability to consumers (Sanchez-Muniz et al 1992; Oluwaniyi and Dosumu, 2008).

However, these processing treatments such as heating, smoking and poaching may denature protein, disrupt the food matrix and cause the formation of maillard compounds, oxidized forms of sulfur amino acids, D-amino acids, and cross-linked peptide chains resulting in lower essential amino acid bioavailability, reduced digestibility and protein quality (Camire et al., 1990; Mensa-Wilmot et al., 2001). Studies have shown that smoking causes some decrease in available lysine which is proportional to the temperature and duration of smoking (Eyo, 2001; Clifford et al., 1980).

Moderate cooking, though often improves biological value and has a number of other benefits. Moderate cooking improves the biological value of protein, and excessive cooking reduces or destroys amino acids. Resent studies have suggested that maximum protein bioavailability is best achieved by poaching (cooking in water at near boiling temperature) (Worldwide Fifflex, 2015). In this method, all amino acids are preserved because the cooking temperature stays at or below the boiling point, in contrast to barbecuing, frying or baking, in which the temperature can get above 400^0F. Besides improving protein quality slightly, poaching is more penetrating (Worldwide Fifflex, 2015).

Protein is a component in the diet of any farmed species, particularly in aquaculture and the pet food industry, thus making an accurate assessment of protein utilization critically important. It is a source of essential amino acids mostly needed for tissue synthesis, maintenance and repair (Young, 2001). Protein quality analysis provides an estimate of the content and bioavailability of indispensable or dietary essential amino acids present in the food (Rasco, 2001; Mensa-Wilmot et al., 2001).

Proteins are digested to release amino acids. The nutritional value of protein is measured by the quality of essential amino acids that it provides. Different foods contain different amounts of amino acids. Generally animal products (such as chicken, beef or fish) contain all of the essential amino acids. Once inside the body, these proteins are used to make new proteins including enzymes and hormones such as adrenalin. Proteins are sometimes also used as an energy source (Better Health Channel, 2015).

Additionally, some fish preservation methods often times involves removal of the head, viscera and other parts of fish, which may have either negative or positive effect on the total nutritive values of the fish (Saliu 2008; Johnson et al., 2010). The effect of fish processing methods is necessary to determine protein quality and it's important in food science, particularly for developing foods with targeted nutritional value in animal husbandry.

However, there remains paucity of scientific information on the effect of different processing methods (poached, wood and charcoal smoked) on protein quality of *T. trachurus* (fillet and SHB) in weaned male wistar rats. *Trachurus trachurus* was chosen in present study, because of its good consumer acceptance and economic availability. The present study, therefore evaluated the effect of processing methods on the digestibility and bioavailability of processed *kote* fillet and SHB; as the protein source in compounded feed using rat bioassay of protein efficiency ratio (PER), net protein ratio (NPR), net protein utilization (NPU), true protein digestibility (TPD) and protein digestibility-corrected amino acids score (PDCAAS).

2. MATERIALS AND METHODS

2.1 Samples 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 and 2 respectively. 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 (Association of Official Analytical Chemists (AOAC), 2002). Total crude fat was determined using the Soxhlet extraction method according to AOAC (2002) as modified by Reinik et al, (2007). The crude fiber content was estimated by acid-base digestion method as described by Association of Official Analytical Chemists (AOAC) (2002). Crude protein content was determined by the Kjeldahl method (Association of Official Analytical Chemists (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 (Figure 1) (Okalebo et al, 2006). Crude protein was estimated by multiplying the nitrogen value by the converting factor of 6.25.

