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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*)

Zulhisyam Abdul Kari [a,*], Muhammad Anamul Kabir [b], Khairiyah Mat [a], Nor Dini Rusli [a], Mohammad Khairul Azhar Abdul Razab [c,*], Nik Shahman Nik Ahmad Ariff [d], Hisham Atan Edinur [c], Mohd Zulkifli Abdul Rahim [c], Siddhartha Pati [e,f], Mahmoud A. O. Dawood [g,h,*], Lee Seong Wei [a]

[a] Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan, Malaysia
[b] Faculty of Fisheries, Sylhet Agricultural University, Sylhet, 3100, Bangladesh
[c] School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Malaysia
[d] Razak Faculty of Technology and Informatics, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 50410, Kuala Lumpur, Malaysia
[e] SIAN Institute, Association for Biodiversity Conservation and Research (ABC), Odisha 756001, India
[f] Centre of Excellence, Khallikote University, Berhampur, Ganjam, Odisha 761008, India
[g] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
[h] The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, 11835, Cairo, Egypt

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ABSTRACT

The development of plant ingredients as an alternative to fish meal (FM) has received sustained interest in the aquaculture sector. The study investigated the replacement of FM with different percentages of dietary fermented soy pulp (FSP) to assess the growth performance, haematology, blood biochemical, liver, and gut morphology of African catfish. Five isonitrogenous (32 % crude protein) diets were prepared with FSP by replacing 0 % FSP (D1), 25 % FSP (D2), 50 % FSP (D3), 75 % FSP (D4) and 100 % FSP (D5) of FM component of the diets. The results showed a significant difference ($p < 0.05$) in growth parameters where the fish fed D3 diet showed the highest weight gain, specific growth rate, and condition factor compared with other diets. The mean values of Red Blood Cell (RBC) and Lymphocytosis (LYM) were significantly highest ($p < 0.05$) in fish fed the D3 diet. The albumin (ALB), globulin (GLOB), and total protein (TP) were significantly lower ($p < 0.05$) in the control diet compared with the experimental diets. In addition, the D3 diet provides the highest total lactic bacteria (LAB) and total bacteria (TB) compared with other diets. FSP as a protein replacement of the FM had a significant effect ($p < 0.05$) on villus length, width, and crypt depth in fish's anterior and posterior gut. The histological study of the intestinal revealed that the gut of the D3 diet had an intact epithelial barrier with goblet cells arrangement and very well-organized villus structure, tunica muscularis compared with the other treatments. In conclusion, the replacement of 50 % FM with FSP could be used in the aquafeed industry for better growth and health status of African catfish and possibly for freshwater species.

1. Introduction

There has been tremendous growth in the aquaculture feed industry in the past few years due to the aquaculture activity, which is the fastest-growing animal production sector in the world with an average annual rate of about 10.3 % since 2010 and produced 89 % of the global total in volume terms in the last 20 years (FAO, 2020). The consistent supply and the cost of acquiring feed are the main issues in aquaculture (Dawood, 2021; Pati et al., 2016). Feed also contributes the most to the cost of sustainable fish farming for all species, including African catfish.

* Corresponding authors at: Faculty of Agro Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia; School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Malaysia; Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt.
E-mail addresses: zulhisyam.a@umk.edu.my (Z. Abdul Kari), khairul.azhar@usm.my (M.K.A.A. Razab), mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood).

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The cost of feed is crucial because it usually comprises 30–70 % of the total operational costs and influences profitability in aquaculture investments (Danial, 2018; Dossou et al., 2021; Muzinic et al., 2006). This makes the research into other protein sources vital as they could replace a fish meal (FM), which gradually decreases production (Amer et al., 2021). Plant proteins' wide availability and reasonable cost have made them the most viable choice as an alternative (Zulhisyam et al., 2020a). The identification and development of plant ingredients as an alternative to FM and its high cost has received sustained interest in the flourishing world aquaculture sector (Azarm and Lee, 2014; Goda et al., 2014; Mzengereza et al., 2021). This will eventually decrease the use of FM. Due to its stable supply, price, and nutritional value, soy pulp (SP) is a potential alternative protein source from the various plant-based feed being researched as a FM substitute (Rahman et al., 2014).

