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# Effects of Herring Fillets and By-Products on Protein Digestibility, Hematology, and Blood Biochemistry in Rats

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***Abstract.*** *Protein bioavailability and digestibility are critical for animal growth and health and are influenced by source and processing methods. This study investigated the effects of diets incorporating processed Clupea harengus (herring) fillets and by-products (heads, bones, viscera) prepared by charcoal, wood, or poaching on growth performance, protein quality, hematology, and blood biochemistry in Wistar rats. Forty rats were randomly allocated to eight dietary groups: charcoal-smoked fillet (CSFBD), wood-smoked fillet (WSFBD), poached fillet (PFD), charcoal-smoked by-products (CSHB), wood-smoked by-products (WSHB), poached by-products (PSF), soybean-based positive control, and basal negative control. Diets were fed for 28 days. Smoked by-product diets (CSHB and WSHB) produced significantly higher final body weights (120.86 ± 0.68 g and 105.52 ± 19.94 g) and weight gains (9.14 ± 1.39 g, $p \leq 0.05$) than the basal diet (59.55 ± 1.37 g and 5.35 ± 0.25 g). Smoked diets improved feed intake, feed conversion ratio, apparent protein digestibility, and biological value. Hematological parameters remained within physiological ranges across groups; the soybean diet showed the highest RBC (7.8 × $10^{12}$/L) and hemoglobin (16.3 g/dL), whereas smoked fish diets recorded slightly lower but normal values (RBC 7.0-7.5 × $10^{12}$/L; hemoglobin 14-15 g/dL). Biochemical profiles revealed that smoked diets reduced serum triglycerides and creatinine, suggesting enhanced lipid metabolism and renal function. Smoking significantly improves the nutritional quality of Clupea harengus fillets and by-products, making them practical, safe, and growth-promoting protein sources for animal nutrition. Inclusion of smoked fish by-products offers a sustainable alternative to conventional plant proteins while supporting metabolic and systemic health.*

***Keywords****: Blood biochemistry, Clupea harengus, Fish by-products, Hematology, Protein digestibility, Smoking processing*

## Citation

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## INTRODUCTION

Proteins are fundamental macromolecule essential for animal growth, development, tissue repair, and metabolic functions (FAO, 2022). The bioavailability and digestibility of dietary proteins are critically influenced by their amino acid composition, structural characteristics, and the processing techniques employed during food preparation (Wang et al., 2021; Li et al., 2022). High-quality protein sources are vital in animal nutrition to optimize growth performance, feed efficiency, and overall health outcomes.

*Clupea harengus* (Atlantic herring), a commercially abundant small pelagic fish, is recognized as a rich source of high-quality protein, characterized by a balanced amino acid profile including essential amino acids such as methionine and lysine and substantial levels of long-chain omega-3 polyunsaturated fatty acids (Santos et al., 2023). These components are integral to physiological functions, including cellular development, immune regulation, and metabolic health.

Processing techniques, including heating, smoking, and enzymatic hydrolysis, alter the structural and physicochemical properties of herring proteins. Such modifications facilitate the breakdown of complex, often insoluble muscle proteins into smaller peptides, thereby enhancing gastrointestinal digestibility and nutrient absorption (Aiello et al., 2025). Additionally, processing reduces anti-nutritional factors like phytic acid and protease inhibitors, which otherwise hinder nutrient utilization (Talabi et al., 2023). The resulting protein hydrolysates exhibit improved solubility, functional properties like emulsification, and palatability, all of which support better feed intake and growth in animals (Elgaoud et al., 2024).

The influence of processed Clupea harengus products extends beyond digestibility; they also modulate systemic health markers, including hematological indices (e.g., red blood cell counts, hemoglobin levels) and blood biochemical parameters (e.g., triglycerides, creatinine, electrolytes). These changes reflect improvements in oxygen-carrying capacity, metabolic efficiency, renal function, and immune status factors that collectively contribute to enhanced growth performance and disease resistance (Lee et al., 2022; Singh et al., 2022).

Despite evidence suggesting positive effects of processing techniques such as smoking (charcoal or wood smoke) and poaching on nutrient quality, the mechanisms underlying their influence on nutrient utilization, blood health, and metabolism remain incompletely understood, particularly in animal models such as rats. Smoking may induce the formation of bioactive compounds that influence lipid metabolism and renal function, while poaching may better preserve heat-sensitive nutrients.

This study aims to clearly evaluate the effects of various processed *Clupea harengus* fillets and by-products, specifically the smoked and poached forms, on protein digestibility, serum hematology, and blood biochemistry in rats. By comparing these processing methods to a soybean-based positive control diet, the research seeks to elucidate how processing influences nutrient availability, animal growth, metabolic health, and immune function. The findings are expected to inform the development of optimized fish-based feed ingredients for improved productivity and health in livestock and aquaculture systems.

## MATERIALS AND METHODS

### Sample Collection

Twenty kilograms (20 kg) of *Clupea harengus* was purchased from two fish distributors in Ilorin, Kwara State (Asake and Heritage Fisheries)—average length: 32.22 cm; Average weight: 197.66 grams. The fish was washed, prepared, and cooked through boiling and smoking (burning wood/charcoal).

