Journal of Environment and Sustainable Development

Research Article https://doi.org/10.55921/DFID5487

INCORPORATING PALM KERNEL MEAL, COWPEA HUSK AND SOYBEAN HUSK AS PROTEIN SOURCES IN CATFISH DIETS: EFFECTS ON GROWTH, HEMATOLOGY AND INTESTINAL HISTOLOGY

Received: 29 May 2025

Accepted: 15 August 2025

Published: 30 September 2025

Christian Larbi Ayisi[1*], Moses Nganwani Tia[2], Samuel Osei Ayeh[3], Cecilia Asemah[1]

Abstract

This study examined the effects of replacing fish meal (FM) with soybean husk (SBH), palm kernel meal (PKM), and cowpea husk (CPH) on growth performance, feed utilization, and intestinal histology of catfish. Four experimental diets were formulated with different proportions of PKM, CPH, and SBH to partially replace fish meal. Juvenile catfish (Clarias gariepinus) were fed these diets for ten weeks. The results indicated that inclusion of these alternative protein sources had significant effects on growth performance and feed utilization compared to FM diet. The weight gain recorded in this study was as follows: SBH (119.5±68.02) > CPH (113.2±53.14) > FM (104.3±56.82) > PKM (86.73±31.51). Feed conversion ratio ranged from 1.25±0.57 (SBH) to 1.52±0.56 (PKM). C. gariepinus fed the diet PKM had the lowest protein efficiency ratio (2.09±0.76), followed by those fed the FM (2.57±1.40) and the CPH diet (2.74±1.28). To a larger extent, the dietary protein sources significantly influenced serum hematology parameters. C. gariepinus fed the FM diet had the highest white blood cells count (133.0±2.51), which was significantly higher than all other groups ($p < 0.0001$). The range of red blood cell values observed in this study was 2.08±0.13 to 2.62±0.13. Histological examination indicated modifications in intestinal morphology, suggesting possible metabolic adjustments to the experimental diets. Fish fed the FM had the highest villus height (441.2±22.6), followed by SBH (398.3±7.51), PKM (279.2±15.65), and CPH (142.3±10.84). Villus width and muscular thickness also followed this pattern, with fish fed the FM diet having the largest villus width (153.7±9.06), significantly greater than all other groups ($p<0.0001$). Overall, incorporating CPH, and SBH into catfish diets appears to be a viable replacement for conventional fishmeal-based diets; however, further studies are required to determine optimal inclusion levels for achieving maximum growth and well-being

Keyword: Alternative protein, Agricultural by-products, Intestinal morphology, Blood biochemistry, Growth.

[1*] Department of Water Resources Development and Aquaculture Management, School of Sustainable Development, University of Environment and Sustainable Development, Somanya, Eastern Region, Ghana,
*Corresponding author: clayisi@uesd.edu.gh
[2] SavaNet, Tamale, Northern Region, Ghana
[3] Department of Fisheries and Aquatic Resources Management, Faculty of Bioscience, University for Development Studies, P. O. Box TL 1882, Tamale- Ghana

Introduction

Total fisheries and aquaculture production (excluding algae) has significantly expanded in the past seven decades going from 19 million tons (live weight equivalent) in 1950 to an all-time record of about 179 million tons in 2018, with an annual growth rate of 3.3 percent (FAO, 2022). Projections of about 96 million metric tons of fish were raised in aquaculture in 2023, compared to 90.6 million metric tons that were (fishing) captured same year (Dauda et al., 2023). Feed is necessary in aquaculture, just like in animal husbandry, to guarantee good production, particularly in intensive culture systems that demand a lot of input (Boyd, 2020).

One of the most significant species of cultured fish and one of the most commonly used fish products is *Clarias gariepinus* (Kari et al., 2021). Farmers are aligning themselves with the culture of catfish farming, which is growing in importance and is catching up to tilapia production (Mbokane et al., 2022). This is because catfish farming provides low-cholesterol animal protein which lowers food insecurity and creates jobs (Montgomery et al., 2022). Fish farming intensified to meet the rising demand for fish has resulted in high stress levels, low wellbeing, and delayed growth performance of aquaculture species (Nasr et al., 2021).

Since feed makes up over 50% of the entire production expenses in the aquaculture business, feed is a crucial component (Mzengereza et al., 2014). The costliest feed ingredient in aquafeeds is the source of protein (Lim et al., 2023). Fishmeal is considered the gold standard protein due to its balanced amino acid content, which is perfect for the healthy growth and development of fish (Perez-Velazquez et al., 2019). Fish by-products and wild-caught fish are used to make fish-meal, an industrial product (Wan, 2015).

Fishmeal is a major source of aquafeed worldwide. However, the rising demand for fish and fish products has caused its price to continuously rise, greatly affecting the market price (Jannathulla et al., 2019). To cut feed costs, several plant-based proteins are now utilized in place of fish-meal, either totally or partially (Jannathulla et al., 2019). Aquafeeds with plant-based protein diets have long been known to perform worse than those with fish-meal (Egerton et al., 2023) due to an inadequate amino acid profile, decreased palatability, and the existence of anti-nutritional factors (ANFs) that have an impact on the performance of farmed fish as a whole (Maundu, 2020). The main objective of using plant-based proteins is to offer fish-meal as a more affordable substitute. Finding a less expensive option isn't the only factor, though (Gutasi, 2021). Moreover, the production of these plant-based proteins needs to be sustainable without affecting the health and growth of fish (Magbanua and Ragaza, 2024). Conventional methods for assessing the general health of fish and their reaction to various proteins and diets include hematological and histopathological studies (Docan et al., 2018). A few of the plant-based protein sources that are used are soybean husk, cowpea husk, and palm kernel meal (Senthilkumaran et al., 2022). These plant sources are more broadly and cheaply accessible worldwide than fish-meal (Senthilkumaran et al., 2022). An agricultural by-product of the oil palm (Elaeis guineensis Jacq) industry, palm kernel meal (PKM) is regarded as an agro-industrial waste (Ng and Chong, 2002; Sangavi et al., 2020). The crude protein content of PKM ranges from 12 to 21%. In addition, PKM is said to be widely available and reasonably priced in many tropical nations (Botello-León et al., 2022). One ingredient that can be purchased in huge quantities for incredibly low costs is soybean husks (Shuaib et al., 2023). Numerous fish species' dietary protein requirements can be covered and met by these plant-based protein sources (Jia et al., 2022). While lysine and methionine are typically absent from plant-based proteins (Gorissen et al., 2018), this is somewhat compensated for by minuscule amounts of crystalline amino acids. Consequently, the aim of this study was to evaluate the effects of soybean husk, cowpea husk, and palm kernel meal as protein sources on growth performance, feed utilization, intestinal histology, and health of catfish using full blood count and liver function test as indicators.

Materials and methods

Formulation of the experimental diets and feeding

Table 1 shows the experimental diets used for this study. In formulating the diets, substantial amount of fish meal was progressively replaced with soybean husk (SBH), cowpea husk (CPH) and palm kernel meal (PKM) until equal amounts of protein (approximately 35%) were obtained. Following procedures previously described by Ayisi et al. (2017), the formulated diets were prepared. In brief, the progressive enlargement method was used to mix all of the dry ingredients (Zhou et al., 2007). In a mixer, the dry ingredients were combined with vegetable oil and distilled water to form a moist dough that was then pelleted using a single screw dry type extruder (Model: LM 70) fitted with a 2-mm die. The dry extruded and sun dried 2-mm pelleted diets were then sealed in plastic bags and kept at room temperature until needed. *C. gariepinus* were fed to apparent satiation, three times daily at 8:00 am, 12 noon and 4:00 pm.

Table 1 Composition of experimental diets

Ingredients	FM	CPH	SBH	PKM
Fish meal	35.00	27.50	22.00	23.50
Soybean husk	30.50	25.00	30.50	30.50
Cowpea husk	0.00	15.50	0.00	0.00
Soybean husk	0.00	0.00	18.00	0.00
Palm kernel meal	0.00	0.00	0.00	15.50
Maize	13.50	13.00	10.50	11.50
Wheat bran	10.00	8.00	8.00	8.00
Premix	1.50	1.50	1.50	1.50
Vegetable oil	4.00	4.00	4.00	4.00
Starch	2.00	2.00	2.00	2.00
Salt	2.00	2.00	2.00	2.00
D-L methionine	0.50	0.50	0.50	0.50
D-L lysine	0.50	0.50	0.50	0.50
H-histidine	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Proximate composition				
Moisture	90.49	90.30	90.73	90.15
Protein	35.8	35.42	35.53	35.75
Lipid	11.51	10.78	11.05	10.93
Ash	10.07	10.04	10.85	10.35

Experimental conditions and design

The experiment was carried out in concrete tanks with dimensions of 1.5 m x 1.5m x 1.2 m (length, breadth and depth). Five hundred healthy *C. gariepinus* with initial weight of approximately 2.5 g were purchased from a commercial farm and transported to the rearing facility. Prior to the onset of the trial, fish were acclimatized to the rearing conditions for 2 weeks and fed on commercial diet with protein content of 32%. At the onset of the trial, the fish were distributed into four groups with duplicates and fed their respective diets. Each tank was stocked with fifty fish.

Measurements of the biological indices

Feed utilization and growth performance were measured following formulas previously used by Mutalib et al. (2023).

Feed intake = The total amount of feed consumed (g) during the total number of trial days is the feed intake (FI).

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g).