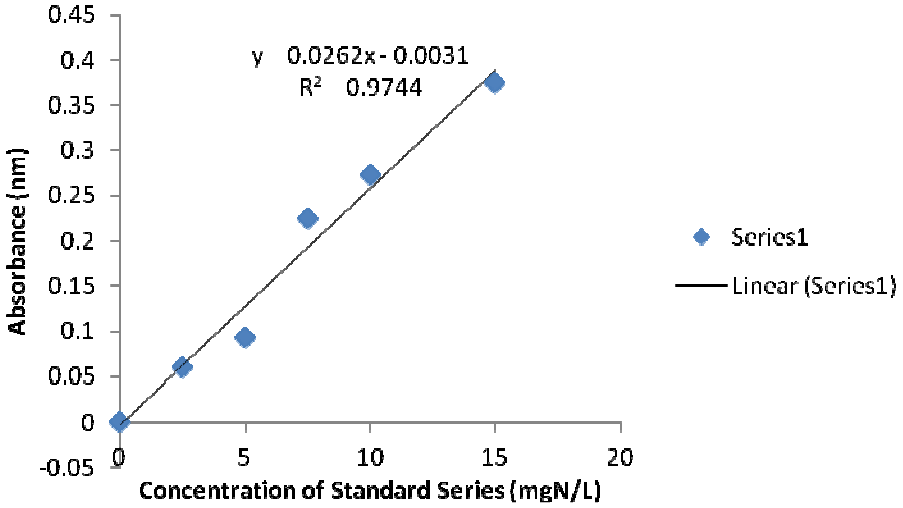

Figure 1: Graph Showing Calibration Curve Used for the Calculation of Nitrogen Content

2.4 Amino acids analysis

About 0.1 g of the fish samples was defatted using chloroform/methanol and then hydrolyzed using heat at 110 °C in an evacuated sealed ampoule for 24 hour. Samples collected were treated with 6 M HCl+15% Phenol (AOAC, 2003). The hydrolysate was allowed to cool down to room temperature and 2 ml was submitted for amino acids analysis using Eppendorf Biotronic, LC 3000 Amino acid analyzer (Eppendorf-Biotronic, Hamburg, Germany). The Dumas dry combustion method was used to evaluate amino acids concentration in processed fish diets (AOAC, 1992).

2.5 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.

2.6 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 feces were collected. At the end of the experimental period, the protein quality was determined via the following parameters; Biological value, net protein ratio (NPR), net protein utilization (NPU), apparent protein digestibility (APD), true protein digestibility (TPD), protein digestibility corrected amino acids score (PDCAAS) and fecal nitrogen content (FNC) were recorded.

2.7 Protein Diets Hydrolysis

Samples were hydrolyzed as described by AOAC (2003). Hydrolysis tubes with samples were placed in glass beakers, and then hydrolyzed in oven at 110^{o}C for 24 hours. The tubes were removed and allowed to cool down to room temperature. The content was poured in 2ml Eppendorf tube and later analyzed for amino acids using LCMS.

2.8 Assessment of biological value (BV)

Biological value of the samples was assessed following the method of FAO/WHO (1991). BV was calculated as:

$$\frac{\text{Nitrogen intake of test animals} - (F\text{-}F_M) \times 100}{\text{Nitrogen intake of test animals}}$$

Where F = faecal nitrogen output by test animals; F_M = faecal nitrogen output by zero-protein treated animals.

2.9 Determination of Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR)

PER was determined by the method of AOAC (1975) and Mitchell & Grundel, (1989). A 10% protein diet from processed fish or soybean-groundnut cake was administered to the weaned rats. The weight gained or protein consumed by the animals was monitored and recorded. PER was then calculated as the ratio of weight gained of the animals to the amount of protein consumed. The net protein ratio was determined as described by Bender (1957) and FAO/WHO (1991). Net protein was calculated as the ratio of weight gain by the animal fed with protein source minus the weight loss of animal fed without protein to the amount of protein consumed by the animal fed with protein. Percent NPR was determined as the ratio of the mean value of NPR from test protein (fish source) to the NPR reference protein.

2.10 Determination of Net Protein Utilization (NPU)

Net protein utilization was done using the standard method described by FAO/WHO (1991). NPU was calculated as the proportion of nitrogen retained to the nitrogen intake or the product of biological value and true digestibility.

2.11 Determination of TPD and Apparent Protein Digestibility (APD)

True protein digestibility (TPD) was determined according to the modified method of Sarwar and Peace (1986) and FAO/WHO (1991). TPD was done by collecting the rat faeces from 8th to 13th days and stored in different containers under refrigerated condition (8^{o}C). At the end of the experiment, the faeces were dried in an air circulating oven at 60^{o}C for 24 hour and then milled in a food processor for the determination of nitrogen concentration using Kjeldahl method (AOAC, 1984). Apparent protein digestibility was done according to the methods described by FAO/WHO (1991). TPD and APD were calculated using the equations described below:

$$\% \text{ TPD} = \frac{[\text{PI} - (\text{FP} - \text{MFP})] \times 100}{\text{PI}}$$

Where; PI = protein intake (g); FP = faecal protein (g); and MFP = the metabolic faecal protein (g). The MFP is calculated from the amount of protein in the faeces of rats fed a protein-free diet (Sarwar, 1996). The amount of protein in the faeces of rats fed the protein-free diet was used as the estimate for MFP.