Sample Processing: A portion was poached (60 °C for 15 min) and charcoal- or wood-smoked in a traditional kiln (charcoal/ firewood), following FAO/UN (2007) and Adeyemi et al. (2013). No additives. Fish were dried in a microwave oven to a constant weight (60 °C). Flesh separated from bones, skin, and head—homogenization of skin, head, bones, and fillets. Homogenized samples were analyzed for anti-nutrient and shelf-life characteristics on a dry matter basis. The fish was dried to constant weight (60 °C) in a microwave oven. The meat was separated from bones, skin, and head—homogenization of skin, head, bones, and fillets. Samples were analyzed for anti-nutrients and shelf-life characteristics.

**Experimental animals**

40 female Wistar albino mice (*Rattus norwegicus*, 40-70 g) were placed in plexiglass cages at 24 °C, on a 12-hour light/dark cycle, with 45-50% humidity. Feed prepared diet/ standard rat chow with feed and water ad libitum for 12 days. All animal experiments were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. The protocol was reviewed and accepted with the valuable resource of the Animal Ethics Committee number UFH/UREC/2013/0012, of the University of Fort Hare, Alice, South Africa. The animals were handled following the National Research Council's Guide for the Care and Use of Laboratory Animals. Rats were randomly assigned to eight treatment groups (± 2.00 g weight difference): I. soybean-groundnut cake meal-based diet (positive control), II. basal diet (negative control), III: cooked clupea fillet. IV: charcoal-smoked clupea fillet. V: wood-smoked clupea fillet. VI: Skin-head & Bone (SHB) (smoked herring by-product) diet. VII: SHB diet s ubjected to carbon smoke. VIII: SHB diet subjected to wood smoke. Rats were weighed before the experiment and daily thereafter. Food and water intake are measured daily. Cages were cleaned on day 4.

**Protein Quality Evaluation**

Parameters used (FAO/WHO, 1991; Johnson & Parsons, 1997; FAO/WHO/UNU, 1985; Meltzer, 1987; Cohen & Michaud, 1993): Biological Value (BV), Net Protein Ratio (NPR), Net Protein Utilization (NPU), Apparent Protein Digestibility (APD), True Protein Digestibility (TPD), Protein Efficiency Ratio (PER), and Protein Digestibility-Corrected Amino Acid Score (PDCAAS).

**Rat Diets Formulation**

Yellow maize (*Zea mays*) from Alice Market, South Africa. Soaked and changed daily for 4 days, then dried to constant weight (40 °C). Ground to powder. Diets were organized according to the FAO/WHO (1991) and Adeyemi et al. (2015) methods. Protein-free diet (negative control). Processed fish species (fillets and SHB) as experimental diets (10% minimum protein). Positive control: soy flour and peanut cake. Both diets included DL-methionine, sucrose, wheat flour, vitamins, and minerals—gross and chemical composition of diets in Table 1.

The analysis was conducted using the FAO/UN (2007) method, with slight modifications by Adeyemi et al. (2013). Raw and processed herring fillet and by-products (SHB) were oven-dried at 60°C until a constant weight was achieved. Following drying, the fillets were manually separated from non-edible components, including skin, head, and bones, prior to analysis. Fillet/SHB ground to powder for protein concentrate. Moisture and ash levels (AOAC, 2002a & d). Total crude fat (AOAC, 2002; Reinik et al., 2007): soxhlet extraction; crude fiber (AOAC, 2002c): Acid-base digestion; crude protein (AOAC, 2002b): kjeldahl technique; Nitrogen

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**Table 1.** Gross Proximate Composition of Test and Control Diets (g/100g DM)*

| Determined analysis (%) | Control Diets | | Test Diets | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | CSSF | WSSF | PSF | CSHB | WSHB | PSHB |
| Protein | 12.85 | 10.10 | 11.07 | 11.07 | 11.07 | 11.07 | 11.07 | 11.07 |
| Fat | 3.31 | 3.30 | 3.48 | 3.48 | 3.48 | 3.48 | 3.48 | 3.48 |
| Crude Fiber | 2.30 | 2.06 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 |
| Ca | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Av. P | 0.16 | 0.24 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Methionine | 0.68 | 0.65 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 |
| Lysine | 0.42 | 0.24 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |
| ME | 2,429.00 | 2,545.30 | 2,261.40 | 2,261.40 | 2,261.40 | 2,261.40 | 2,261.40 | 2,261.40 |

*Av. P= Available phosphorus; ME= Metabolizable Energy. *CSSF: charcoal smoked Sawa fillet; CSHB: charcoal smoked Sawa SHB; WSSF: wood smoked Sawa fillet; WSHB: wood smoked Sawa SHB; PSF: poached Sawa fillet; PSHB: poached Sawa SHB; RSF: Raw Sawa fillet; RSHB: Raw Sawa SHB.