To reduce the use of antibiotics in intensive fish production, SP potentially contains various biologically active compounds that can enhance the growth and health of fish, and it is originating from soybean since it's a by-product of bean curd (tofu) manufacturing (Antonio et al., 2008; Villanueva et al., 2011; Rahman et al., 2014; Kim et al., 2016; Zulhisyam et al., 2020b). Limitations related to plant protein used in fish feed are well known. Anti-nutritional factors (ANFs) present in the plant-based feed are the primary constraint (National Research Council, 2011). As a solution, research into solid-state fermentation (SSF) has focused on developing bio-processes for bio-remediation and biological detoxification of agro-industrial wastes to eliminate or reduce these wastes by fermentation (Dawood and Koshio, 2020). The reduction of ANFs and improving the nutritional benefits of plant-based protein sources such as soybean by-products may be achieved through the fermentation approach (Shiu et al., 2013; Azarm and Lee, 2014; Jiang et al., 2018; Zulhisyam et al., 2020b). Several recent research has looked into fermented soybean meal (FSBM) usage in animal feed (Rahman et al., 2014) and aquaculture (Azarm and Lee, 2014; Jiang et al., 2018; Yamamoto et al., 2010) to replace FM. Soybean (SBM) is a plant protein source that possesses a lower amount of ANFs such as trypsin inhibitor while containing only nutritional elements of high protein content, small-sized peptide, and concentrations of free amino acids (Shiu et al., 2013). In addition, SP's composition and quality can also be enhanced by using microbes like *Lactobacillus* spp., *Bacillus* spp., *Aspergillus* spp., and yeast to ferment it (Rashad et al., 2011; Shi et al., 2013). Recently, Zulhisyam et al. (2020b) reported that lactic acid bacteria might have the ability to multiply throughout the SP fermentation process, and FSP may be the best carrier to deliver probiotics like *Lactobacillus* spp. to the fish gut.

It is demonstrably apparent that the diets given to African catfish are insufficient due to a lack of nutrients and questionable quality (Sinyangwe et al., 2017). Large-scale aquaculture can't be achieved without first ensuring that African catfish can be farmed in sufficient quantities and that the fish produced are of acceptable quality. A poor farming system and insufficient fish feed quality are currently the main problems in African catfish farming management. To overcome these problems, aquaculture nutritionists are exploring alternative approaches for feed administration, such as including alternative protein sources from plants and animals in the diet. Thus, the present research addresses the above issues by determining the effect of FSP inclusion at different percentages (0 %, 25 %, 50 %, 75 %, and 100 %) to evaluate the growth performance, haematology, blood biochemical, liver, and gut morphology of African catfish production.

2. Materials and methods

2.1. Animal ethics approval

The protocols and design of this research have been approved by the Animal Ethics Committee of Universiti Malaysia Kelantan and UMK Biosafety and Biosecurity Committee (Ref: UMK/FIAT/ACUE/PG3/2018). During the experiments, all the protocols and methodology were followed according to the comment and approval by the committee.

2.2. Experimental fish and husbandry condition

Two thousand African catfish fry (Length: 8.4 ± 1.2 cm; Weight: 8.1 ± 0.8 g each) in total were procured from Prima Mekar Enterprise, a fish farm in Jeli, Kelantan, and the fingerlings were given commercial fish feed that had 43 % crude protein and 6 % crude lipid content. The experimental fish were acclimatized for ten days before starting the research in three tanks (1000 L). When the experiment was started, the fish were then stocked in 15 indoor fiberglass tanks (360 L) at a density of 70 fish/tank. The duration of the feeding experiment was 70 days for all treatments. The fish were fed to satiation thrice daily in the early morning, late afternoon, and night (i.e., 6.30 a.m., 7 p.m., and 11 p.m.), and the weight of feed was appropriately recorded. Throughout the experiment, the pH (7.5 ± 0.2), morning temperature (26.9 ± 2.9 °C), dissolved oxygen (5.8 ± 0.68 mg/L), total ammonia (1.28 ± 0.39 mg/L), nitrite (0.17 ± 0.7 mg/L), alkalinity (62.6 ± 11.2 mg $CaCO_3$/L) and hardness (94.7 ± 1.5 mg $CaCO_3$/L) of the water were maintained during the study.

2.3. Preparation of fermented soy pulp (FSP) and experimental diets

Fermented soy pulp (FSP) was prepared in various levels to replace FM at 0 % FSP (D1), 25 % FSP (D2), 50 % FSP (D3), 75 % FSP (D4) and 100 % FSP (D5). The D1 was used as a control diet and contain 0 % FSP. In brief, SP was made through fermentation method and mixed with Sigma^R commercial *Lactobacillus acidophilus* powder with a concentration of 10^{10} CFU/g and 1 % liquid molasses as suggested by Aderolu et al. (2013) and kept into an HDPE container for three weeks for every 1 kg production. The fine powder of FSP was finely grounded and mixed with other ingredients; fish meal, soybean meal, fish oil, rice brain, vitamin-mineral premix, and carboxymethyl cellulose as a binder in HDPE container with respective percentage levels. Five isonitrogenous (crude protein 32 %) diets were formulated according to a standard procedure. The mixture was then pelleted by passing it through a mincer of 2 mm die to produce 2 mm diameter size of pellets. The pellets were then packed in polythene bags and kept safe dry for use. Table 1 shows the formulation and chemical analysis (AOAC, 2003) of experimental diets, and Table 2 presents the levels of essential amino acids in the diets

Table 1
Composition and proximate analysis (dry matter basis) of the five experimental diets fed to African catfish.