$$\% \text{ APD} = \frac{\text{I} - \text{F}}{\text{I}} \times 100$$

Where; I = nitrogen intake (g); F = Faecal nitrogen (g)

2.12 Determination of Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The PDCAAS index was computed for the three experimental products from their essential amino acid profile determined by acid hydrolysis of test proteins separation and quantification by HPLC (FAO/WHO/UNU, 1985; Meltzer, 1987; Cohen & Michaud, 1993). The methodology compared essential amino acids profile of a test protein corrected for digestibility to the FAO/WHO, (1989) essential amino acid requirement pattern for a 2–5 year old child.

$$\text{Amino acid score} = \frac{\text{Mg of amino acid in 1 g of test protein}}{\text{Mg of amino acid in 1 g of reference protein}}$$

PDCAAS = True digestibility × lowest amino acids score

2.13 Determination Of Fecal Nitrogen Content (Fnc)

Total nitrogen content was assessed by micro-Kjeldahl method (AOAC, 1984) modified by Okalebo et al. (2002). A known weight (0.5 g) of the fecal sample was mixed with 12 ml of (9 ml Conc Nitric acid + 3 ml Conc. HCl) digestion mixture. The resulting mixture was digested using Buchi 425 digester from Switzerland for 1 hour until clear solution was observed. The mixture was allowed to cool and then made up to 50 ml with distilled water. The resulting solution was left to stand in the dark for 2 hours for full color development, and then absorbance was measured at 650 nm. A calibration curve was plotted and used to read off the nitrogen concentration of the solution. Percentage nitrogen was calculated using the equation $Y= 0.026x - 0.003$ and $R^2= 0.974$ from the calibration curve at various concentration of the standards.

2.14 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 (SPSS, 2005). Results were considered statistically

significant at a minimum of $p < 0.05$ with Duncan's multiple range test (Duncan 1955).

3 RESULT

3.1 Proximate Analysis of Experimental 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 Amino Acids Composition

The results of total essential amino acids (ΣEAAs), total semi essential (ΣSEAAs) and total non - essential amino acids (ΣNEAAs) in the fish samples were presented in Table 2 and 3. There are various amino acids (AA) compositions in raw and processed fish diets, seventeen AAs were identified in the fish fillet out of which seven are essential amino acids (EAAs). All processed fillet was significantly higher in EAAs composition as compared with RKF but significantly lower to the dietary recommended value for the children.

The values of essential amino acid contents are presented in decreasing order of magnitude 227.19 > 216.31 > 200.45 for PKF, WSKF and CSKF respectively. On the other hand, the levels of EAAs in the PSHB (90.29) was markedly reduced but remained unchanged ($p>0.05$) in WSSHB (110.57) and CSSHB (103.25) compared to the RSHB (108.83). The processed fillet contained high amounts of SEAAs of which cysteine was highest ($p<0.001$) in CSKF and tyrosine in WSKF compared to the unprocessed sample whereas processed SHB diets had a significant increase ($p < 0.05$) in SEAAs compared to the RSHB.

The levels of alanine, glutamic acid, and glycine ($p<0.05$) increased in WSKF and CSKF, compared to the RKF while PKF had high levels of alanine, aspartic acid, glutamic acid, glycine and proline. Both wood and coal smoking processing methods improved the value of cysteine but decreased by poaching. Tyrosine (80.21mg/g) was remarkably higher in WSKF followed by poaching (21.71 mg/g) and coal smoking (19.42 mg/g). RKF contained least amount of NEAAs but appreciably increased by wood smoking followed by poaching and then coal smoking. The processing methods demonstrated similar trend of influence on both the fillet and SHB. Meanwhile, wood smoking had the best improvement on the total amino acids investigated followed by coal smoking.

3.3 Protein Quality And Amino Acids Digestibility Parameters

The results of true protein digestibility (TPD) and protein quality indices of experimental diets based on rat growth are shown in Table 4. Biological value (BV) as one of the parameters used to assess protein quality in rat fed processed fillet (100%) and SHB (93.24-97.66%) compared favourably with animal fed control diet (99.98%). APD values (41.5-55.8 %) significantly increased in experimental animals fed fillet or SHB as compared with the control (37.89 %). Poaching of SHB diet markedly increased the APD values but lowest in the fillet whereas coal smoking had the best result in rats treated with processed fillet. There was no significant difference observed in animal groups treated with WSKF and WSSHB. The data obtained on TPD was comparable in experimental animals fed with processed fillet or SHB based diets.