Nitrogen (%) calculated by:

$$Y = 0.026x - 0.003, R^2 = 0.974$$

(Okalebo et al., 2006)

$$\text{Crude protein} = \text{Nitrogen} \times 6.25$$

**Amino Acid Analysis**

Amino Acid Analysis was performed using the methods reported by AOAC (1992; 2003), as modified by Talabi et al. (2023). A sample of 0.1 g of fish tissue was defatted with a chloroform/methanol mixture, then hydrolyzed at 110 °C in an evacuated, sealed ampoule for 24 hours. The hydrolysate was then treated with 6 M hydrochloric acid containing 15% phenol, in accordance with AOAC (2003) guidelines. After cooling, 2 mL of the hydrolysate was analysed using an Eppendorf Biotronic LC 3000 Amino Acid Analyzer (Eppendorf-Biotronic, Hamburg, Germany). The amino acid concentrations in processed fish diets were determined according to AOAC (1992) protocols, using Dumas dry-combustion methodology.

**Determination of Protein Digestibility**

Animal experiments were conducted using a randomized cluster sampling of 40 Wistar albino rats. The animals were assigned to three dietary treatment groups (positive control, negative control, and experimental diet) and kept in plexiglass cages maintained at 23°C with 55% humidity, under a 12-hour light/dark cycle. Food and water were provided ad libitum, in compliance with the moral standards of the Fort Hare and NIH guidelines, with reference number UFH/UREC/2013/0012.

The rats were randomly assigned to eight groups, each comprising five animals, with mean weights differing by approximately ±2.00 g. Daily individual weights, food intake, and water consumption were recorded. The cages were cleaned every 4 days, and spilled food and stools were collected and oven-dried at 40 °C for frequent weighing. Fecal nitrogen was determined using the method of Okalebo et al. (2002). The dietary remedy lasted for 14 days, during which feeding, water intake, feed conversion ratio, feed performance ratio, fecal output, and survival rates were measured, as described by Jobling (1983) and Edemy and Mohammed (2011). Euthanasia was performed using chloroform anesthesia.

**Determination of Biological Value (BV)**

The BV was calculated following FAO/WHO (1991) and Adeyemi et al. (2015), using the formula:

BV = (N retained/N absorbed)×100

where N retained was obtained by subtracting fecal nitrogen (F) from nitrogen intake, and F was derived from nitrogen excreted by test animals. FM represented nitrogen excreted by protein-free animals.

**Determination of Protein Efficiency Ratio (PER) and Relative PER (RPER)**

The PER was calculated as the ratio of weight gain to protein consumed, following AOAC (1975), Mitchell & Grundel (1989), and Johnson & Parsons (1997). The RPER was determined as:

RPER = (Mean PER of test protein×100)/
Mean PER of reference protein

**Calculation of Net Protein Ratio (NPR) and Relative NPR (RNPR)**

The NPR was determined based on Bender (1957), modified by FAO/WHO (1991) and Adeyemi et al. (2015), as:

NPR = (Weight gain of test animal – weight loss by protein-free animals)/protein consumed by test animals.

RNPR was calculated as:

RNPR = (Mean NPR of test protein × 100)/
Mean NPR of reference protein.

**Net Protein Utilization (NPU)**

NPU was calculated according to FAO/WHO (1991) as:

NPU = (N retained/N intake)×100

which can also be expressed as BV multiplied by true digestibility.

**Apparent Protein Digestibility (APD)**

APD was calculated following FAO/WHO (1991):

APD (%) = [(I – F)/I] × 100

where I is nitrogen intake and F is fecal nitrogen.

**True Protein Digestibility (TPD)**

TPD was calculated as following formula:

TPD (%) = {[PI – (FP – MFP)]/PI}×100

where PI is protein intake, FP is fecal protein, and MFP is metabolic fecal protein, estimated from animals fed a protein-free diet following Sarwar (1986). Fecal samples were collected every four days, dried at 60°C, and ground into powder. Nitrogen concentration was determined using AOAC (1984) & Adeyemi et al. (2015).

**Protein Digestibility Corrected Amino Acid Score (PDCAAS)**

PDCAAS was determined based on FAO/WHO/UNU (1985), Meltzer (1987), and Cohen & Michaud (1993). The amino acid score was calculated as the ratio of milligrams of amino acid per gram of test protein to that in the reference protein, and PDCAAS was obtained by multiplying the true digestibility by the lowest amino acid score.

**Fecal Nitrogen Content (FNC)**

Fecal nitrogen content was measured using a modified micro-Kjeldahl method (Okalebo et al., 2002). A 0.5 g fecal sample was digested with 12 mL of a digestion mixture (9 mL $HNO_3$ and 3 mL HCl) in a Buchi 425 digester. Nitrogen concentration was determined via a calibration curve:

$Y = 0.026x – 0.003$, $R^2 = 0.974$

**Biochemical Evaluation of Diets on Rat Tissues**

Rats were fasted for 12 hours prior to the experiment, then fed the test diets for 14 days. Following this period, animals were sacrificed under chloroform anesthesia. Blood was collected via cardiac puncture, and plasma was prepared for analysis. Serum electrolytes like sodium, chloride, potassium, and calcium,

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along with serum creatinine and blood-related measurements such as hemoglobin, red blood cell counts, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, hematocrit, red cell distribution width, white blood cell count, and the different types of white blood cells were tested using standard lab techniques. The electrolytes and creatinine levels were checked using Beckman Coulter Synchron® Clinical Systems and UniCel® DxC machines.