Protein efficiency ratio (PER) = Wet weight gain (g) / Protein intake (g)

Weight gain (WG) = Final weight (g) – Initial weight (g)

Protein intake = protein content of feed x feed intake

Specific growth rate (SGR) (%) = (ln final weight (g) - ln initial weight (g)) /T × 100, where T is the number of feeding experimental (feeding) days

Blood biochemical analysis

After 10 weeks of feeding trial, blood from experimental fish were sampled as previously described by Abarike et al. (2018). Using a 2 mL disposable syringe, whole blood was drawn and then poured into two separate tubes—one for the liver and kidney toxicity test and the other for the haematological analysis—both of which contained EDTA to prevent the blood from clotting. The blood was subsequently sent to the laboratory of E-MED Diagnostic Services for the haematological parameters and plasma constituents to be determined using a haematological analyzer.

Proximate composition

Samples from whole body fish and experimental diets were sent to the laboratory for protein, lipid, moisture, and ash analyses. The AOAC (2003) standard methods, as reported by Ayisi et al. (2021), were employed to analyze the aforementioned parameters. Briefly, samples were oven dried at 105 °C to a constant weight to determine moisture content. Following digestion with concentrated H_2SO_4, crude protein was measured using the Kjeldahl method and a Kjeltec Auto 2300 Analyzer. Protein is expressed as a percentage of dry weight (% DW). The crude lipid content was measured by homogenizing the samples and extracting the lipids (Folch et al., 1957). The samples were burned at 550 °C for 4-6 hours in a muffle furnace to determine the amount of crude ash.

Histological examination

Six fish per group had sections of their intestines removed, which were then quickly preserved in a Bouin solution for histological examinations. Samples of tissue were promptly preserved in a Bouin solution. After that, fixed samples were subjected to a series of alcohol solutions with increasing grades (70–100 percent) to dehydrate them. Following the dehydration procedure, tissues underwent xylene deparaffinization, paraffin embedding, 5 µm sectioning, and Hematoxylin and Eosin (H&E) staining (Kim, 2013; Ismail, et al. 2020). A Leica light microscope was used to examine the slides, and an eyepiece camera was used to capture the images. ImageJ version 1.54 was used to determine the target cells in each section (National Institutes of Health, USA). An average of ten target cells per section served as the basis for each section's measurements.

Statistical analysis

The gathered data was statistically analyzed using Graph Pad Prism (V.5.03). The data is presented in tables using the standard error of the mean (SEM), which is represented as the mean ±. To compare treatment means, One-way Analysis of Variance and Tukey's multiple tests were applied to all the data. Differences are considered significant for all data at the 0.05 probability level ($P<0.05$). Pearson's correlation was conducted to ascertain the relationship between selected parameters. Results of correlation analysis is presented in figures.

Results

Effects of dietary protein sources on growth and feed utilization

Table 2 is a summary of the effects of different protein sources on the growth and feed utilization of *C. gariepinus*. FW and FL were significantly influenced by different protein sources (p = 0.0002 and 0.0044 respectively). The highest FW (122.0±68.12 g) and FL (25.07±4.00 cm) were observed in *C. gariepinus* fed diet SBH. Similarly, feeding *C. gariepinus* with diet SBH recorded the highest WG (119.5±68.02 g) and was significantly higher than *C. gariepinus* fed diet PKM with a WG of 86.73±31.51g (p<0.0001). There was no significant difference in feed intake (p = 0.1369). There was, however, a significant difference in feed conversion ratio (p=0.001). *C. gariepinus* fed with diets FM, CPH, SBH and PKM recorded FCRs of 1.27±0.53, 1.27±0.57, 1.25±0.57 and 1.52±0.56, respectively. There was no significant difference in protein intake (p = 0.9514). Protein intake (PI) ranged between 40.43±1.92 (FM) and 41.35±1.83 (PKM). *C. gariepinus* fed with diets CPH and SBH recorded PIs of 41.21±0.81 and 41.21±1.95, respectively. Protein efficiency ratio (PER) was significantly altered among groups (p = 0.0002). Feeding *C. gariepinus* with diet SBH recorded the highest PER (2.90±1.65) and was more significant than all other groups. The least PER (2.09±0.76) was observed in *C. gariepinus* fed with diet PKM, followed by diet FM (2.57±1.40) and diet CPH (2.74±1.28).

Table 2 Effects of different protein sources on growth and feed utilization of *C. gariepinus*

	FM	CPH	SBH	PKM	p-value
IW	2.65±0.96	2.53±0.79	2.56±1.01	2.50±0.79	0.6207
FW	106.9±56.84^{ab}	115.8±53.29^{b}	122.0±68.12^{b}	89.21±31.42^{a}	0.0002
FL	24.43±3.82^{ab}	24.84±3.56^{b}	25.07±4.00^{b}	23.31±2.95^{a}	0.0044
WG	104.3±56.82^{ab}	113.2±53.14^{b}	119.5±68.02^{b}	86.73±31.51^{a}	<0.0001
FI	105.0±5.00	116.3±2.30	116.0±9.53	115.7±5.13	0.1369
FCR	1.27±0.53^{a}	1.27±0.57^{a}	1.25±0.57^{a}	1.52±0.56^{b}	0.0013
SGR	5.18±0.80^{ab}	5.36±0.69^{ab}	5.43±0.83^{b}	5.08±0.69^{a}	0.0073
PI	40.43±1.92	41.21±0.81	41.21±1.95	41.35±1.83	0.9514
PER	2.57±1.40^{b}	2.74±1.28^{b}	2.90±1.65^{c}	2.09±0.76^{a}	0.0002

IW: Initial weight, FW: Final weight; FL: Final length; WG: Weight gain; FI: Feed intake; FCR: Feed conversion ratio; SGR: Specific growth rate; PI: Protein intake; PER: Protein efficiency ratio.

Proximate composition of whole body

A summary of the proximate composition of the whole body of *C. gariepinus* fed different protein sources is shown in Table 3. This study revealed a non-significant difference in protein content between the initial fish and the four experimental groups (p= 0.1441).

Table 3 Proximate composition of whole-body *C. gariepinus* fed different experimental diets

	Initial	FM	CPH	SBH	PKM	p-value
Moisture (% DM)	75.81±1.45	75.48±0.93	78.18±1.20	77.19±1.27	76.12±2.13	0.6769
Protein (% DM)	16.44±0.20	15.80±0.35	15.62±0.57	15.19±0.31	16.38±0.40	0.1441
Lipid (% DM)	10.17±0.26	9.05±0.24	9.88±0.30	9.84±0.32	9.88±0.21	0.0792
Ash (% DM)	3.78±0.38	3.93±0.28	3.68±0.22	3.64±0.24	3.78±0.38	0.9264

It is worth noting that the initial protein content was higher than that of the experimental groups. Protein content of fish fed diets FM, CPH, SBH, and PKM was

15.80±0.35, 15.62±0.57, 15.19±0.31, and 16.38±0.40, respectively. There was no significant difference in ash content (p = 0.9264). Whereas the initial fish had ash content of 3.78±0.38, *C. gariepinus* fed diet FM had ash content of 3.93±0.28. *C. gariepinus* fed diets CPH, SBH, and PKM recorded ash contents of 3.68±0.22, 3.64±0.24, and 3.78±0.38, respectively. Similarly, there was a non-significant difference in moisture content of *C. gariepinus* fed different protein sources, as well as the initial fish (p = 0.6769). The initial moisture content was 75.81±1.45, while that of *C. gariepinus* fed diets FM, CPH, SBH, and PKM was 75.48±0.93, 78.18±1.20, 77.19±1.27, and 76.12±2.13, respectively. The lipid content of experimental groups and the initial fish did not vary significantly (p = 0.0792). Lipid content as recorded in this study ranged between 9.05±0.24 and 10.17±0.26.

Hematology

Table 4 shows hematology parameters of *C. gariepinus* fed four different protein sources. To a larger extent, different protein sources affected hematological parameters of catfish. In this study, WBC, RBC, HGB, HCT, MCH, PDW and MCHC of *C. gariepinus* were significantly altered by different protein sources ($p < 0.05$). Similarly, RDW, LYM, and GRA were all significantly altered by different protein sources ($p < 0.05$). There was however, no significant differences in MON, PCT, MVP, and PLT ($p > 0.05$). The highest WBC (133.0±2.51) was recorded in *C. gariepinus* fed diet FM and was significantly higher than all other groups ($p < 0.0001$). *C. gariepinus* fed diets CPH, SBH, and PKM recorded WBC of 107.6±3.44, 108.6±2.84, and 109.0±4.93, respectively. RBC recorded in this study ranged between 2.08±0.13 and 2.62±0.13. The least RBC was recorded in *C. gariepinus* fed diet and was significantly lower than *C. gariepinus* fed diets CPH and PKM (p = 0.0133). RBC recorded in this study ranged between 2.08±0.13 (FM) and 2.62±0.13 (CPH). HGB was induced in *C. gariepinus* fed diet SBH (8.66±0.29) and was significantly higher than *C. gariepinus* fed diet FM which recorded HGB of 6.47±0.19 ($p < 0.0001$). Also, feeding *C. gariepinus* with diet CPH recorded GRA level of 2.82±0.14 and was significantly lower than *C. gariepinus* fed diet FM (< 0.0001). Feeding *C. gariepinus* with plant protein sources led to elevated levels of LYM and were all significantly higher than *C. gariepinus* fed diet FM ($p < 0.0001$). LYM as recorded in this study ranged between 88.49±1.84 (FM) and 110.2±2.47 (SBH). RDW, MCHC, and PDW ranged from 5.21±0.28 to 8.00±0.25, 28.73±0.67 to 35.77±0.95, and 7.33±0.14 to 9.58±0.48, respectively. In the case of PDW and MCHC, the least values were recorded in *C. gariepinus* fed diet FM with the highest recorded in *C. gariepinus* fed SBH. In the case of RDW, the highest was recorded in *C. gariepinus* fed diet FM and the least observed in *C. gariepinus* fed SBH. There was significant difference in HCT, MCV, and MCH levels. Higher levels of HCT and MCH were observed in *C. gariepinus* fed plant protein diets (CPH, SBH, and PKM) and were significantly higher than *C. gariepinus* fed FM ($p < 0.05$).