The PER and NPR values of S-GBD were 0.08 and 0.07 respectively (Table 4). The coal and wood smoking fillet diet statistically improved the PER value while poaching was insignificantly different from the control. Similar trend was observed in rats fed with processed SHB diets. Both wood and coal smoking processing methods caused significant improvement on the NPR values but reduced by poaching especially in the fillet formulated diet. The NPU values of CSKFBD (100.02%), WSKFBD (100.56%) and PKFBD (100.01%) and S-GBD was 99.97% indicating that there was no significant difference among processed fillets and the control (Table 4). Coal smoking and poaching methods drastically reduced the NPU values in SHB treated groups while wood smoking had a positive effect as compared with the control.

The PDCAAS value for treated groups and control was small but that of wood smoking fillet was higher than others. PDCAAS values in animals treated with SHB diet was not improved as compared with control group. Feacal Nitrogen levels of rats fed on the test and control diets are shown in Figures 2 and 3 respectively. Rats fed with processed test diet, showed lower ($p < 0.05$) fecal nitrogen content compared to the positive control.

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]

*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.

Table 2: Amino acids composition (mg/g) of experimental fillet based diets

GROUP (mg/g)	RKF	CSKF	WSKF	PKF	Pre-School (2-5yrs) FAO / WHO (1991)
Lysine	36.46	44.48	44.40	47.09	58
Methionine	16.38	18.33	20.23	20.93	25
Threonine	20.75	24.29	28.02	28.35	34
Isoleucine	19.17	23.03	23.25	26.34	28
Leucine	39.06	42.47	46.10	47.70	66
Phenylalanine	18.17	18.66	22.85	22.47	63
Valine	25.81	29.19	31.42	34.31	35
Total essential amino acids	**175.80**	**200.45**	**216.31**	**227.19**	**309**
Histidine	4.82	21.63	22.98	21.55	19
Serine	16.70	20.60	22.57	24.37	-
Arginine	29.76	67.45	70.83	75.44	-
Cysteine	0.86	0.96	1.05	0.76	-
Tyrosine	16.61	19.42	80.21	21.71	-
Semi essential amino acids	**68.75**	**130.06**	**197.64**	**143.83**	**19**
Alanine	29.25	30.75	32.31	34.38	-
Aspartic acid	41.59	45.92	47.71	51.96	-
Glumic Acid	73.70	82.60	88.57	91.07	-
Glycine	28.67	28.77	30.76	32.39	-
Proline	19.30	20.05	21.06	22.47	-
Nonessential amino acids	**202.48**	**208.09**	**220.41**	**232.27**	**-**
Total amino acids	**447.03**	**538.60**	**634.36**	**603.2**	**328**

*CSKF: charcoal smoked *Kote* fillet; WSKF: wood smoked *Kote* fillet; PKF: poached *Kote* fillet; RKF: Raw *Kote* fillet.

Table 3: Amino acids composition (mg/g) of experimental SHB based diets

GROUP (mg/g)	RSHB	CSSHB	WSSHB	PSHB	Pre-School (2-5yrs) FAO/WHO (1991)
Lysine	23.12	21.32	22.39	16.38	58
Methionine	10.2	9.94	10.42	8.46	25
Threonine	13.69	13.53	14.84	12.43	34
Isoleucine	11.53	11.45	11.83	9.69	28
Leucine	23.36	20.76	22.09	19.71	66
Phenylalanine	11.10	11.26	13.29	10.45	63
Valine	15.83	14.99	15.71	13.17	35
Total essential amino acids	**108.83**	**103.25**	**110.57**	**90.29**	**309**
Histidine	1.65	8.63	10.72	8.38	19
Serine	13.98	14.97	15.42	13.00	-
Arginine	21.90	86.77	91.66	77.16	-
Cysteine	0.53	0.23	0.40	0.13	-
Tyrosine	9.98	38.51	44.21	8.36	-
Alanine	22.29	21.96	23.67	18.95	-
Total Semi essential amino acids	**70.33**	**171.07**	**186.08**	**125.98**	**19**
Glutamic acid	45.27	46.36	45.99	36.53	-
Aspartic acid	26.25	28.3	28.04	21.90	-
Glycine	34.07	37.16	39.83	33.00	-
Proline	19.63	21.30	22.12	18.82	-
Total Nonessential amino acids	**125.22**	**133.12**	**135.98**	**110.25**	**-**
Total amino acids	**304.38**	**407.44**	**423.63**	**326.52**	**328**

*CSHB: charcoal smoked *kote* SHB; WSHB: wood smoked *kote* SHB; PSHB: poached *kote* SHB; RSHB: Raw *kote* SHB.