### Statistical Analysis

All data were expressed as means ± standard error (n = 3). Statistical significance was assessed using one-way ANOVA followed by Duncan's multiple range test at a significance level of $p \leq 0.05$. Analyses were performed using SPSS version 14.0 (SPSS Inc., 2005).

## RESULTS AND DISCUSSION

Figure 1 shows the proximate analysis of nutrients analyzed. The results show that moisture ('M') had the highest concentration across all groups, especially in WSSFBD and PSFBD, indicating these diets provided sufficient moisture to support growth and development in all animals. Nutrients such as protein (P) and carbohydrate (C) were also present at adequate levels, suggesting that all diets, regardless of processing method, supported growth and development effectively. The lower levels of crude fiber and ash across the samples indicate minimal impact on nutrient adequacy and minimal

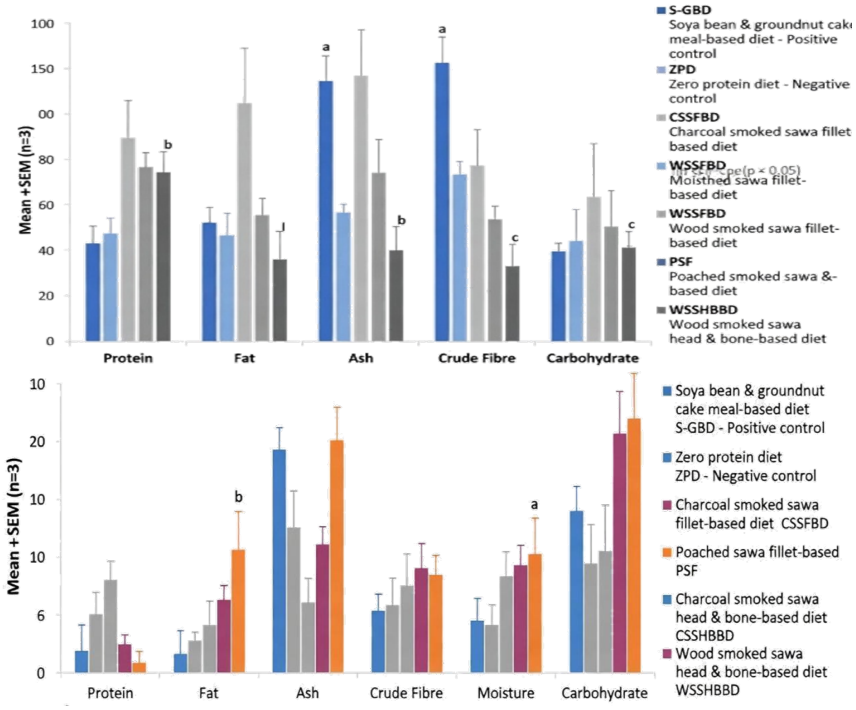


**Figure 1: *Proximate analysis of feed fillet & cut-offs.**

* Data Mean ± SEM, n=3. Values with different superscripts along a row are significantly different ($p<0.05$). CSSFBD: charcoal smoked sawa fillet-based diet; WSSFBD: wood smoked sawa fillet-based diet; PSFBD: poached sawa fillet-based diet; RSFBD: Raw sawa fillet-based diet; CSSHBBD: charcoal smoked Sawa SHB-based diet; WSSHBBD: wood smoked Sawa SHB-based V; PSHBBD: poached Sawa SHB-based diet; RSHBDB: Raw Sawa SHB-based diet; S-GBD: Soy bean & Groundnut cake meal-based diet (Positive control); ZPD: Zero protein diet (Negative control). CF= Crude fibre; M= Moisture; C=Carbohydrate; P= Protein.

processing effects. These findings imply that, despite differences in diet preparation, all diets contained sufficient nutrients to meet the animals' growth needs, which is critical for their health and development. This aligns with the literature from Paez et al. (2016) and FAO/IAEA (2015), which emphasizes the importance of balanced nutrient profiles in diets for optimal growth.

Figure 2 shows the result of the Protein Digestibility of Rats Fed on Control, Poached, Charcoal, and Wood-smoked Clupea harengus Fillet & cut-offs for 14 days. Smoked diets enhanced protein digestibility indices, as evidenced by significantly higher true protein digestibility (TPD) and net protein utilization (NPU), indicating improved amino acid absorption and utilization. Specifically, smoked Clupea harengus diets (CSSFBD and WSSFBD) demonstrated near-complete amino acid utilization, likely attributable to heat-induced protein denaturation and peptide formation that facilitate enzymatic breakdown and improve digestibility (Marmon, 2012; Aiello et al., 2025). In contrast, soybean-based diets showed comparatively lower digestibility scores, reflecting less efficient amino acid absorption.