Kidney function test

Table 5 shows kidney function of *C. gariepinus* fed diets with different protein sources. With the exception of serum potassium (p = 0.0005) and chloride (p = 0.0047), there were no significant modifications in creatinine (p = 0.3945), urea (p = 0.8884) or sodium (p = 0.4724). C. gariepinus fed diets with SBH and PKM diets recorded the highest concentrations of potassium (6.04±0.23 and 6.09±0.28, respectively), while the highest chloride concentrations were observed in C. gariepinus fed diets FM (102.7±3.58) and CPH (109.2±11.31). Creatinine concentration ranged between 33.25±2.49 and 39. 21±5.20, while urea ranged between 2.79±0.26 and 3.00±0.19.

Table 4 Erythrogram of *C. gariepinus* fed four different protein sources.

	FM	CPH	SBH	PKM	p-value
WBC	133.0±2.51^{b}	107.6±3.44^{a}	108.6±2.84^{a}	109.0±4.93^{a}	< 0.0001
RBC	2.08±0.13^{a}	2.62±0.13^{b}	2.50±0.14^{ab}	2.61±0.06^{b}	0.0133
HGB	6.47±0.19^{a}	8.06±0.21^{b}	8.66±0.29^{b}	8.21±0.35^{b}	< 0.0001
HCT	27.43±1.19^{a}	31.26±0.79^{b}	32.99±0.78^{b}	32.74±1.07^{b}	0.0011
MCV	138.3±1.82^{b}	113.1±3.89^{a}	116.6±4.66^{a}	117.4±3.46^{a}	< 0.0001
MCH	35.43±0.66^{a}	42.71±0.92^{b}	44.18±1.05^{b}	43.13±1.22^{b}	< 0.0001
PDW	7.33±0.14^{a}	9.25±0.34^{b}	9.58±0.48^{b}	7.63±0.34^{a}	< 0.0001
MCHC	28.73±0.67^{a}	34.86±0.47^{b}	35.77±0.95^{b}	34.29±0.88^{b}	< 0.0001
RDW	8.00±0.25^{c}	5.65±0.21^{ab}	5.21±0.28^{a}	5.80±0.27^{b}	< 0.0001
PLT	46.74±1.46	48.50±2.63	42.80±1.64	49.58±1.72	0.0862
MVP	6.10±0.22	5.12±0.18	5.38±0.31	5.68±0.37	0.1102
GRA	4.63±0.16^{c}	2.82±0.14^{a}	2.85±0.22^{a}	3.28±0.23^{b}	< 0.0001
LYM	88.49±1.84^{a}	103.8±2.97^{b}	110.2±2.47^{b}	109.9±2.98^{b}	< 0.0001
PCT	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.0898
MON	14.59±0.27	15.31±0.50	15.81±0.51	14.60±0.63	0.2552

Table 5 Variation in the kidney function test of blood of *C. gariepinus* fed different protein sources.

	FM	CPH	SBH	PKM	p-value
Creatinine	33.25±2.49	35.68±3.57	39.21±5.20	34.92±5.98	0.3945
Urea	2.89±0.24	2.79±0.26	3.00±0.15	3.00±0.19	0.8884
Sodium	168.7±8.44	159.5±19.77	157.4±21.43	159.5±19.63	0.4724
Potassium	5.16±0.36^{ab}	4.31±0.33^{a}	6.04±0.23^{b}	6.09±0.28^{b}	0.0005
Chloride	102.7±3.58^{ab}	109.2±11.31^{b}	95.41±6.06^{a}	99.05±11.10^{a}	0.0047

Liver function

Table 6 represents variations observed in the liver function of *C. gariepinus* fed different protein sources. AST and ALT levels were enhanced in *C. gariepinus* fed plant protein sources. The highest AST (121.7±6.71) and ALT (109.5±10.36) were observed in *C. gariepinus* fed diet CPH, with the least AST (82.13±4.77) and ALT (77.73±7.49) observed in *C. gariepinus* fed diet FM. Both AST and ALT were significantly different amongst groups (p = 0.0001 and 0.0140, respectively). There was no significant alteration in ALP (0.3750), irrespective of protein source. Feeding *C. gariepinus* with diet CPH recorded a GGT of 2.63±0.30, and was significantly higher than *C. gariepinus* fed diets SBH and PKM. In all cases, the highest concentrations of TB (0.93±0.11; p=<0.0001), DTB (0.69±0.12; p=0.0001), IDTB (0.35±0.07; p=0.0159) and TP (41.52±9.09; p=0.0002) were observed in *C. gariepinus* fed diet PKM. There was a significant difference in ALB levels among treatments (p=0.0039). It is worth noting that the highest concentration of ALB (16.42±3.42) was recorded in *C. gariepinus* fed diet SBH.

Table 6 Variation in the liver function test of blood of *C. gariepinus* fed different protein sources.

	FM	CPH	SBH	PKM	p-value
AST	82.13±4.77^{a}	121.7±6.71^{b}	114.4±12.54^{b}	109.2±7.18^{b}	0.0001
ALT	77.73±7.49^{a}	109.5±10.36^{b}	92.19±5.03^{ab}	82.0±3.30^{a}	0.0140
ALP	16.80±5.05	16.62±3.74	14.15±3.73	16.15±2.82	0.3750
GGT	2.00±0.30^{a}	2.63±0.30^{b}	1.54±0.15^{a}	1.81±0.18^{a}	0.0237
TB	0.48±0.04^{a}	0.40±0.03^{a}	0.64±0.05^{a}	0.93±0.11^{b}	<0.0001
DTB	0.29±0.02^{a}	0.26±0.02^{a}	0.43±0.08^{ab}	0.69±0.12^{b}	0.0001
IDTB	0.18±0.03^{a}	0.15±0.02^{a}	0.26±0.02^{ab}	0.35±0.07^{b}	0.0159

TP	31.40±4.59^{b}	27.18±8.83^{a}	38.61±6.63^{bc}	41.52±9.09^{c}	0.0002
ALB	13.35±3.49^{a}	12.15±2.05^{a}	16.42±3.42^{b}	15.75±2.37^{ab}	0.0039

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transferase; TB: Total bilirubin; DTB: Direct total bilirubin; IDTB: Indirect total bilirubin; TP: Total protein; ALB: Albumin

Intestinal histology

Table 7 and figure 1 summarizes changes in the mid-intestinal structures of *C. gariepinus* fed different protein sources. Villus height, villus width, and muscular thickness were significantly altered by protein source. The highest villus height was in fish fed diet FM (441.2±22.6), followed by SBH (398.3±7.51), PKM (279.2±15.65), and CPH (142.3±10.84). This pattern was also observed for villus width and muscular thickness, with the highest villus width (153.7±9.06) in fish fed diet FM, significantly higher than other groups (p<0.0001).

Table 7 Mid-intestinal histopathological examination of *C. gariepinus* fed diets with different protein sources

Item (μm)	FM	CPH	SBH	PKM	p-value
Villus height	441.2±22.6^{d}	142.3±10.84^{a}	398.3±7.51^{c}	279.2±15.65^{b}	<0.0001
Villus width	153.7±9.06^{b}	56.87±9.45^{a}	69.42±3.65^{a}	41.59±5.40^{a}	<0.0001
Muscular thickness	114.2±2.925^{d}	54.96±4.15^{a}	96.28±5.23^{c}	71.51±1.773^{b}	<0.0001

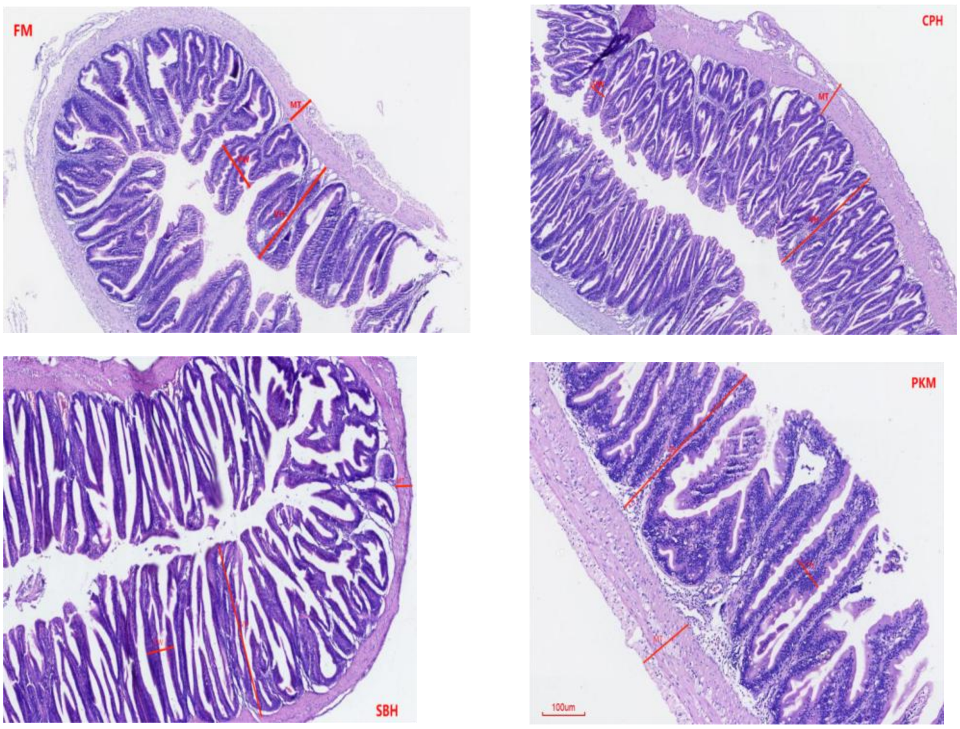

Fig 1. Morphological appearance of the mid intestines of *C. gariepinus* fed different plant protein diets (FM, CPH, SBH and PKM) under the light microscope. All photos were taken at 40X; the scale bar represents 100 µm.