Table 4: Parameters for protein digestibility of rats fed with experimental diets

Diets	BV	PER	NPR	NPU	TPD	APD	PDCAAS
γS-GBD	100.98±0.02^{a}	0.08±0.00^{c}	0.07±0.01^{b}	99.97±0.05^{a}	99.98±0.02^{a}	37.89±7.95^{c}	0.70±0.00^{ab}
†S-GBD	99.98±0.02^{a}	0.08±0.00^{b}	0.07±0.01^{b}	99.97±0.05^{a}	99.98±0.02^{a}	37.89±7.95^{c}	0.70±0.00^{a}
CSKFBD	100.01±0.01^{a}	0.14±0.01^{a}	0.12±0.02^{a}	100.02±0.02^{a}	100.01±0.01^{a}	55.80±4.40^{a}	0.50±0.00^{c}
CSHBBD	93.24±1.61^{c}	0.11±0.01^{a}	0.11±0.01^{a}	93.18±1.62^{b}	99.93±0.02^{a}	48.75±3.00^{b}	0.66±0.01^{b}
WSKFBD	100.04±0.00^{a}	0.12±0.02^{b}	0.10±0.02^{a}	100.09±0.01^{a}	100.04±0.00^{a}	48.16±0.71^{b}	1.00±0.00^{a}
WSHBBD	97.66±1.11^{a}	0.12±0.01^{a}	0.12±0.01^{a}	97.63±1.12^{a}	99.98±0.01^{a}	48.93±1.33^{b}	0.66±0.01^{b}
PKFBD	100.01±0.01^{a}	0.10±0.02^{b}	0.06±0.01^{b}	100.01±0.02^{a}	100.01±0.01^{a}	41.50±0.71^{bc}	0.80±0.00^{ab}
PSHBBD	94.45±0.96^{b}	0.10±0.02^{a}	0.10±0.02^{a}	94.40±0.97^{b}	99.95±0.01^{a}	55.14±2.50^{a}	0.56±0.01^{bc}

*Data= Mean ± SEM, n=3. Values with different superscripts across the column 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); BV= Biological value; PER= protein efficiency ratio; RPER=Relative PER; NPU= Net protein Utilization; RNPU= Relative NPU; NPR= Net Protein Ratio; TPD= true protein digestibility; APD= Apparent Protein Digestibility; PDCAAS= Protein Digestibility-Corrected Amino Acid Scores.†stands for SHB group and γ stands for fillet fed group.

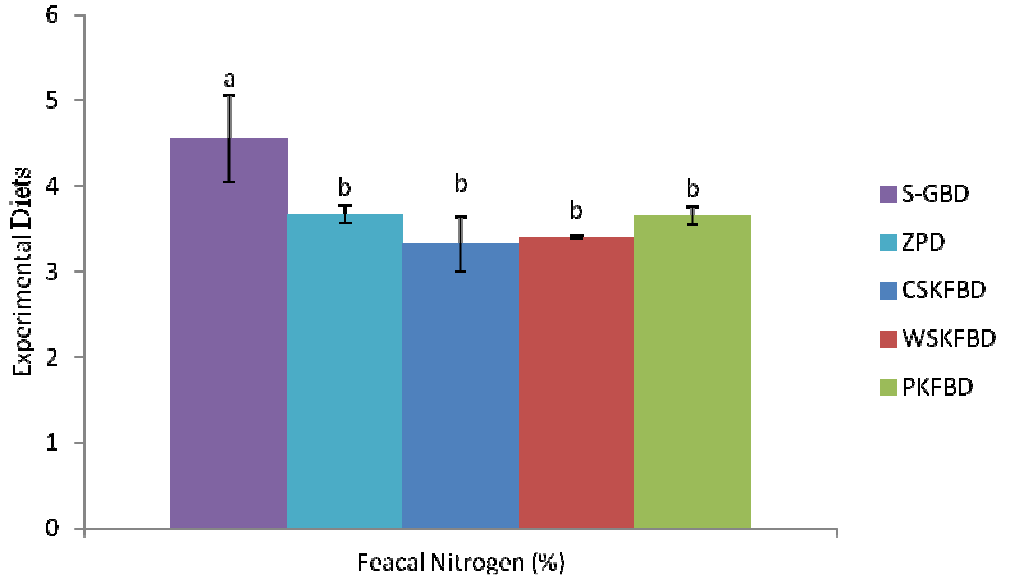

Figure 2: Feacal nitrogen contents of rats fed fish fillet based diets

*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)