	Diets				
Ingredients (g/kg)	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
Fish meal	360	270	180	90	0
Fermented soy pulp[a]	0	90	180	270	360
Soybean meal	360	360	360	360	360
Wheat	170	170	170	170	170
Vitamin-mineral premix	20	20	20	20	20
Fish oil	30	30	30	30	30
Vegetable oil	30	30	30	30	30
Binder[b]	30	30	30	30	30
Total	1000	1000	1000	1000	100
Proximate composition (%)					
Moisture	5.33	5.57	7.45	8.46	9.45
Protein	32.31	32.16	31.75	31.91	32.14
Lipid	5.41	4.14	4.41	4.63	4.87
Fiber	4.52	4.64	4.77	4.94	5.19
Ash	5.72	5.76	6.32	6.2	6.33
Carbohydrate	45.72	44.73	43.54	41.56	40.29

[a] FSP were added with 0.001 g *Lactobacillus acidophilus* and 1 % molasses for every 1 kg.
[b] Carboxymethyl Cellulose (CMC).

Table 2
The amino acid composition of the test diets (g/100 g) is expressed as a percent of protein fed to African catfish. Data expressed as mean ± standard deviation (SD).

	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
EAA (%)					
Arginine	7.58 ± 0.05	5.91 ± 0.24	4.99 ± 1.21	4.20 ± 1.15	4.78 ± 0.71
Histidine	2.30 ± 2.07	6.69 ± 1.55	6.45 ± 0.05	5.93 ± 4.22	4.65 ± 2.07
Isoleucine	0.26 ± 1.98	0.06 ± 1.38	0.32 ± 0.09	0.30 ± 0.05	0.25 ± 1.19
Leucine	1.83 ± 0.05	1.80 ± 0.05	2.08 ± 0.05	2.70 ± 2.07	2.22 ± 4.32
Lysine	1.66 ± 0.05	1.45 ± 1.45	1.28 ± 0.05	1.35 ± 0.32	1.48 ± 0.05
Phenylalanine	1.15 ± 2.07	3.20 ± 0.05	2.40 ± 0.05	2.15 ± 0.24	2.95 ± 1.55
Threonine	0.90 ± 2.87	0.29 ± 0.22	0.96 ± 1.98	0.68 ± 0.31	1.06 ± 0.05
Valine	0.51 ± 0.05	2.09 ± 0.05	2.34 ± 2.07	2.51 ± 2.76	2.54 ± 1.78
Methionine	1.02 ± 0.05	1.06 ± 3.31	1.16 ± 0.66	1.75 ± 0.67	1.98 ± 0.05
NEAA (%)					
Alanine	1.66 ± 0.05	1.67 ± 1.54	1.62 ± 0.24	1.80 ± 0.95	2.22 ± 1.54
Aspartic acid	2.61 ± 1.55	2.62 ± 0.05	2.40 ± 0.84	2.25 ± 0.05	1.97 ± 0.05
Glutamic acid	4.74 ± 1.54	4.71 ± 1.98	3.09 ± 0.24	3.80 ± 2.07	3.79 ± 1.31
Glycine	1.92 ± 0.05	1.58 ± 0.05	1.22 ± 2.07	1.31 ± 1.11	1.48 ± 2.24
Proline	1.28 ± 4.01	0.89 ± 0.07	0.72 ± 0.55	0.84 ± 0.05	0.79 ± 0.05
Serine	1.31 ± 0.05	0.69 ± 0.55	0.96 ± 1.98	0.68 ± 1.94	0.57 ± 2.95
Tyrosine	0.13 ± 1.99	0.18 ± 1.98	0.16 ± 0.33	0.45 ± 0.05	0.25 ± 0.05

Note: Different superscripts in each row indicate a significant difference ($p < 0.05$).
Abbreviations: EAA, Essential amino acid; NEAA, Non-essential amino acid.

(Kabir et al., 2015) expressed as a percentage of protein.

2.4. Growth performances parameters

The African catfish were made to fast for 24 h, and their total length and weight measurements were taken under anaesthetics at 0.1 g/L water MS-222 at the beginning and at the end of the trial. Each trial consisted of three biological replicates of the rearing tanks. The collected samples were analyzed to estimate the growth performances using the following formulae:

Survival rate (%) = (Number of surviving fish/Total number of fish at the start of the experiment) x 100

Weight gain (%) = (Final weight - initial weight)/Initial weight) x 100

Specific growth rate (%) = (Final Mean weight – Initial Mean weight)/Length of feeding trial (days)

Hepatosomatic index (%) = 100*(Weight of liver/Total body weight)

Visceral somatic index (%) = (Viscera Weight/Fish Weight) x 100

Feed Conversion Rate (FCR) = (Total Feed Consumption/Weight Gain of Fish)

Condition factor (CF) = (Weight of fish body (g) X 100)/(Length of fish, L)^3 (cm)

2.5. Proximate composition analysis for fish body muscle, liver, and intestine

Standard protocols established by the Association of Official Analytical Chemists (AOAC, 1997) were used to analyze the body composition of the fish. Lipid extraction from the samples by homogenization was used to determine the crude lipid content in the body compositions (Folch et al., 1957).