Correlation between growth, feed utilization and serum biochemical parameters

Fig 2 shows correlation linear (R) Pearson correlation between growth, feed utilization and serum biochemical parameters. PDW correlated significantly with growth and feed utilization parameters. PDW correlated positively with FCR (0.005), and negatively with WG (0.024), ADWG (0.0247), PER (0.0247), and FW (0.0244). There was however no significant correlation between other serum biochemical parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, GRA, LYM and PCT) and growth performance, and feed utilization.

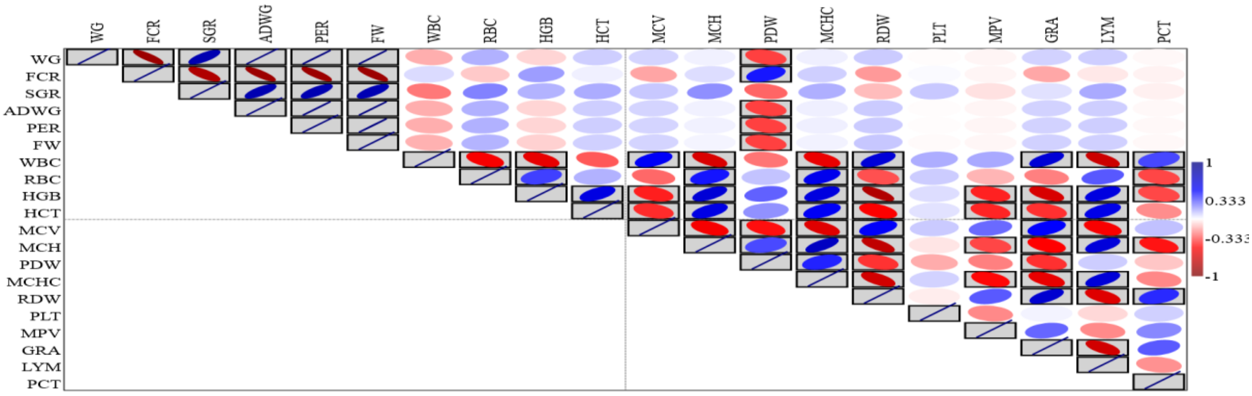

Fig 2. Correlation between growth, feed utilization and serum biochemical parameters

Correlation between growth, feed utilization and changes in intestinal morphology

Figure 3 shows correlation between growth, feed utilization and changes in intestinal morphology. No significant correlation was observed between intestinal structures (villi height, villi width and muscular thickness) and growth performance, and feed utilization.

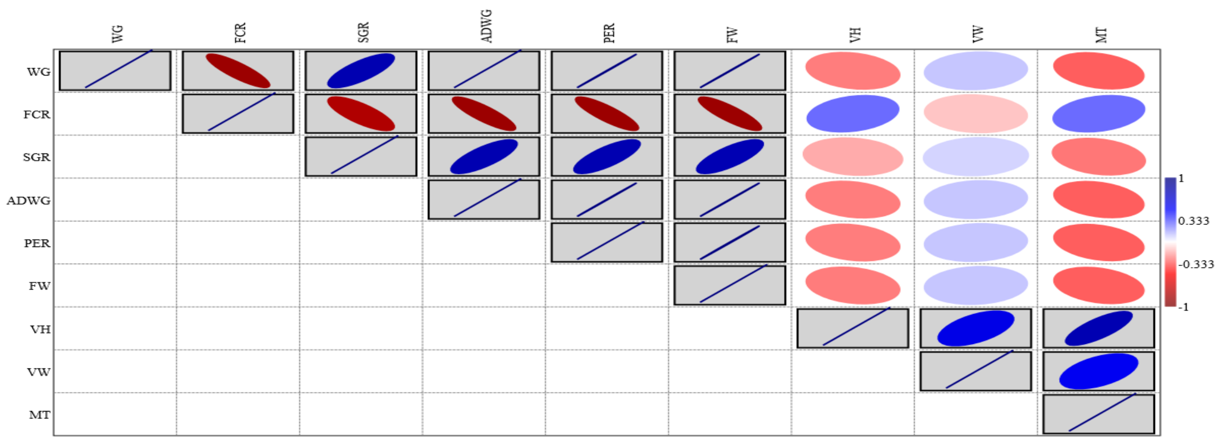

Table 8 shows P-values and R-values for Pearson correlation between intestinal structures and growth and feed utilization. There was a moderate positive correlation between villus width and ADWG (r = 0.4969, p = 0.1106), weight gain (r = 0.4969, p = 0.1106), and PER (r = 0.4969, p = 0.1106). Also, similar observation (moderate positive correlations) was observed between muscular thickness and ADWG (r = 0.0510), PER (r = 0.0510), and FW (r = 0.0516). Notably, villus height showed negative, though non-significant, correlations with several parameters including weight gain FCR (r = 0.0748, p = 0.2886), (r = 0.1197, p = 0.2533), and SGR (r = 0.3180, p = 0.1641).

Table 8 P-values and R-values for Pearson correlation between intestinal structures and growth and feed utilization.

Parameter	P-value	R-value
Weight gain and Villus height	-0.25332	0.1197
Weight gain and Villus width	0.11059	0.4969
Weight gain and muscular thickness	-0.31467	0.0510
FCR and Villus height	0.28857	0.0748
FCR and Villus width	-0.11235	0.4900
FCR and muscular thickness	0.28831	0.0750
SGR and Villus height	-0.16413	0.3180
SGR and Villus width	0.08291	0.6110
SGR and muscular thickness	0.26546	0.1024
ADWG and Villus height	-0.25332	0.1197
ADWG and Villus width	0.11059	0.4969
ADWG and muscular thickness	0.31467	0.0510
PER and Villus height	-0.25332	0.1197
PER and Villus width	0.11059	0.4969
PER muscular thickness	0.31467	0.0510
FW and Villus height	-0.25491	0.1173
FW and Villus width	0.11103	0.4952
FW muscular thickness	-031388	0.0516

Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), average daily weight gain (ADWG), protein efficiency ratio (PER), and final weight (FW).

Discussion

Contrasting results have been reported with respect to the effects of substituting fish meal with plant-based protein in diets of different species (Cheng et al., 2010; Bu et al., 2018; Dossou et al., 2018; Al-Thobaiti et al., 2018; Egerton et al., 2020; Kari et al., 2021; Cai et al., 2022; Roslan et al., 2024; Serrano et al., 2024). This study reports that FL, FW, WG, SGR, ADWG and FCR were significantly altered by the source of protein, with *C. gariepinus* fed with CPH and SBH performing better. This is in agreement to previous studies that reported that substituting FM with plant-based meals did not compromise growth (Sheikhzadeh et al., 2012; Kpundeh et al., 2015; Guo et al., 2016). It is not surprising SBH performed better than the control diet and PKM. This is because soy products, with their high digestibility, high protein content, and well-balanced amino acid composition, have long been used as the most effective substitute for fish-meal in aquaculture diets (Macusi et al., 2023).

In order to estimate the amount of feed required for the fish's growth cycle, the feed conversion ratio (FCR) is a crucial metric for aquaculture farmers to calculate the activity's profit margin (Kari et al., 2021). Based on actual feeding practices, the FCR can be used to predict the feed cost (Roslan et al., 2024). The FCR (1.25±0.57) of *C. gariepinus* fed SBH diets was the highest.

This suggests that this group was able to utilize feed more effectively (Aglago et al., 2021). This finding suggests that the best course of action for African catfish future growth in the aquafeed industry may be to replace FM with SBH. Serrano et al. (2024) observed no significant alteration in PER when FM was replaced with Anean lupin in diets of Rainbow trout.

When introducing a new feed, it is crucial to investigate the proximate composition of body tissue (Rani et al., 2016; Roslan et al., 2024). There were no discernible changes in proximate composition of this study. The absence of a significant difference between the dietary groups implies that FM, CPH, SBH, and PKM were successfully incorporated into *C. gariepinus* diets to achieve nutritional balance. The non-significant differences in whole body proximate composition observed in this study is consistent with earlier findings that no appreciable changes in proximate composition were observed in summer flounder (Enterria et al., 2011) or blunt snout bream (Ahmed et al., 2019) fed varying protein sources. Similarly, there was no discernible change in the proximate composition of rockfish when plant protein sources were substituted for FM in their diets (Kim and Cho, 2024). Also, there was no significant alteration in the proximate composition of the whole body when *C. gariepinus* was fed diets with fish meal replaced by plant protein (Nasr et al., 2021). In contrast to this study, the proximate composition of pearl gentian grouper (Chen et al., 2019) and red drum (Minjarez-Osorio et al., 2016) changed when different plant-based protein sources were substituted for fish meal.