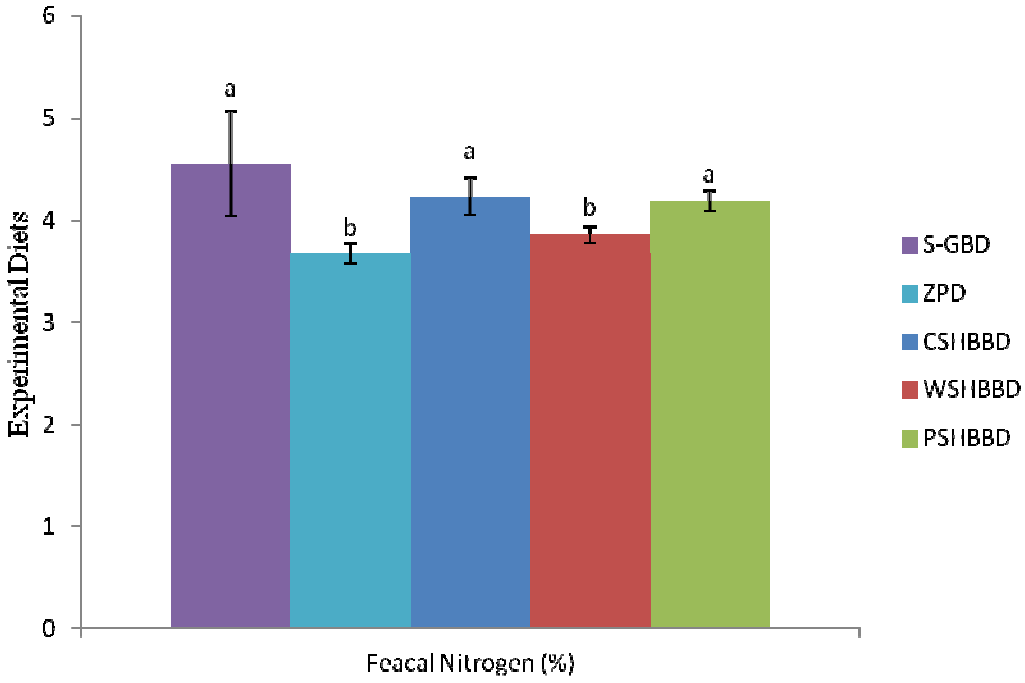

Figure 3: Feacal nitrogen contents of rats fed fish SHB based diets

*Values are means of 3 determinations ± SEM. Bars with the same colour but different letters are significantly different ($p < 0.05$). CSHBBD = coal smoked *kote* SHB meal based diet; WSHBBD= wood smoked *kote* SHB meal based diet; PSHBBD= poached *kote* SHB meal based diet; Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control)

4. DISCUSSION

The role of formulated fish diets on protein digestibility and bioavailability in weaned male Wistar rats showed that compounded feeds conformed to the recommended feeding standards (Food and Agricultural Organization

Protocol, 1991; Aduku, 2005) and were adequate to meet growth requirements. Although the crude fat content in WSCF was significant high but less than 30% required for animal growth (Delorme and Gordon, 1983; Benevenga et al., 1995). The analyzed nutrients and chemical components in test and control diets fell within the acceptable recommendation range for laboratory animal (Benevenga et al, 1995). Consequently, the values of the nutrient composition obtained in the experimental diets were similar to those reported by Benevenga et al, (1995) and comparable with soy-groundnut based diets.

The observed weight gained in rats fed on CSKFBD further lends credence to the low levels of RDCs earlier observed in the coal smoked fillet. RDCs have been reported to reduce the nutritional values of fish (Liggins and Furth, 1997; Adeyemi et al., Private communication). Furthermore, the weight gained in rats fed on CSKFBD could be attributed to the high concentration of potassium reported by Adeyemi et al. (2013). Our data confirmed with other researchers who opined that body weight gain of the animals was not a reflection of feed intake; because the rats fed with negative control meal based diet, consumed the highest amount of feed but gained the least ($p < 0.05$) weight. However, growth rate/net weight gain of rats correlated with the dietary protein consumption irrespective of dietary lipid content.