2.6. Bacterial load determination in the gut

In brief, the sterile saline solution was used to dilute the homogenate to 10^{-7} serially. A 10 μL quantity of the diluted sample was spread onto de Man, Rogosa, and Sharp agar (MRS, HiMedia, India) or Tryptic Soy Agar (TSA, HiMedia, India) in triplicate plates to determine total viable lactic acid bacteria (aerobic activity) and total counts of bacteria, respectively. The plates were placed in an incubator at 37 °C for 36 h. After a colony became visible, the plates were removed, and viable cell counts were expressed as colony-forming units (CFU/g feed).

2.7. Histological examination of the gut and liver

To conduct the histological analysis on the gut and liver, three fish from each replicate tank were collected and dissected. A 10 % neutral-buffered formalin solution was used to fix the tissues immediately and separately. 1 mm transverse sections from each gut and liver segments were cut from each replicate fish. These sections were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin blocks. Transverse sections of 5–8 μm were then cut and mounted on glass slides and oven-dried overnight at 40 °C. Haematoxylin and Eosin were used to stain the sections. The tissue sections were evaluated under a light microscope (Olympus BX43). An image capture analysis system (Cellsens software, Netherlands) was used for digitizing each fish section's images and the images used to determine villus length, width, and crypt depth.

2.8. Haematological parameters

A group of 3 fish were collected at random from each feeding treatment. The fish were placed in the plastic tanks and deprived of food for 5 h. The fish were then each lightly anesthetized by Tricaine Methanesulfonate (MS-222) while their blood were taken to minimize the stress. A 1 cc sterile syringe was used to take blood from the mid-ventral line behind the anal fin and collected it in heparinized tubes with green and yellow caps. For the complete blood count test, the blood samples (150 μL per treatment) were used for determining the blood haematological parameters using the automatic haematology analyzer (Mythic 18 Vet, US). For the biochemical blood tests, a 150 μL sample of serum from each treatment was drawn into pipette tips and dropped on cassettes containing the reagent of the individual chemistry (Brand: IDEXX, USA). All individual chemistries were automatically detected by the VetTest analyzer (Brand: IDEXX, USA) except for globulin. The subtraction of albumin from total protein results in globulin level.

2.9. Statistical analysis

All data were tested for normality before being analyzed in this study. SPSS software 20.1 was used to carry out one-way analysis of variance (ANOVA) to test for significant differences ($p < 0.05$) among the diets' treatment groups and control group. Duncan's multiple range test was used to compare differences among the treatment means when significant F-values were observed at ($p < 0.05$) level. The data are presented as mean ± SD.

3. Results

3.1. Fish growth performance

The results of growth performance were shown in Table 3. There were significant differences ($p < 0.05$) in the growth parameters in the experimental diet groups in terms of final weight, weight gain (%), specific growth rate (%), and condition factor. The highest weight gain, specific growth rate, and condition factor occurred in the D3 diet with mean and standard deviation values of 1552.41 ± 81.67 %, 1.73 ± 0.03 %, and 0.86 ± 0.06, respectively, in comparison with other treatments. African catfish in the D3 diet had a significantly lower ($p < 0.05$) food conservation rate (FCR) compared with the other experimental diets. However, more than 90 % overall survival rate of the African catfish displayed no significant differences ($p > 0.05$) in the mean values among the different diets with the highest is at D3 diet with mean and standard deviation of 96.57 ± 1.43 %.

3.2. Body tissue proximate composition

The body muscle, liver, and proximate intestine composition are shown in Table 4. There were significant differences in the muscle, intestine, and liver lipid contents among the FSP levels. However, the level did not follow any specific trend when the FSP level was raised to 100 %. As the FSP level rose from 25 % to 100 %, ash content in the muscle, liver, and intestine increased as well and was significantly different among the treatments. However, fish fed with D5 produced the significantly highest ash content in all body tissues of African catfish.

3.3. Bacterial load determination in the gut

Table 5 illustrates the fish gut total bacteria (TB) and lactic acid bacteria (LAB) estimation against the different treatment groups. Total bacteria (TB) mean value was significantly higher ($p < 0.05$) in the 50 % FSP. The lowest TB and LAB mean values were found in the fish fed D3 diet with mean and standard deviation values of 1.37±0.06 and 1.64±0.05 CFU/g.

Table 3
Growth performance of African catfish fed with experimental diets for 70 days. Data expressed as mean ± standard deviation (SD) of three replicates.

Item	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
IW (g)	23.83 ± 2.21	23.70 ± 2.06	24.10 ± 1.05	24.00 ± 2.08	23.70 ± 1.73
FW (g)	242.33 ± 2.08^{d}	306.33 ± 5.50^{b}	397.66 ± 3.51^{a}	277.66 ± 3.78^{c}	177.66 ± 1.52^{e}
WG (%)	992.98 ± 101.09^{c}	1200.27 ± 133.01^{b}	1552.41 ± 81.67^{a}	1064.03 ± 122.59^{bc}	652.58 ± 60.53^{d}
SGR (%)	1.44 ± 0.06^{c}	1.59 ± 0.06^{b}	1.73 ± 0.03^{a}	1.52 ± 0.06b^{c}	1.25 ± 0.05^{d}
VSI	2.87 ± 0.36^{c}	3.70 ± 0.16^{ab}	3.55 ± 0.22^{b}	4.10 ± 0.55^{ab}	4.20 ± 0.12^{a}
HSI	1.39 ± 0.1	1.84 ± 0.5	1.58 ± 0.1	1.68 ± 0.4	2.01 ± 0.3
CF	0.76 ± 0.09^{ab}	0.84 ± 0.07^{a}	0.86 ± 0.06^{a}	0.82 ± 0.05^{a}	0.69 ± 0.04^{b}
SR (%)	90.95 ± 2.97	95.23 ± 3.59	96.57 ± 1.43	92.38 ± 2.97	91.90 ± 2.18
FCR	1.62 ± 0.06^{b}	1.22 ± 0.03^{d}	1.01 ± 0.01^{e}	1.39 ± 0.03^{c}	2.19 ± 0.05^{a}