The age or size of the fish as well as the varying rates of nutrient deposition in tissues among various species are the reason for the variations observed in the effects of replacing FM with different plant-based proteins as reported in different studies (Mzengereza et al., 2022).

The activities of aminotransferases, such as γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), catabolize amino acids and transfer amino groups to α-keto acids. These processes also mirror the balance of amino acids in the diet (Cheng et al., 2010). AST and ALT are known to be important indicators for protein metabolism and/or ammonia excretion (Zhang et al., 2019). The transaminases (ALT and AST) and ALP catalyze trans-amination reactions to help the body metabolize other macromolecules and xenobiotics. They also regulate physiological functions. As a result, changes in their behavior enable the immediate detection of harm to the liver and kidney functions and serve as indicators of the organisms' ability to maintain the integrity of these tissues (Bharti and Rasool, 2021). As a result, we also looked at the enzyme activity (IU/L) of ALT, AST, and ALP in the serum of *C. gariepinus* that were fed diets varying in protein sources in this study. In this study, substituting fish oil with plant protein sources (Palm kernel meal, Soybean husk and Cowpea husk) resulted in an increase of AST concentration. This is in line with previous report by Zhang et al (2020) which documented an increase in AST levels when fish meal was replaced with rapeseed meal in diets for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*). Feeding *C. gariepinus* with plant-based protein diets led to an increase in both ALT and AST. The heightened levels of ALT and AST in these groups could potentially result from altered protein catabolism metabolism or hepatic cell membrane lysis, which allows these enzymes to seep into the fish's bloodstream (Bharti and Rasool, 2021). The hepatic cell's degeneration could be the cause of the rise in AST (Abalaka, 2013). These cytoplasmic serum enzymes are only released into the bloodstream following the damage that has been done to these cells (Jafarpour, and Fard., 2016). Elevated levels of ALT and AST in the plasma are indicative of liver impairment and cellular or hepatocellular damage resulting from anti-nutritional factors (ANFs) present in plant-based ingredients in the liver, heart, or muscle (Kari et al., 2021).

Contrary to this study, Cai et al. (2022) observed no significant differences in the concentration levels of TP when gibel carp were fed diets with different protein compositions. The increase in total protein as observed in *C. gariepinus* fed SBH and PKM could be due to an increase in the non-specific immune response (Mohamad and Abasali, 2010). When diagnosing different liver and kidney diseases, albumin is required. Albumin, the most prevalent protein in blood plasma, serves as a transporter for a variety of compounds in circulation, thereby maintaining the health of the body (van de Wouw and Joles, 2022). In this investigation, ALB levels were raised when *C. gariepinus* was fed SBH and PKM. However, *C. gariepinus* fed CPH showed a marked decline. As noted by Andrews et al. (2011), a decrease in some essential plasma levels may be the cause of this drop in ALB levels.

In this study, feeding *C. gariepinus* with plan-based protein generally led to hyperbilirubinemia blood conditions. An obstruction of the bile ducts is most likely the cause of the hyperbilirubinemia (Bharti and Rasool, 2021). The haematological variable known as gamma-glutamyltransferase (GGT) enzyme is clinically measured as a biomarker for liver health (Rastiannasab et al., 2016). GGT modifies and breaks down movable proteins. The ability of *C. gariepinus* fed diet CPH to catalyze the transfer of glutathione's gamma-glutamymoiety to an acceptor, which could be an amino acid, peptide, or water, may be explained by the higher GGT content seen in this group (Adamu and Nwadukwe, 2013).

Haematological variables such as white blood cells (WBC), red blood cells (RBC), hemocrit (Hct) and hemoglobin (Hb) are increasingly being used to monitor fish health (Maftuch, 2018). WBC in blood aid in an organism's immune responses; RBC controls the amount of oxygen delivered to bodily tissues; Hb is essential for metabolism; and haematocrit (Ht) establishes the volume of RBC (Esmaeili, 2021). According to Fagbenro et al. (2010), blood analysis is an important indicator for assessing the physiological condition of cultured fish as well as the effects of diets and other stressors on the health of fish. Hematological test results can be impacted by food, feeding schedule, age, sex, and environmental factors (Teixeira et al., 2000; Ibrahim et al., 2022). The assessment of various physical conditions in fish is frequently done through hemato-biochemical studies (Pradhan et al., 2012; Sharmin et al., 2016; Jahan et al., 2019; Billah et al., 2022; Howlader et al., 2023). Feeding *C. gariepinus* with plant protein sources (CPH, SBH, and PKM) led to lower levels of RBC. This is in agreement with a previous report that suggested that higher levels of soybean meal fed to beluga lowered RBC (Hosseini and Khajepour, 2013). *C. gariepinus* fed diet SBH recorded the highest HGB in the blood. Better oxygen transport in tissues leading to improved growth may be the cause of the highest level of HGB seen in C. gariepinus fed diet (Esmaeili, 2021; Hossain et al., 2022; Howlader et al., 2023). One of the most important indicators of fish health is WBC count (Khieokhajonkhet et al., 2023). In this study, feeding *C. gariepinus* with plant-based protein lowered WBC levels in the blood and could be inferred to have compromised the cellular defense of *C. gariepinus*. According to Clauss et al. (2008), an increase in WBC counts within the normal range indicates that fish have stronger cellular defenses against infections. There was significant alteration on levels of HGB, HCT, MCHC, MCV, and MCH in this study. This is contrary to earlier report that documented no significant differences in theses parameters when fish meal was replaced with kernel meal and sunflower meal (Kumar et al., 2010; Rahmdel et al., 2018). Replacing FM with SBH, CPH and PKM significantly altered HCT levels. This indicates that *C. gariepinus* suffered negative effects when FM was substituted with these plant protein sources because higher hematocrit values are thought to pose a risk to farmed fish's health (Hardy, 2010). However, as previously noted by Lall and Kaushik (2021), the lower level of haematocrit seen in *C. gariepinus* fed FM diet is indicative of the inhibitory impact of phytate and glucosinolates on the bioavailability of important minerals for cell synthesis, such as iron and zinc.

The hemoglobin level in each red blood cell is known as the mean corpuscular hemoglobin concentration (MCHC), and it can be used to measure animal anemia (Habotta et al., 2022; Zakaria et al., 2022). A MCHC value of less than 28 g/dL frequently denotes anemia. All groups' MCHC values in this study ranged from 28.73 to 35.77 g/dL, indicating that none of the experimental fish were anaemic. There was an increase in MCHC and MCH levels in *C. gariepinu* fed plant-based diets. Elevation of MCH, a hemoglobin marker in erythrocytes, and MCHC suggest that feeding *C. gariepinus* plant-based diets may affect hematopoiesis and damage red blood cells in circulation (Hassan et al., 2023). The highest mean corpuscular volume (MCV) content was observed in *C. gariepinus* fed FM diet. This may be due to the inhibition of erythropoiesis which to a larger extent is confirmed by the least RBC observed in this study (Ahmed and Ahmad, 2020).

In this study, there was no significant difference in levels of creatinine and urea and is similar to previous report by Nasr et al. (2021). With the exception of ALP (p= 0.3750), all other parameters were significantly different. In this study, substituting fish oil with plant protein sources (palm kernel meal, soybean husk and cowpea husk) resulted in an increase of AST concentration. This collaborates with previous report by Zhang et al (2020) which reported an increase in AST levels when fish-meal was replaced with rapeseed meal in diets for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*). The hepatic cell's degeneration could be the cause of the rise in AST (Abalaka, 2013). These cytoplasmic serum enzymes are only released into the bloodstream following the damage that has been done to these cells (Jafarpour, and Fard., 2016). Contrary to this study, Cai et al. (2022) observed no significant differences in the concentration levels of TP when gibel carp were fed different diets with different protein compositions. In this study, there was a decrease in GGT concentration when C. Gariepinus was fed SBH and could imply healthy liver. This is because, a decrease in Gamma-glutamyl transferase (GGT) concentration in plasma may have a positive impact on liver health (Bongiorno et al., 2022).

A metabolic waste product excreted with urine, creatinine levels rise in blood when renal malfunction occurs (Bharti and Rasool, 2021). Elevated levels of urea and creatinine may be caused by decreased kidney clearance or renal tissue breakdown, glomerular dysfunction, or both (Amin and Hashem, 2012). In this study, there was no significant elevation of urea and creatinine levels and signify no renal malfunction. The non-significant effects of different protein sources on creatinine levels observed in this study is comparable to previous report by Ismail, et al. (2020) when fish mean was replaced with soybean or/and corn gluten meal in Nile tilapia (*Oreochromis niloticus*) diets.