Variations observed in the fecal output (F.O) could be has a result of the crude fibre content of the experimental diets. This result corresponds with the finding of Lopez-Guisa et al. (1988) and Nishina et al. (1991) who reported that additions of insoluble, un-degradable sources of fiber such as cellulose, oat hulls, wheat bran, and corn bran to rat diets at concentrations up to 20% do not affect growth. This is because the non-fermentable crude fiber sources dilute the nutrient density of the diet and thus reduce feed intake. On the contrary, the crude fiber in the SHB diets supported growth and development in the rats fed with SHB diets.

Water is an essential nutrient, without which animal or man life cannot survive. It is known to aid food digestion. The water intake of experimental rats was directly proportional to the weight gained observed in animals fed with experimental diets. Protein and carbohydrates digestion to usable and absorbable forms has been demonstrated to depend absolutely on water as part of the biochemical reaction (McKinzie, 2009).

Biological value (BV) is a scale of measurement used to determine the percentage of dietary nitrogen utilized by the body from protein intake (Hoffman & Falvo, 2004).

This parameter also determine how readily digested protein can be used for protein synthesis and thus justifies variation of BV in different foods owing to food preparation and diet consumed (FAO/WHO, 1991; Hernandez, 1996). Our data on BV (100%) was significantly higher compared to the control groups and 75% reported by Corinne (1986) in the fillet but concurred with the findings of JSSM (2004). The decreased BV observed in animals treated with CSHBBD and PSHBBD diets could be linked to deficiency of certain essential amino acids or vitamins (A and E) or minerals as demonstrated in our previous studies (Adeyemi et al, 2013). Lack of critical EAAs in the diet has been indicated to prevent protein synthesis and thus reduce its biological value which may perhaps affect the correct function of cells in experimental animal (Schaafsma, 2000: JSSM, 2004). Although SHB dietary formulated had lower EAAs but could be a significant source to substantiate daily recommended dietary value for growth and development in man or animal.

Protein quality is dependent on the availability of essential amino acids in a given protein source. Determining protein quality analysis is important particularly for developing foods with targeted nutritional value for animal feeding and husbandry. PER is a measure to show how well the protein sources in the diet could provide essential amino acid requirements for the animal growth (Dimes et al., 1994; Babara, 2001). Our observation on the PER values in rats fed with Atlantic Horse mackerel fillet and SHB meal based diets are in close agreement to the reports of Siddiqui (1988); Anderson et al. (1995); Singh and Singh (2011). In addition, among the animals fed with the fillet or SHB meal based diets, CSKFBD and WSHBBD had significant PER values than the positive control group which could be associated to high phosphorus content earlier reported in the CSKF, CSHB, WSHB, and PSHB (Adeyemi et al., 2013).

Phosphorus has been reported to play essential roles for growth, maintenance and repair of body tissues and nucleic acids synthesis (Bressani 1984; Berner & Shike, 1988). Naturally protein quality is maximized in animal products, and increases when various protein sources are combined contiguously as observed in the present investigation. However, the S-GBD (soybeans-groundnut) recorded the lowest PER values corresponding to PSHBBD. The discrepancy observed in soybeans-groundnut diet could be due to anti-nutritional and anti-chymotrypsin factors as well as low level of methionine present in soy beans (Pongmaneerat et al., 1993). Generally, animals treated with processed fillet had the best PER values which may perhaps be due to the presence of protease: an enzyme that aid digestion of fishes in animals.

NPR is often used to correct PER values for the amount of protein required for cell maintenance and is often run in conjunction with a PER (Friedman, 1996). Our results on NPR and RNPR levels for animals fed with test diets lend credence to the PER and RPER values earlier observed in this study. NPR score was highest in animal fed with test diets. The higher RNPR values of rats fed with the positive control diet compared to those fed on the CSHBBD may be due to decrease in anti-nutritional factors during heat treatment process in the soy bean meal or the levels of PAHs. Several studies confirmed that heat treatment can be efficient in reducing

trypsin inhibitor activity (Evans, 1966; Sgarbieri 2002, 1980; Mendez et al., 1993).