Figure 3 depicts the results of the hematological parameters of Rats Fed on Control, Poached, Charcoal, and wood-smoked *Clupea harengus* Fillet and cut-offs for 14 days. It was observed that the rats fed soybean diets maintained higher red blood

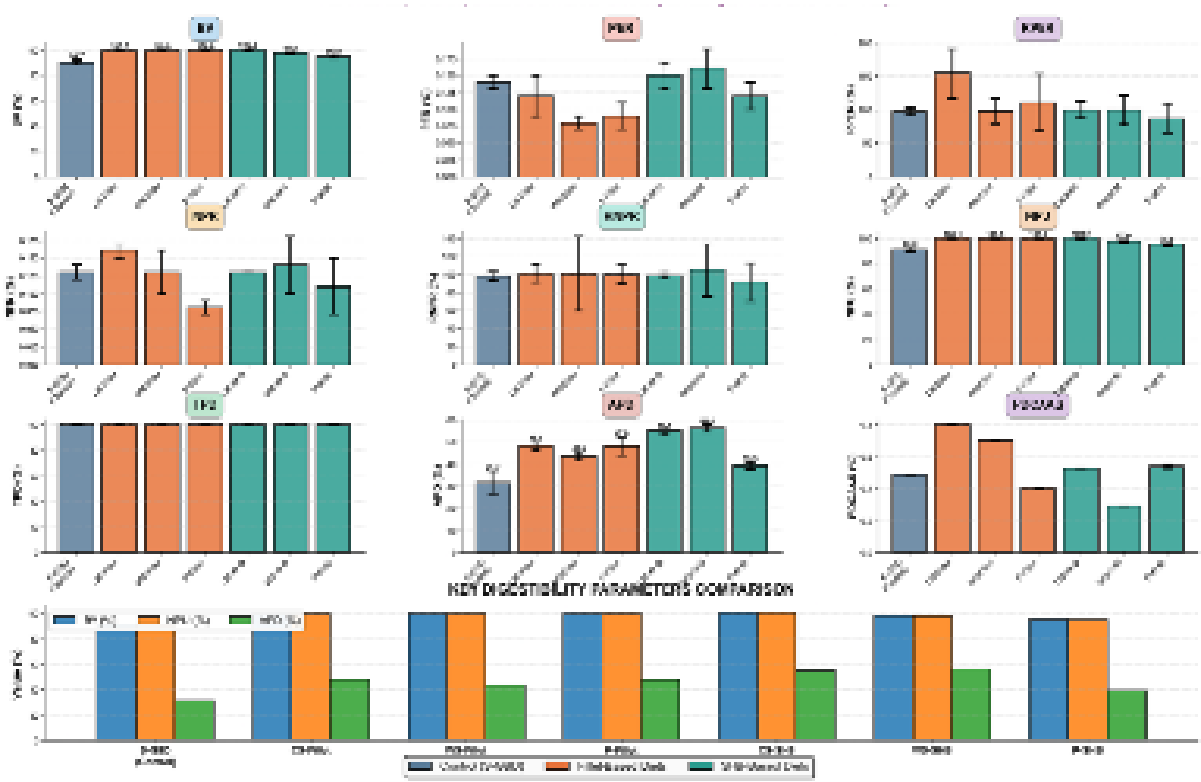


**Figure 2: *Protein digestibility of rats fed on control, poached, charcoal and wood smoked *Clupea haregus* fillet & cut-offs for 14 days.**

* ($p < 0.05$); CSSFBD: charcoal smoked sawa fillet meal based diet; WSSFBD: wood smoked sawa fillet meal based diet; PSFBD: poached sawa fillet meal based diet; S-GBD: CSSSHBBD: charcoal smoked sawa skin, head & bone meal based diet; WSSSHBBD: wood smoked sawa skin, head & bone meal based diet; PSSHBBD: poached sawa skin, head & bone meal based diet; 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; PDAAS= Protein Digestibility-Corrected Amino Acid Scores

cell counts (RBC: approximately $7.8\times10^{12}$/L) and hemoglobin (Hb: 16.3 g/dL), indicative of sustained oxygen transport capacity and overall hematopoietic health. Smoked fish diets resulted in slightly lower RBC counts (14-15 g/dL), but these remained within normal physiological ranges (Liu et al., 2017). The biological mechanism underlying these differences may relate to the nutritional composition of the diets: soybean is a rich source of bioavailable iron and sulfur amino acids (methionine and cysteine), which are essential cofactors for hemoglobin synthesis (Talabi et al., 2023). Conversely, smoking processing can induce oxidative modifications in proteins, potentially affecting mineral bioavailability and hematopoiesis (Gutteridge & Halliwell, 2010). Nonetheless, the observed hematological values suggest that smoked diets support adequate oxygen-carrying capacity, although slight reductions compared to soybean diets may reflect subtle effects of thermal processing on nutrient bioavailability. Biochemically, smoked diets led to lower serum triglycerides and creatinine levels, indicating improved lipid metabolism and renal function, possibly due to the bioactive peptides and high-quality proteins generated during smoking (Adeyemi et al., 2015). The heat treatment may also reduce anti-nutritional factors, enhancing overall metabolic health (Adeyemi et al., 2015; Talabi et al., 2023).