When introducing a novel feed, it is important to evaluate the length and width of the villus in order to gain an understanding of the processes involved in the digestion and absorption of proteins (Roslan et al., 2024). During feeding trials, the histological structures of the digestive organ are assessed to provide crucial information on the fish's capacity to digest food as well as potential health effects of the new diets (Dzifa et al., 2022). The measurement of intestinal villi height, muscle layer thickness, and goblet cell count are used to assess the health of the intestines, including absorptive capacity and digestive functions (Khojasteh, 2012; Pirarat et al., 2011). A decrease in the height and width of intestinal villi as well as muscular thickness as observed in fish fed CPH, SBH as well as PKM diets may indicate a reduction in the digestive tract's absorption area and nutrient absorption (Mahmoud et al., 2020). The reduction in VH, VW and MT as observed in this study may also be attributed to the presence of anti-nutritional factors in the plant-based protein sources which could lead to lots of deteriorations in intestinal morphology (Zhang et al., 2013). The reduced villus length as observed in *C. gariepinus* fed diets CPH, SBH and PKM as compared to FM could imply the inability of *C. gariepinus* to adapt and protect the guts as a response to the anti-nutritive factors in plant-based protein sources (Mzengereza et al., 2020). The perceived lower nutrient absorption did not correlate positively with growth and nutrient utilization and call for further studies to elucidate the clear relationship. This study is in agreement with previous studies that reported decrease in villi height and width when FM were replaced with plant-based protein sources (Roslan et al., 2024). On the contrary, earlier reports showed that substituting FM with plant-based protein in diets of fish resulted in an increase in the length of villus (Ur´an, et al., 2008; Bansemer et al., 2015; Matuli´c et al., 2020). Kari et al. (2021) observed that histopathological changes of the intestines may vary depending on the feed utilized in the experiments and the species under consideration and that might have accounted for the variation in impacts of diets in different studies. The correlation analysis of the histological structures of the intestines and growth, and feed utilization observed in this study did not have any influence on growth and feed utilization.

Conclusion

This study concludes that inclusion of soybean husk as alternative protein sources had significant positive effect on growth performance or feed utilization compared to fish meal diet. Also, feeding *C. gariepinus* with plant-based protein sources significantly altered haematological parameters, function of the liver and kidney. Histological examination, also, indicated significant modifications in tissue morphology, pointing to possible metabolic adjustments to the novel dietary formulations. All things considered, adding PKM, CPH, and SBH to catfish diets as protein sources seems like a viable replacement for conventional fishmeal-based diets; however, more study is required to determine the ideal levels of inclusion for optimum development and well-being as well as their comprehensive effects on health and nutritional composition of fish.

References

Abalaka, S.E. (2013). Evaluation of the haematology and biochemistry of Clarias gariepinus as biomakers of environmental pollution in Tiga dam, Nigeria. Br. Arch. *Biol. Technol., 56,* 371-376.

Abarike, E.D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., Tang, J., Jun, L., & Kuebutornye, F.K.A. (2018). Effects of a commercial probiotic BS containing Bacillus subtilis and Bacillus licheniformis on growth, immune response and disease resistance in Nile tilapia, Oreochromis niloticus. *Fish & Shellfish Immunology, 82*, 229-238. https://doi.org/10.1016/j.fsi.2018.08.037.

Adamu, K.M., & Nwadukwe, F.O. (2013). Growth And Physiological Profiles Of Hybrid Catfish Fed Practical Diet In Different Water Regimes. *Wayamba Journal of Animal Sciences, 13*,1377084656

Aglago, A., Ayisi, C.L., & Ampofo-Yeboah, A. (2021). Effect of stocking density of juvenile Bagrid catfish *Chrysichthys nigrodigitatus* (Siluriformes: Claroteidae) on growth performance, feed utilization, proximate composition and water quality. *Iran. J. Ichthyol., 8*(4): 294-302

Ahmed, M., Liang, H., Chisomo Kasiya, H., Ji, K., Ge, X., Ren, M., Liu, B., & Zhu, X. (2019). Complete replacement of fish meal by plant protein ingredients with dietary essential amino acids supplementation for juvenile blunt snout bream (*Megalobrama amblycephala*). *Aquac. Nutr. 25*, 205-214. doi: 10.1111/anu.12844

Al-Thobaiti, A., Al-Ghanim, K., Ahmed, Z., Sulimana, E.M., & Mahboob S. (2018). Impact of replacing fish meal by a mixture of different plant protein sources on the growth performance in Nile Tilapia (*Oreochromis niloticus* L.) diets. *Braz. J. Biol., 78*(3): 525-534

Amin, K.A. and Hashem, K.S. (2012). Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. *BMC Vet. Res. 8*: 45.

Andrews, S.R., Sahu, N., Pal, A., Mukherjee, S., & Kumar, S. (2011). Yeast extract, brewer's yeast and spirulina in diets for Labeo rohita fingerlings affect haematoimmunological responses and survival following *Aeromonas hydrophila* challenge. *Res. Vet. Sci. 91*, 103-109.

AOAC. (2003). Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Washington D.C., USA.

Ayisi, C.L., Alhassan, E.H., & Sarfo, F. (2021). Substitution Of Fish Oil With Palm Kernel Oil In Diets Of *Oreochromis Niloticus* Fry: Effects On Growth, Feed Utilization And Economic Estimates. *Indonesian Aquaculture Journal, 16* (2), 99-107.

Ayisi, C.L., Zhao, J. & Rupia, E.J. (2017). Growth performance, feed utilization, body and fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing elevated levels of palm oil. *Aquaculture and Fisheries, 2*(2), 67-77. https://doi.org/10.1016/j.aaf.2017.02.001

Bansemer, M.S., Forder, R.E.A., Howarth, G.S., Suitor, G.M., Bowyer, J., & Stone, D.A.J. (2015). The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritis in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperature. *Aquac. Nutr. 21*, 300–310. https://doi.org/10.1111/anu.12160.

Bharti, S., & Rasool, F. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. T*oxicology Reports 8*:443–455

Billah S.M., Sumi K.R., Howlader S., Sarkar S., Ferdous Z., Islam S.M., & Shahjahan M. (2022). Effects of supplemental L-methionine for total replacement of fish meal by soybean meal on growth, feed utilisation and health status of stinging catfish, Heteropneustes fossilis fry. *Aquacult. Fish Fish. 2022*:1–9.

Bongiorno, V., Gariglio, M., Zambotto, V., Cappone, E.E., Biasato, I., Renna, M., Forte, C., Coudron, C., Bergagna, S., Gai, F., & Schiavone, A. (2022). Black soldier fly larvae used for environmental enrichment purposes: can they affect the growth, slaughter performance, and blood chemistry of medium-growing chickens? *Front. Veterinary Sci. 9*, 1064017. https://doi.org/10.3389/fvets.2022.1064017

Botello-León, A., Martínez-Aguilar, Y., Viana, M. T., Ortega-Ojeda, M., Morán-Montaño, C., Pérez-Corría, K., Méndez-Martínez, Y., & Velázquez-Martí, B. (2022). Efecto del palmiste en la nutrición de alevines de tilapia (Oreochromis niloticus). *Revista MVZ Córdoba*, *27*(2), e2527. https://doi.org/10.21897/rmvz.2527

Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., ... & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society, 51*(3), 578-633.

Bu, X.Y., Wang, Y.Y., Chen, F.Y., Tang, B.B., Luo, C.Z., Wang, Y., Ge, X.P., &Yang, Y.H. (2018). An evaluation of replacing fish-meal with rapeseed meal in the diet of *Pseudobagrus ussuriensis*: growth, feed utilization, non-specific immunity, and growth related gene expression. *J. World Aquac. Soc. 49*, 1068–1080.

Cai W, Liu H, Han D, Zhu X, Jin J, Yang Y & Xie S. (2022). Complete Replacement of Fishmeal With Plant Protein Ingredients in Gibel Carp (*Carassius auratus* gibelio) Diets by Supplementation With Essential Amino Acids Without Negative Impact on Growth Performance and Muscle Growth-Related Biomarkers.Front. Mar. Sci. 8:759086. doi: 10.3389/fmars.2021.759086

Chen, Y., Ma, J., Huang, H., & Zhong, H. (2019). Effects of the replacement of fish-meal by soy protein concentrate on growth performance, apparent digestibility, and retention of protein and amino acid in juvenile pearl gentian grouper. *PloS One 14*, e0222780. doi: 10.1371/journal.pone.0222780.

Cheng, Z.Y., Ai, Q.H., Mai, K.S., Xu, W., Ma, H.M., Li, Y., & Zhang, J.M. (2010). Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture 305*, 102-108

Clauss T., Dove A., & Arnold, J. (2008). Hematologic disorders of fish. *Veterinary Clinics of North America Exotic Animal Practice, 11*(3):445-462.

Dauda, A. B., Dasuki, A., Oshoke, J. O., Nababa, A. S., & Bello, O. M. (2023). Status of Fish Farming in Katsina State, Nigeria: The Current Practices, Operation, Management and Constraints to its Development. *Fudma Journal of Agriculture and Agricultural Technology*, *9*(4), 9-17.

Docan, A., Grecu, I., & Dediu, L. (2018). Use of hematological parameters as assessment tools in fish health status. *J. Agroaliment. Process. Technol, 24*(4), 317-324.

Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A.O., El Basuini, M.F., Olivier, A., & Zaineldin, A.I. (2018). Growth performance, blood health, antioxidant status and immune response in red sea bream (*Pagrus major*) fed Aspergillus oryzae fermented rapeseed meal (RM-Koji). *Fish Shellfish Immunol. 75*, 253–262.