Note: Different superscripts in each row indicate a significant difference ($p < 0.05$).
Abbreviations: IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; VSI, visceral somatic index; HIS, hepatosomatic index; CF, condition factor; SR, survival rate; FCR, feed conversion ratio.

Table 4
Intestine, liver, and muscle proximate composition (g/kg) of fish fed different dietary FSP protein supplement levels (n = 3). Data expressed as mean ± SD.

	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
Intestine					
Moisture	5.54 ± 0.71^{a}	5.70 ± 0.22^{b}	5.80 ± 0.28^{c}	5.78 ± 0.43^{b}	5.83 ± 0.82^{d}
Protein	57.93 ± 0.66^{d}	63.07 ± 1.15^{c}	66.05 ± 0.16^{ab}	67.18 ± 0.63^{a}	65.22 ± 0.80^{b}
Lipid	28.53 ± 0.63^{a}	23.65 ± 0.79^{b}	21.14 ± 0.24^{c}	19.52 ± 1.06^{d}	20.83 ± 0.48^{c}
Ash	5.43 ± 0.43^{ab}	5.37 ± 0.51^{ab}	5.33 ± 0.58^{b}	5.92 ± 0.27^{ab}	6.29 ± 0.52^{a}
Liver					
Moisture	5.77 ± 0.43^{a}	5.65 ± 0.40^{ab}	5.32 ± 0.20^{ab}	5.02 ± 0.15^{b}	5.35 ± 0.47^{ab}
Protein	54.37 ± 0.74^{c}	56.67 ± 1.35^{b}	59.11 ± 0.95^{a}	58.68 ± 1.28^{a}	56.13 ± 0.53^{bc}
Lipid	33.89 ± 0.29^{a}	31.26 ± 0.86^{b}	28.34 ± 0.67^{d}	29.46 ± 0.93^{cd}	30.39 ± 1.00^{bc}
Ash	4.54 ± 0.37^{c}	4.90 ± 0.21^{bc}	5.35 ± 0.75^{abc}	5.55 ± 0.60^{ab}	5.88 ± 0.31^{a}
Muscle					
Moisture	4.90 ± 0.19^{a}	2.99 ± 0.11^{c}	3.87 ± 0.19^{b}	4.60 ± 0.52^{a}	4.74 ± 0.48^{a}
Protein	77.83 ± 0.91^{bc}	82.00 ± 1.30^{a}	80.28 ± 3.14^{ab}	77.80 ± 0.98^{bc}	74.76 ± 0.95^{c}
Lipid	10.61 ± 0.62^{a}	9.72 ± 0.54^{ab}	8.93 ± 0.35^{b}	9.55 ± 0.68^{ab}	10.35 ± 1.13^{a}
Ash	4.72 ± 0.43^{c}	3.75 ± 0.16^{d}	4.71 ± 0.40^{c}	5.81 ± 0.35^{b}	7.98 ± 0.22^{a}

Note: Different superscripts in each row indicate a significant difference ($p < 0.05$).

Table 5
Fish gut total lactic acid bacteria (LAB) and total bacteria (TB) count from fish fed different dietary FSP protein supplement levels (n = 3). Data expressed as mean ± SD.

Parameters	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
TB (CFU/g intestine) $\times 10^{9}$	1.37 ± 0.06^{e}	4.43 ± 0.07^{d}	6.43 ± 0.44^{a}	5.60 ± 0.23^{b}	5.10 ± 0.11^{c}
LAB (CFU/g intestine) $\times 10^{7}$	1.64 ± 0.05^{c}	5.21 ± 0.32^{b}	6.10 ± 0.20^{a}	6.00 ± 0.11^{a}	5.35 ± 0.10^{b}

Note: Different superscripts in each row indicate significant differences ($p < 0.05$).

3.4. Histomorphology of mid intestine and liver

The distal intestines histopathological examination showed changes in the mid intestine of fish fed with different FSP diets. These included the normal lamina propria, lamina epithelial mucosae, stratum compactum, tunica muscularis, and villus arrangement structure, as well as goblet cells in the fish gut. The results showed that fish gut from the 50 % FSP diet had an intact epithelial barrier with goblet cells arrangement and very well-organized villus structure, tunica muscularis compared to the other treatment groups. However, moderate histopathological changes were discovered in fish fed with 25 % and 75 % FSP in comparison with the control and 100 % FSP groups, but regularly distributed lamina epithelial mucosae, tunica muscularis, and large goblet cells were present (Fig. 1).