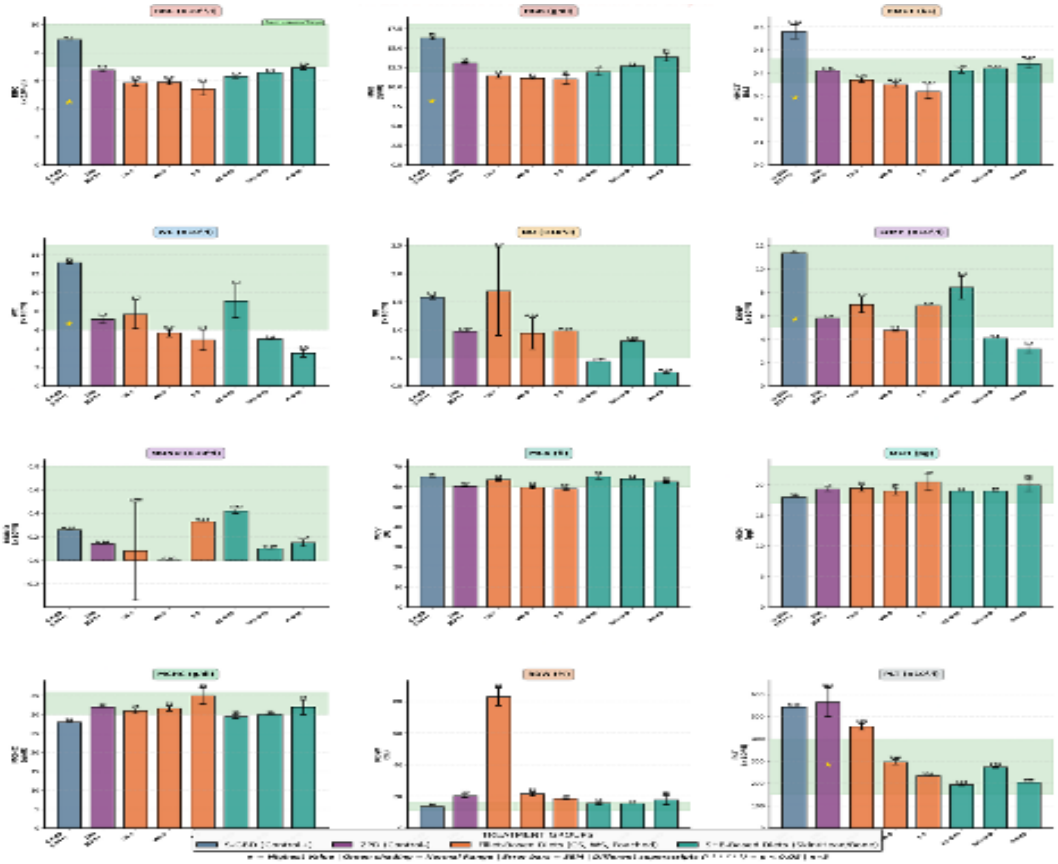

**Figure 3: *Heamatological parameters of rats fed on control, poached, charcoal and wood smoked *Clupea harengus* fillet & cut-offs.**

*($p < 0.05$). CSSF: charcoal smoked sawa fillet; WSSF: wood smoked sawa fillet; PSF: poached sawa fillet; CSSSHB: charcoal smoked sawa skin, head & bone; WSSSHB: wood smoked sawa skin, head & bone; PSSHB: poached sawa skin, head & bone; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control); S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control). RBC = Red blood cell; WC=White blood cell.

In contrast, soybean diets better preserved haematological stability and metabolic parameters, supporting their role in maintaining systemic health, likely owing to their stable nutrient profile rich in iron, vitamins, and antioxidants. These findings highlight that processing methods such as smoking improve protein digestibility by inducing physicochemical modifications, protein denaturation, and peptide formation, thereby facilitating enzymatic breakdown and amino acid absorption (Marmon, 2012; Aiello et al., 2025). However, systemic health markers such as RBC and WBC counts are influenced by the nutritional composition of the diets; soybeans' higher iron content sustains erythropoiesis, whereas smoked fish, despite enhanced digestibility, may have subtle effects on hematopoietic parameters due to heat-related mineral modifications.

Smoked diets significantly improved protein digestibility indices, as evidenced by higher True Protein Digestibility (TPD) and Net Protein Utilization (NPU). These improvements likely result from heat-induced protein denaturation and peptide formation during smoking, which can enhance enzymatic protein breakdown and amino acid absorption (Marmon, 2012; Aiello et al., 2025). TPD reflects the efficiency of protein digestion. It correlates positively with the extent of weight loss during smoking, suggesting that controlled thermal processing optimizes protein bioavailability. In contrast, soybean-based diets exhibited comparatively lower digestibility scores, indicating less efficient amino acid absorption. These results align with previous findings that plant proteins often contain anti-nutritional factors inhibiting protease activity, thereby reducing digestibility (Gonzalez et al., 2021).