Dzifa, M.G., Ayisi, C.L., & Alhassan, E.H. (2022). Substitution of fish Meal with Shea Nut Meal in Diets of Nile Tilapia Fry on Growth, Feed Utilization, Tissue histology and Economic Analysis. *Pakistan Journal of Zoology, 54*(3): 1385.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J., McGinnity, P., Culloty, S., Ross, R.P., & Stanton, C. (2020). Replacing fish-meal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. *Sci Rep 10*, 4194. https://doi.org/10.1038/s41598-020-60325-7

Enterria, A., Slocum, M., Bengtson, D. A., Karayannakidis, P. D., & Lee, C.M. (2011). Partial replacement of fish meal with plant protein sources singly and in combination in diets for summer flounder, *Paralichthys dentatus*. *J. World Aquac. Soc 42*, 753–765. doi: 10.1111/j.1749-7345.2011.00533.x

Esmaeili, M. (2021). Blood performance: a new formula for fish growth and health. *Biology 10*, 1–17, https://doi.org/10.3390/biology10121236.

Fagbenro, O., Adeparusi, E., & Jimoh, W. (2010). Haematological profile of blood of African catfish (*Clarias gariepinus*, Burchell 1822) fed sunflower and sesame meal-based diets. *J Fish Aquat Sci 8*, 80–86.

Folch, J., Lees, M., Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem. 226* (1), 497–509.

Gorissen, S. H., Crombag, J. J., Senden, J. M., Waterval, W. H., Bierau, J., Verdijk, L. B., & van Loon, L. J. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino acids, 50*, 1685-1695.

Guo, J., Guo, B., Zhang, H., Xu, W., Zhang, W. & Mai, K. (2016). Effects of nucleotides on growth performance, immune response, disease resistance and intestinal morphology in shrimp Litopenaeus vannamei fed with a low fish meal diet. Aquac. Int. https://doi.org/10.1007/s10499-015-9967-7.

Gutasi, A. (2021). Benefit and drawbacks of fish meal substitution in aquaculture diets. (Diploma Thesis, University of Veterinary Medicine Vienna).

Habotta, O.A., Dawood, M.A., Kari, Z.A., Tapingkae, W., &Van Doan, H. (2022). Antioxidative and immunostimulant potential of fruit derived biomolecules in aquaculture. *Fish Shellfish. Immunol., 130*, 317–322.

Hardy, R.W. (2010). Utilization of plant proteins in fish diets: Effects of global demand and supplies of fish-meal. *Aquac. Res., 41*, 770–776.

Hassan, M., Melad, A.A.N., Yusoff, N.A.H., Tosin, O.V., Norhan, N.A-S., & Hamdan, N.A. (2023). Melaleuca cajuputi leaf extract accelerates wound healing in African catfish, *Clarias gariepinus*. *Aquaculture Reports, 31*, 101682. https://doi.org/10.1016/j.aqrep.2023.101682.

Hossain, M.K., Hossain, M.M., Mim, Z.T., Khatun, H., Hossain, M.T., & Shahjahan, M., (2022). Multi-species probiotics improve growth, intestinal microbiota and morphology of Indian major carp mrigal *Cirrhinus cirrhosus*. *Saudi J. Biol. Sci. 9*, 103399.

Hosseini S.A., & Khajepour F. (2013). Effect of partial replacement of dietary fish meal with soybean meal on some hematological and serum biochemical parameters of juvenile beluga, *Huso huso*. *Iran. J. Fish. Sci. 12*(2):348–356.

Howlader, S., Sumi, K.R., Sarkar, S., Billah, S.M., Ali, M.L., Howlader, J., & Shahjahan, M. (2023). Effects of dietary replacement of fish meal by soybean meal on growth, feed utilization, and health condition of stinging catfish, *Heteropneustes fossilis*. *Saudi J Biol Sci. 30*(3):103601. doi: 10.1016/j.sjbs.2023.103601.

Ibrahim, R.E., Amer, S.A., Shahin, S.A., Darwish, M.I.M., Albogami, S., Abdelwarith, A.A., Younis, E.M., Abduljabbar, M.H., Davies, S.J., & Attia, G.A. (2022). Effect of fish meal substitution with dried bovine hemoglobin on the growth, blood hematology, antioxidant activity and related genes expression, and tissue histoarchitecture of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Reports, 26*, 101276. https://doi.org/10.1016/j.aqrep.2022.101276.

Ismail, T., Hegazi, E., Dawood, M.A.O., Nassef, E., Bakr, A., Paray, B.A., & Van Doan, H. (2020). Using of betaine to replace fish meal with soybean or/and corn gluten meal in Nile tilapia (*Oreochromis niloticus*) diets: Histomorphology, growth, fatty acid, and glucose-related gene expression traits. *Aquaculture Reports, 17*, 100376. https://doi.org/10.1016/j.aqrep.2020.100376.

Jafarpour, M., & Fard, A.N. (2016). The effects of aqueous extract of Melissa officinalis on some blood parameters and liver of *Oncorhynchus mykiss*. *Aquac. Aquar. Conserv. Legis. 9*, 748–758.

Jahan A., Nipa T.T., Islam S.M., Uddin M.H., Islam M.S., & Shahjahan M. (2019). Striped catfish (*Pangasianodon hypophthalmus*) could be suitable for coastal aquaculture. J. Appl. Ichthyol. 35(4):994–1003.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., & Dayal, J. S. (2019). Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. *Aquaculture Research, 50*(12), 3493-3506.

Jia, S., Li, X., He, W., & Wu, G. (2022). Protein-sourced feedstuffs for aquatic animals in nutrition research and aquaculture. *Recent Advances in Animal Nutrition and Metabolism*, 237-261.

Kari, Z.A., Kabir, M.A., Mat, K., Rusli, N.D., Razab, M.K.A.A., Ariff, N.S.N.A., Edinur, H.A., Rahim, M.Z.A., Pati, S., Dawood, M.A.O. & Wei, L.S. (2021). The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). *Aquaculture Reports, 21*,100815. https://doi.org/10.1016/j.aqrep.2021.100815.

Khieokhajonkhet, A., Roatboonsongsri, T., Suwannalers, P., Aeksiri, N., Kaneko, G., Ratanasut, K., Inyawilert, W., & Phromkunthong, W. (2023). Effects of dietary supplementation of turmeric (*Curcuma longa*) extract on growth, feed and nutrient utilization, coloration, hematology, and expression of genes related immune response in goldfish (*Carassius auratus*). *Aquaculture Reports, 32*, 101705.https://doi.org/10.1016/j.aqrep.2023.101705.

Khojasteh, S.M.B. (2012). The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. *Inter. J. Aquat. Sci. 3* (2), 71–88

Kim, J., & Cho, S.H. (2024). Substitution effect of fish meal with various plant protein sources on growth performance and feed utilization in rockfish (*Sebastes schlegeli*) diets including jack mackerel meal used as feed stimulants. *Front. Mar. Sci. 11*:1339471. doi: 10.3389/fmars.2024.1339471

Kim, S. (2013). Christopher Layton and Bancroft John, D. Bancroft's Theory and Practice of Histological Techniques, 7th ed.

Kpundeh MD, Qiang J, He J, Yang H, & Xu P. (2015). Effects of dietary protein levels on growth performance and haemato-immunological parameters of juvenile genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. *Aquaculture International. 23*(5):1189-1201.

Kumar, V., Makkar, H.P., Amselgruber, W., & Becker, K. (2010). Physiological, haematological and histopathological responses in common carp (*Cyprinus carpi*o L.) fingerlings fed with differently detoxified Jatropha curcas kernel meal. *Food Chem. Toxicol. 48*, 2063–2072.

Lall S.P., & Kaushik S.J. (2021). Nutrition and Metabolism of Minerals in Fish. *Animals.* *11*(9):2711. https://doi.org/10.3390/ani11092711

Lim, C., Lee, C. S., & Webster, C. D. (Eds.). (2023). Alternative protein sources in aquaculture diets. *CRC Press*.

Macusi, E.D., Cayacay, M.A., Borazon, E.Q., Sales, A.C., Habib, A., Fadli, N., & Santos, M.D. (2023). Protein Fishmeal Replacement in Aquaculture: A Systematic Review and Implications on Growth and Adoption Viability. *Sustainability*, *15*, 12500. https://doi.org/10.3390/su151612500.

Maftuch, M. (2018). Haematological Analysis of Nile tilapia (Oreochromis niloticus) and Striped Catfish (*Pangasius hypophthalmus*) using hematology analyzer tool software at Fish Breeding Center Jojogan, Tuban, East Java. *Res. J. Life Sci. 5*, 107-115, https://doi.org/10.21776/ub.rjls.2018.005.02.4.

Magbanua, T. O., & Ragaza, J. A. (2024). Selected dietary plant-based proteins for growth and health response of Nile tilapia *Oreochromis niloticus*. *Aquaculture and Fisheries, 9*(1), 3-19.

Matuli´c, D., Bariˇsi´c, J., Aniˇci´c, I., Tomljanovi´c, T., Safner, R., Treer, T., Gao, J., Glojnari´c, I., & Co ˇ ˇz-Rakovac, R., 2020. Growth, health aspects and histopathology of brown bullhead (*Ameiurus nebulosus* L.): replacing fishmeal with soybean meal and brewer's yeast. *Sci. Rep. 10*, 1104. https://doi.org/10.1038/s41598-020-57722-3.

Maundu, A. M. (2020). Digestibility, growth and economic performance of Nile tilapia (*Oreochromis niloticus*) fed on a mixture of plant protein diets in cages (Doctoral dissertation, Kenyatta University,).