Fig. 2 shows the liver cell histomorphology of fish fed with the experimental diets. The histopathological investigation of the liver cells showed that the liver cells of the fish groups had changed with different levels of FSP diets, involving the sinusoid, vacuole, nucleus, and erythrocytes. The liver cells of the D3 diet fish had better nuclei and

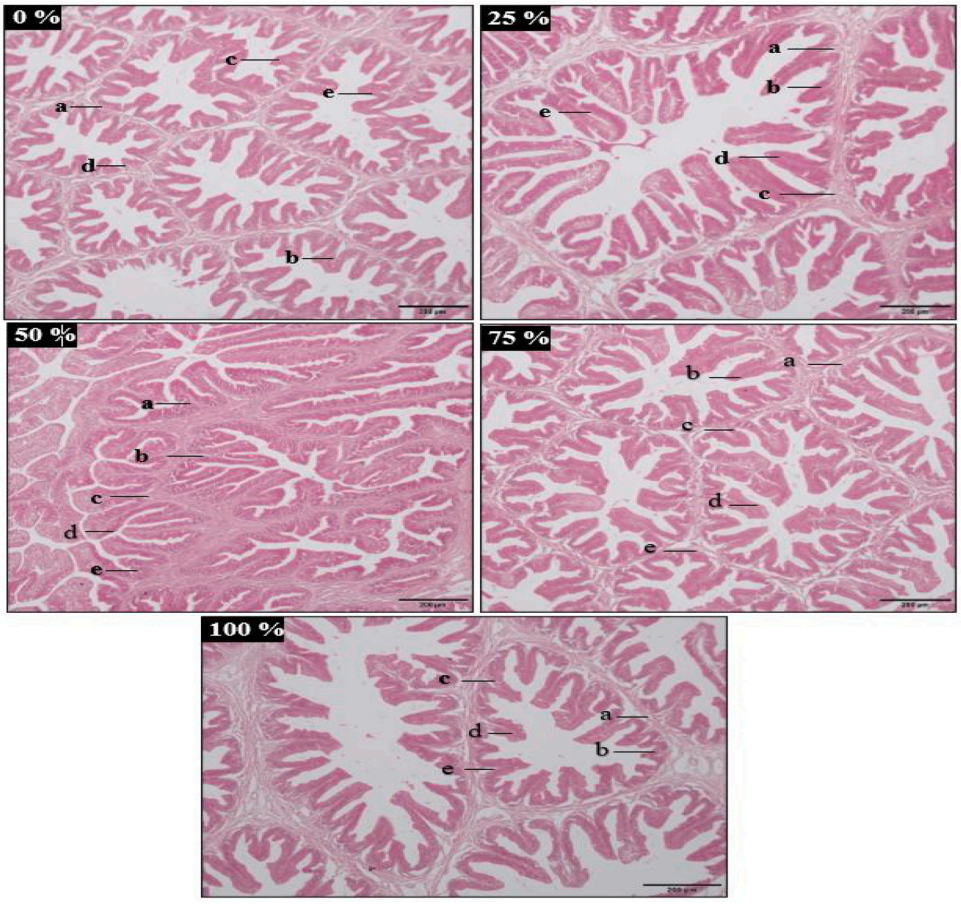

Fig. 1. Morphological appearance of the distal intestines of African catfish with different percentages of FSP diets (0 %, 25 %, 50 %, 75 %, and 100 %) under the light microscope (Olympus BX43). All photos were taken at 40X; the scale bar represents 200 μm. The histopathological changes were observed in lamina propria (a), lamina epithelial mucosae (b), stratum compactum (c), goblets cell (d), tunica muscularis (e).

cytoplasm structure compared with the other groups of feed treatments. However, nuclei and cytoplasm were atrophied, and hepatic cell cords were disordered in fish fed with D4 and D5 diets compared with other treatment groups. Increasing the FSP level also increased the number of vacuolar cytoplasms, though fish fed with up to 50 % FSP diet didn't display these abnormalities.

3.5. Villus length, width, and crypt depth estimation

The histomorphology of the anterior and posterior gut of African catfish was subjected to light microscopic analysis, and Table 6 illustrates the results. Villus length, width, and crypt depth in fish's anterior and posterior gut were significantly ($p < 0.05$) affected by FSP protein supplementation in the experimental diets.

3.6. Blood haematology

The hematological indices of African catfish fed with different levels of FSP diets are shown in Table 7. The fish fed with D4 and D5 diets had a significantly lower ($p < 0.05$) mean value of white blood cell (WBC) compared with the D3 diet but no significant difference over the D1 and D2 diets. The Lymphocytosis (LYM) count increased significantly higher in the D3 diet. However, there was no significant difference in Monocytes (MON) among the groups of experimental diets. There were significant variances in the blood Granulocytosis (GRA) levels among the test diets, but no specific trend was observed. The mean value of red blood cell (RBC) was significantly higher ($p < 0.05$) in the fish-fed D3 diet group compared with the other diets. The haematocrit (HCT) had no significant difference among the diets. However, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) varied significantly among the test diets. There was no specific trend to the variations, but the numerical mean value of the MCV, MCH, and MCHC were smaller in the control group compared with the other diets.