In addition, rats fed soybean diets maintained higher red blood cell counts (RBC: approximately $7.8\times10^{12}$/L) and hemoglobin (Hb: around 16.3 g/dL), indicative of adequate erythropoiesis and oxygen transport capacity (Liu et al., 2017). Hematocrit (HCT) values were also higher in the soybean group, supporting stable blood oxygen-carrying capacity. White Blood Cell (WBC) counts and differential counts (neutrophils, lymphocytes, monocytes) were elevated in soybean-fed rats, suggesting robust immune function (Adeyemi et al., 2015; Talabi et al., 2023). Conversely, rats fed smoked fish diets showed modest reductions in RBC, Hb, and HCT levels ($7.0\text{-}7.5\times10^{12}$/L for RBC and 14-15 g/dL for Hb). However, these remained within normal ranges, indicating preserved but slightly diminished erythropoietic activity. The slight decrease may relate to heat-related effects on mineral bioavailability, particularly iron, which is essential for hemoglobin synthesis (Gutteridge & Halliwell, 2010). The immune cell counts (WBC, neutrophils, lymphocytes) were also marginally lower in the smoked diet groups, suggesting a mild suppression of immune responses (Adeyemi et al., 2013), potentially due to oxidative stress or thermal degradation of immune-modulatory nutrients.

Figure 4 shows the results of the blood chemistry parameters of rats fed on control, poached, charcoal, and wood-smoked *Clupea harengus* fillet & cut offs. Elevated serum creatinine, often a marker of impaired renal clearance, was observed in some groups fed unprocessed or poorly processed diets, suggesting that proper thermal treatment during smoking may reduce renal stress by decreasing the load of nephrotoxic compounds (Jiang, 2025). Conversely, higher serum urea levels in certain groups could reflect increased protein catabolism or altered renal handling, which warrants further study. Mechanistically, oxidative stress induced by thermal processing such as smoking may impair immune cell function or production (Witeska et al., 2023). Nutrient deficiencies, especially of bioavailable iron and sulfur amino acids (methionine, cysteine), could further influence erythropoiesis and immune competence (Adeyemi et al., 2015). While these interpretations are hypotheses supported by literature, definitive causal relationships require further investigation. Serum biochemical parameters provide additional

insights into metabolic and renal functions. Smoked diets resulted in lower serum triglyceride and creatinine levels, suggesting an improved lipid profile and renal efficiency (Adeyemi et al., 2013; Brien et al, 2020). Serum electrolyte analysis showed that smoked fish diets led to alterations in sodium, chloride, and potassium levels. These changes could be due to thermal modifications of mineral content or bioavailability, possibly affecting electrolyte balance and hydration status (O'Brien et al., 2020). For instance, elevated chloride levels in smoked diet groups may contribute to better cellular hydration and osmotic regulation, though this remains a hypothesis.

Higher total serum protein, albumin, and globulin levels in soybean-fed rats suggest better maintenance of nutritional status and immune capacity, consistent with the stable intake of bioavailable nutrients. In contrast, smoked diets, despite their superior digestibility, did not significantly elevate serum protein levels, possibly due to differences in amino acid composition or bioavailability influenced by processing conditions. Lipid parameters, including cholesterol and triglycerides, were differentially affected by diet type. Omega-3 fatty acids prevalent in smoked fish products are known to exert hypolipidemic effects by modulating hepatic lipid synthesis and lipoprotein clearance (Adeyemi et al., 2013). The observed lipid profile changes may reflect these mechanisms, although precise causal attributions require further targeted studies. Figures 3 and 4 show that hematological parameters, including RBC, Hb, and HCT, are highest in the control group (S-GBD), indicating optimal erythropoiesis, likely due to adequate iron intake. Processed diets, especially smoked fish variants, showed moderate reductions in these parameters, with the most significant preservation in the PSSHB group, suggesting that specific processing methods may mitigate adverse effects on hematopoiesis (Adeyemi et al., 2015).

White blood cell counts and differential leukocyte counts showed similar trends. The reductions in lymphocytes and neutrophils ($p \leq 0.05$) in some smoked diet groups may suggest a mild suppression of the innate immune response, potentially due to oxidative stress or loss of immune-modulating nutrients during processing (Leliefeld et al., 2016). These hypotheses require verification through additional mechanistic studies. Serum biochemical markers, such as Alkaline Phosphatase (ALP), were elevated in some processed diet groups, suggesting hepatic or skeletal stress (Figure 4). Lower serum albumin and globulin levels in certain groups might reflect nutritional deficiencies or impaired hepatic protein synthesis (Jiang et al., 2025). Serum urea and creatinine levels varied across diets, with elevated urea in the zero-protein diet indicating dehydration or altered nitrogen metabolism, and increased creatinine levels suggesting compromised renal function. These parameters underscore the importance of balanced protein intake and proper processing to minimize renal stress (Nwoked et al., 2025). The thermal processing during smoking appears to enhance protein digestibility by altering the physicochemical properties of fish proteins, thereby supporting improved growth performance. However, systemic health markers, such as hematological indices and renal function, are influenced by the nutritional quality and mineral bioavailability of the diet. While smoked fish diets show promise as alternative protein sources, potential oxidative stress and mineral losses associated with processing conditions should be carefully managed to optimize health outcomes.