NPU is the ratio of amino acid converted to proteins from amino acids supplied. This is somewhat affected by the salvage of essential amino acids within the body, but is profoundly affected by the level of limiting amino acids within a foodstuff (Young and Pellet, 1991). There was no significant difference observed on NPU and TPD in rats fed with the processed fillet to those fed with the control diets. Rats fed with the SHB diet recorded lower NPU and TPD values than those with the S-GBD. The differences in digestibility observed in the SHB may have risen from intrinsic differences in the nature of food protein and presence of dietary factors. These factors modify digestion or chemical reactions that alter the release of amino acids from proteins by enzymatic processes (Hopkins, 1981). Nonetheless results of NPU and TPD supported our earlier observation in the BV values for animals fed with the SHB diets. The observed data on APD in animals fed with SHB diets could be attributed to protein digestibility enzymes (Nelson and Cox, 1982; Sultana et al., 2010). Current finding is similar to the reports of Eid and Matty (1989) and thus signifies the importance of processed SHB as a good ingredient in animal feed.

PDCAAS is a method of evaluating the protein quality based on amino acid requirements for man and the ability to digest it (FAO/WHO, 1993; Moughan and Rutherford, 1996; Gilani and Estatira, 2002). The highest PDCAAS value observed in WSKFBD indicated significant amount of amino acids. However, the lowest PDCAAS values observed in CSKFBD, CSHBBD and WSHBBD with the best weight gain, FCR and FER may be because the chemical score does not take into account protein digestibility (James, 2007; Young and Pellett, 1991). Our findings corroborate with the reports of Moughan & Rutherford (1996) and Sarwar & Sepehr (2002) who demonstrated that there are large differences between digestibility of protein and the individual amino acids, especially products that contain anti-nutritional factors. Furthermore present results showed that PDCAAS level for all diets (test and control) were inversely proportional to the digestibility and availability of their nutrients in the rats, as measured by the weight gained, FCR, FER, PER, NPR and NPU. During heating or prolonged storage of protein at high temperatures, lysine may undergo Maillard reaction with reducing sugars or other aldehyde compounds (Carpenter et al., 1989).

This might have caused a severe deterioration in protein digestibility and availability, PER, NPR and NPU values in rats fed with SHB diets compared to those in the positive control group. In addition, amino acids that move past the ileum may be an important route for bacterial consumption of amino acids, and amino acids that reach the colon would not likely be utilized for protein synthesis, even though they do not appear in the feces (Schaarfsma, 2000). Anti-nutritional factors like trypsin inhibitors, lectins, and tannins present in certain protein sources as well as age, have been reported to increase losses of endogenous proteins at the terminal ileum (Salgado et al., 2004; Sarwar, 1997).

This reason may be responsible for the significantly high PDCAAS levels in rats fed with the test diets, but poor digestibility in terms of PER and NPR compared to those fed with S-GBD. Also, earlier observations in present study indicated that WSKFBD and PSKBD had significant high RDC and PAH contents in the processed SHB. Products of Maillard reaction, inhibits the activity of several enzymes, such as trypsin (E.C. 3.4.21.4), pepsin (E.C. 3.4.23.1), carbo-peptidase A (E.C. 3.4.22.17) and amino-peptidase N (E.C. 3.4.11.2) (Schmidt et al, 2003). This is particularly true for lysine, which forms complexes at high-temperatures with carbohydrates (Sarwar-Gilani and Sepehr, 2003; Cordova-Murueta et al, 2007).

5. CONCLUSION

It can be concluded that protein quality of rats fed with the experimental diets showed that CSKFBD was the best utilized diet, in terms of PER, NPR and NPU, followed by WSKFBD and PSKBD. The wide variability of nutrient profile and content between fish processing methods strengthened the importance of producing data derived from a selection of effect of cooking methods on fish varieties. Also, the SHB can be gathered and utilized as little costs for human food and animal feed, thus reducing costs of feeds due to high priced casein and soybean meal.

It is generally recognized that the abilities of rats and humans to digest a variety of foods are similar therefore required clinical data to confirm the adverse effect of these anti-nutrients on protein digestibility of products containing anti-nutritional factors, as indicated in the present investigation. The decreased level of fecal nitrogen content observed in rats fed with experimental diets may be an indication of better utilization of the nutrients than rats fed with control diets. Although the poaching method compared favorably well, the coal smoked process was the best in terms of protein quality and amino digestibility, especially in the rats fed on the SHB diets.

6. ACKNOWLEDGEMENT

The authors thank National Research Foundation (NRF) of South Africa for their financial support.

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