3.7. Blood biochemical parameters

The parameters of blood biochemistry are shown in Table 8. The values of albumin (ALB), globulin (GLOB), and total protein (TP) were significantly lower ($p < 0.05$) in the D1 diet compared with the experimental diets. However, the urea (BUN) significantly differed among the groups but did not follow any specific trends. Creatinine (CREA), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), and total bilirubin (TBIL) levels increased significantly with the increase in FSP level in the diets. However, ALT, AST, GLU mean values were significantly lower in the D1 diet than in other diets. However, the cholesterol (CHOL) value was significantly different among the treatment groups but did not follow any specific trends.

4. Discussion

In the present study, the fish-fed D3 diet resulted in the highest weight gain (%) and specific growth rate (SGR) compared with other experimental diets. Probiotics like *Lactobacillus acidophilus* have been

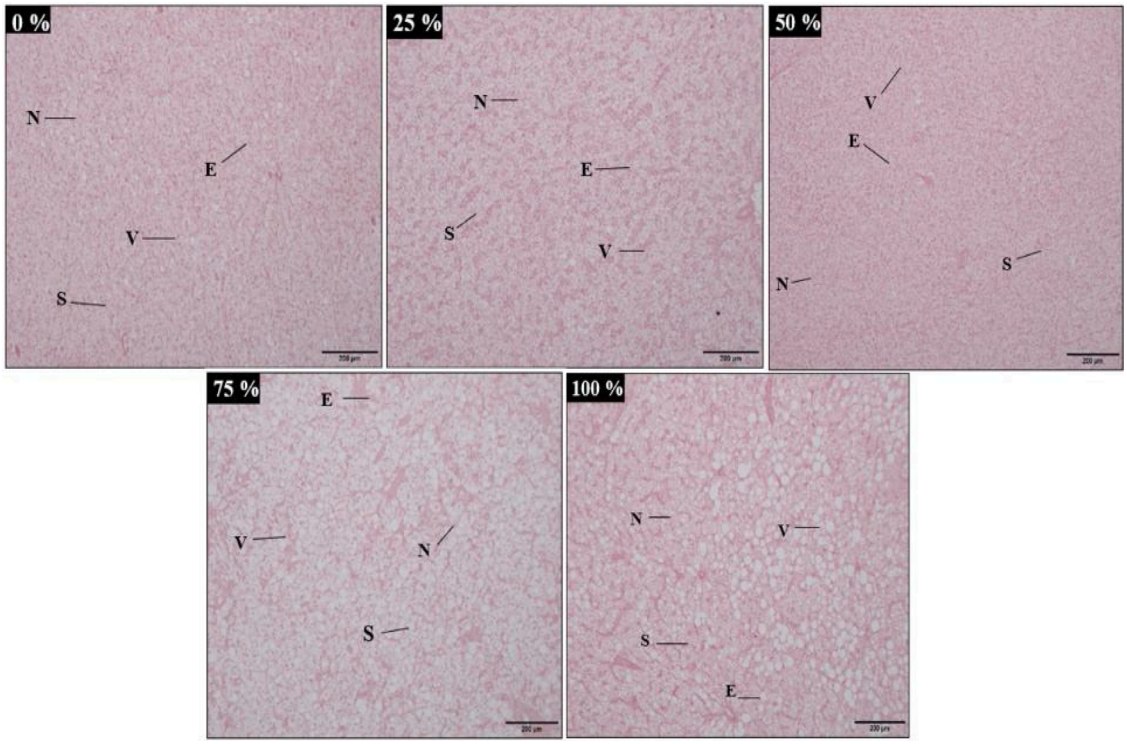

Fig. 2. Morphological appearance of the liver of African catfish with different percentages of FSP diets (0 %, 25 %, 50 %, 75 %, and 100 %) under the light microscope (Olympus BX43). All photos were taken at 40X; the scale bar represents 200 μm. The histopathological changes were observed in Sinusoid (S), Vacuole (V), Nucleus (N), and Erythrocytes (E).

Table 6
Gut morphology from fish fed different dietary FSP protein supplement levels (n = 3). Data expressed as mean ± SD.