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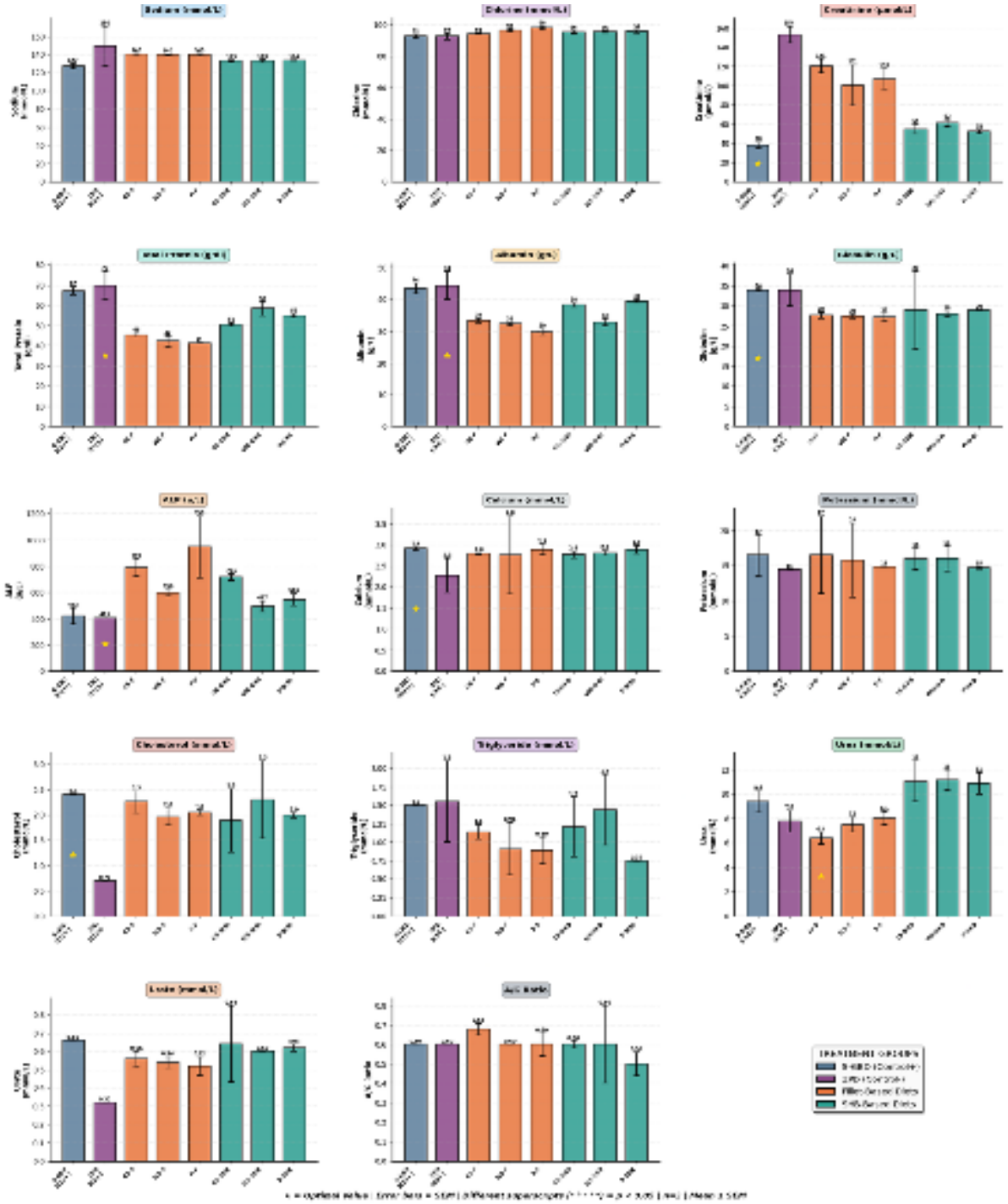

**Figure 4: *Blood chemistry parameters of rats fed on control, poached, charcoal and wood smoked *Clupea harengus* fillet & cut-offs.**

*Values with different superscripts along a row are significantly different ($p<0.05$). CSSF: charcoal smoked sawa fillet; WSSF: wood smoked sawa fillet; PSF: poached sawa fillet; ; CSSSHB: charcoal smoked sawa skin, head & bone; WSSSHB: wood smoked sawa skin, head & bone; PSSHB: poached sawa skin, head & bone; S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control); S-GBD: Soy bean & Groundnut cake meal based diet (Positive control); ZPD: Zero protein diet (Negative control).

## CONCLUSION

Processed *Clupea harengus* (smoked fish) diets, particularly those derived from skin, head, and bone meal, significantly improved protein digestibility, growth, and nutrient availability in rats compared to soybean-based diets. Smoked fish diets yielded higher final weights and weight gain, exhibiting superior protein quality (higher BV and PER) and better metabolic health (lower triglycerides and creatinine). Soybean diets maintained favorable red blood cell counts and albumin levels, while smoked fish diets supported electrolyte balance.

## AUTHOR CONTRIBUTION

**O.T.T.** contributed to study conception, design, experimental execution, preparation of the initial manuscript draft, funding acquisition. **S.O.O.** was responsible for methodology development, supervision of the research, data analysis, manuscript writing, and revisions. **O.O.** participated in the experimental work, provide guidance on data interpretation, and performed data collection and analysis (hematology/ blood chemistry). **F.D.O.** contributed to the design and execution of experiments, data interpretation, and manuscript revision. **O.O.A.** was involved in data interpretation (metabolism/biochemistry), data collection and analysis, and provide editorial input. All authors approved the final version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the research or the research funding.

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