Mbokane, E.M., Mbokane, L.M., Motimele, S.S., & Hlophe-Ginindza, S.N. (2022). Successes and Challenges of Catfish Farming in the Small-Scale Industry in Southern Africa. IntechOpen. doi: 10.5772/intechopen.106380

Minjarez-Osorio, C., Castillo-Alvarado, S., Gatlin, D. M., González-Félix, M. L., PerezVelazquez, M., & Rossi, W. (2016). Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. *Aquaculture 453*, 122–129. doi: 10.1016/j.aquaculture.2015.11.042

Mohamad, S., & Abasali, H. (2010). Effect of plant extracts supplemented diets on immunity and resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio*). *Agric. J. 5* (2), 119–127.

Montgomery, S., Subasinghe, R.P., Siriwardena, S.N., & Shelley, C.C. (2022). Nigerian aquaculture: An investment Framework for Improved Incomes, New Jobs, Enhanced Nutritional Outcomes and Positive Economic Returns. Monographs, The WorldFish Center, number 41029,

Mutalib, A.A., Alhassan, E.A., & Ayisi, C.L. (2023). Evaluating the Impact of Varied Probiotic Levels (*Bacillus Subtilis* 200) on Feed Utilization, Growth Performance, and Proximate Composition in African Catfish (*Clarias gariepinus*). *Journal of Energy and Natural Resource Management, 9*(2): 52-63.

Mzengereza, K., Ishikawa, M., Koshio, S., Shadrack, R.S., Zhang, Y., Dossou, S., Kotani, T., Shahin, S.A., Zaineldin, A.I., Waqalevu, V., Dawood, M.A.O., Hassan, A.M., Al-Sharif, M.M., &El-Basuini, M.F. (2022). Responses of growth, blood health, pro-inflammatory cytokines genes, intestine and liver histology in Red Seabream (*Pagrus major*) to camelina meal. *Aquaculture Reports, 24*, 101175. https://doi.org/10.1016/j.aqrep.2022.101175.

Mzengereza, K., Msiska, O. V., Kapute, F., Kang'ombe, J., Singini, W., &

Kamangira, A. (2014). Nutritional value of locally available plants with potential for diets of Tilapia Rendalli in pond aquaculture in NkhataBay, Malawi. *J Aquac Res Development, 5*(6), 265. doi: 10.4172/2155-9546.1000265

Nasr, M.A.F., Reda, R.M., Ismail, T.A., & Moustafa, A. (2021). Growth, Hemato-Biochemical Parameters, Body Composition, and Myostatin Gene Expression of *Clarias gariepinus*. Fed by Replacing Fishmeal with Plant Protein. *Animals, 11*, 889. https://doi.org/10.3390/ani11030889

Ng, W.-K., & Chong, K.-K. (2002). The nutritive value of palm kernel meal and the effect of enzyme supplementation in practical diets for red hybrid Tilapia (*Oreochromis sp.*). *Asian Fish Sci. 15*, 167-176.

Perez-Velazquez, M., Gatlin III, D. M., González-Félix, M. L., García-Ortega, A., de Cruz, C. R., Juárez-Gómez, M. L., & Chen, K. (2019). Effect of fishmeal and fish oil replacement by algal meals on biological performance and fatty acid profile of hybrid striped bass (*Morone crhysops*□× *M. saxatilis*□). *Aquaculture*, *507*, 83-90.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., & Maita, M. (2011). Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. *Res. Vet. Sci. 91*(3), 92–97.

Pradhan, S.C., Patra, A.K., Sarkar, B., & Pal A. (2012). Seasonal changes in hematological parameters of *Catla catla* (Hamilton 1822). *Comp. Clin. Path. 21*(6):1473-1481.

Rahmdel, K.J., Noveirian, H.A., Falahatkar, B., & Lashkan, A.B. (2018). Effects of replacing fish meal with sunflower meal on growth performance, body composition, hematological and biochemical indices of common carp (*Cyprinus carpio*) fingerlings. *Arch. Polish Fish., 26*, 121-129.

Rani, P., Kumar, V., Rao, K.R., & Shameem, U. (2016). Seasonal variation of proximate composition of tuna fishes from Visakhapatnam fishing harbor, east coast of India. *Int. J. Fish. Aquat. Stud. 4* (6), 308-313.

Rastiannasab, A., Afsharmanesh, S., Rahimi, R., & Sharifian, I. (2016). Alternations in the liver enzymatic activity of Common carp, *Cyprinus carpio* in response to parasites, *Dactylogyrus spp*. and *Gyrodactylus spp*. *J. Parasit. Dis. 40*,1146–1149, https://doi.org/10.1007/s12639-014-0638-9.

Roslan, N.A., Sukri, S.A.M., Wei, L.S., Shahjahan, Md., Rohani, Md.F., Yea, C.S., Kabir, M.A., Guru, A., Goh, K.W., Kallem, P., & Kari, Z.A. (2024). Replacement of fish-meal by fermented spent coffee ground: Effects on growth performance, feed stability, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). *Aquaculture Reports, 36*, 102073. https://doi.org/10.1016/j.aqrep.2024.102073.

Sangavi, S., Sawant, P.B., Ande, M.P., Syamala, K., & Chadha, N.K. (2020). Dietary inclusion of non-conventional palm kernel meal enhances growth, digestive enzyme activities and carcass characteristics of juvenile rohu (*Labeo rohita*). *Aquaculture Reports, 18*, 100502. https://doi.org/10.1016/j.aqrep.2020.100502.

Senthilkumaran, A., Babaei-Ghazvini, A., Nickerson, M. T., & Acharya, B. (2022). Comparison of protein content, availability, and different properties of plant protein sources with their application in packaging. *Polymers, 14*(5), 1065.

Serrano, E., Lefillanca, J.K., Carrasco, J., Davies, S.J., & Arias, A.J.H. 2024. Evaluation of andean lupin (*Lupinus mutabilis*) seed meal as a dietary component on growth performance, feed utilization, nutrient digestibility, and liver histology of rainbow trout (*Oncorhynchus mykiss*) Juveniles. *Aquaculture Reports, 34*, 101919. https://doi.org/10.1016/j.aqrep.2024.101919.

Sharmin S., Salam M.A., Haque F., Islam M.S., & Shahjahan M. (2016). Changes in hematological parameters and gill morphology in common carp exposed to sub-lethal concentrations of malathion. *Asian J. Med. Biol. Res. 2*(3):370–378.

Shuaib, M., Hafeez, A., Chand, N., & Tahir, M. (2022). Effect of Dietary Inclusion of Soybean Hull on Production Performance and Nutrient Digestibility During Peak Egg Production Period with Different Phases in Laying Hens. *Pakistan J. Zool., 55*(1): 397-405. https://dx.doi.org/10.17582/journal.pjz/20211105091115.

Teixeira, M.A., Chaguri, Ld.C.A.G., Carissimi, A.S., Souza, N.Ld, Mori, C.M.C., Gomes, V. M.W., Poli Neto, A., Nonoyama, K., & Merusse, J.L.B. (2000). Hematological and biochemical profiles of rats (*Rattus norvegicus*) kept under microenvironmental ventilation system. *Braz. J. Vet Res. Anim. Sci. 37*, 341-347.

Ur´an, P.A., Schrama, J.W., Rombout, J.H.W.M., Obach, A., Jensen, L., Koppe, W., Verreth, J.A.J., 2008. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. *Aquac. Nutr. 14*, 324-330. https://doi.org/10.1111/j.1365-2095.2007.00534.x.

van de Wouw, J., & Joles, J.A. (2022). Albumin is an interface between blood plasma and cell membrane, and not just a sponge. *Clinical Kidney Journal, 15*(4): 624–634, https://doi.org/10.1093/ckj/sfab194.

Wan, M. S. (2015). Investigation of alternative ingredients for the replacement of fish meal in formulation of feed for Malaysian mahseer fingerlings, Tor Tambroides (Doctoral dissertation, UTAR).

Zakaria, M.K., Kari, Z.A., Van Doan, H., Kabir, M.A., Che Harun, H., Mohamad Sukri, S.A., Goh, K.W., Wee, W., Khoo, M.I., & Wei, L.S. (2022). Fermented Soybean Meal (FSBM) in African Catfish (*Clarias gariepinus*) Diets: Effects on Growth Performance, Fish Gut Microbiota Analysis, Blood Haematology, and Liver Morphology. *Life, 12*, 1851. https://doi.org/10.3390/life12111851

Zhang, J.-X., Guo, L.-Y., Feng, L., Jiang, W.-D., Kuang, S.-Y., Liu, Y., Hu, K., Jiang, J., Li, S.-H., Tang, L., & Zhou, X.-Q. (2013). Soybean β-conglycinin induces inflammation and oxidation and causes dysfunction of intestinal digestion and absorption in fish. *PLoS ONE 8* (3), e58115. https://doi.org/10.1371/journal.pone.0058115.

Zhang, X., Wang, H., Zhang, J., Lin, B., Chen, L., Wang, Q., Li, G., & Deng, J. (2020). Assessment of rapeseed meal as fish meal alternative in diets for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*). *Aquaculture Reports 18*, 100497

Zhang, X.D., Zhang, J.W., Wang, H.Z., Lin, B.B., Chen, L.S., Li, G.B., Wang, Q.M., & Deng, J. M. (2019). Evaluation of soybean meal as alternative to fish meal in diet for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*). *Aquacul. Nutr. 0*, 1-14.

Zhou, J. B., Zhou, Q. C., Chi, S. Y., Yang, Q. H., & Liu, W.C. (2007). Optimal dietary protein requirement for juvenile ivory shell, *Babylonia areolata*. *Aquaculture, 270*, 186e192.