		Diets (g/kg)				
Item	Intestine Region	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
Villus length (μm)	Anterior	142.80 ± 13.86^{c}	193.00 ± 4.01^{b}	211.83 ± 7.26^{b}	237.23 ± 16.99^{a}	126.37 ± 5.10^{c}
	Posterior	122.87 ± 6.37^{d}	182.00 ± 5.29^{c}	198.00 ± 2.64^{b}	222.05 ± 12.39^{a}	114.83 ± 4.71^{d}
Villus width (μm)	Anterior	78.13 ± 7.82^{b}	78.44 ± 3.23^{b}	136.65 ± 3.07^{a}	129.26 ± 5.45^{a}	56.39 ± 1.50^{c}
	Posterior	63.06 ± 5.05^{b}	64.83 ± 4.56^{b}	111.13 ± 12.32^{a}	116.44 ± 8.79^{a}	41.49 ± 0.82^{c}
Crypt depth (μm)	Anterior	55.16 ± 1.98^{c}	57.83 ± 0.93^{c}	93.21 ± 2.87^{b}	99.30 ± 1.63^{a}	46.78 ± 1.12^{d}
	Posterior	47.21 ± 4.47^{b}	52.39 ± 2.09^{b}	81.17 ± 5.93^{a}	81.09 ± 7.25^{a}	35.22 ± 1.06^{c}

Note: Different superscripts in each row indicate significant differences ($p < 0.05$).

reported to produce bioactive materials, which have intrinsic biogenic effects. According to Stanton et al. (2005), fermentation can produce bioactive microbial metabolites like peptides, vitamins, and fatty acids, which can improve the digestion process for better growth performances compared to a non-fermented diet. The present study results were supported by previous research related to the importance of dietary probiotics and prebiotics on fish growth performances (Talpur et al., 2014; Akter et al., 2015). The overall fish survival rate in this study was above 90 %, and the highest is achieved at the D3 diet. Feed conversion ratio (FCR) is an essential parameter to estimate the feed needed for the growing cycle of the fish, and it is vital to the farmers to determine the profit of aquaculture activity. There were reducing trends of FCR from the experimental diet but increased significantly at D4 and D5 diets during the study period. The lowest FCR was observed at the D3 diet with a mean and standard deviation of 1.01 ± 0.01. All experimental diets with around 32 % crude protein and 4 % lipid in this study resulted the FCR value is under 2 (Table 2) except for the D5 diet. Kapka-Skrzypczak et al. (2012) indicated that the bioactive role of dietary probiotics could reduce the FCR, resulted in its more suitable and economical feed formulation as 30–70 % of total production cost in the aquaculture industry (Webster et al., 2001). This result indicates that this level of FSP replacement at 50 % may be the best option in the aquafeed industry for African catfish future growth and health status.

This study showed that increasing the inclusion level of FSP resulted in a positive growth response in fish. However, the hepatosomatic index (HSI), which reflected the accumulation of lipids and glycogen within the liver (Krogdahl et al., 2005), revealed an opposite response. Dimitroglou et al. (2010) observed a similar result when they administered fish meal-based diets supplemented with FSP to the gilthead sea bream. While FSP did not significantly affect the HSI values, the FSP concentrations above 75 % generally decreased the relative lipid content even though the muscle lipid contents were similar to the control group. Fish fed the D3 diet also had a significant increase in muscle protein compared with the control diet, as corroborated in a study on Atlantic salmon smolts (*Salmo salar*) (Dimitroglou et al., 2011). In general, the high FSP levels (D4 and D5) drastically reduced the viscerosomatic index (VSI) compared with the control, indicating that the fish had more meat on its body. Therefore, feeding with an FSP diet has resulted in a better value. In contrast, the muscle nutritional content is significant because it needs to meet the nutritional needs for fish growth and immune system development (Furuita et al., 2002; Kabir et al., 2015).

In all protein levels analyzed in the study, the muscle protein content

Table 7
Blood parameters from fish fed with different dietary FSP protein supplement levels (n = 3). Data expressed as mean ± SD.

Item	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
WBC (10^3/µl)	119.30 ± 2.08^{ab}	119.26 ± 2.31^{ab}	122.83 ± 1.80^{a}	117.80 ± 1.60^{bc}	114.93 ± 2.51^{c}
LYM (%)	88.50 ± 4.03^{c}	98.13 ± 4.46^{b}	106.29 ± 1.76^{a}	96.43 ± 0.55^{b}	99.93 ± 2.76^{b}
MON (%)	14.06 ± 2.64^{a}	12.70 ± 4.12^{a}	13.33 ± 1.62^{a}	17.46 ± 1.23^{a}	12.56 ± 1.62^{a}
GRA (10^3/µl)	4.33 ± 0.45^{a}	3.06 ± 0.50^{bc}	2.55 ± 0.13^{c}	3.46 ± 0.42^{b}	3.76 ± 0.37^{ab}
RBC (10^3/µl)	2.21 ± 0.17^{bc}	2.38 ± 0.08^{b}	2.66 ± 0.15^{a}	2.19 ± 0.15^{bc}	1.96 ± 0.07^{c}
HGB (g/dl)	6.03 ± 1.70^{b}	7.96 ± 1.76^{ab}	9.23 ± 0.70^{a}	9.96 ± 1.20^{a}	8.20 ± 1.87^{ab}
HCT (%)	27.34 ± 2.29^{a}	27.91 ± 4.80^{a}	28.23 ± 1.67^{a}	30.20 ± 3.04^{a}	26.70 ± 3.05^{a}
MCV (µm^3)	125.13 ± 0.65^{a}	126.23 ± 2.44^{a}	128.80 ± 2.42^{a}	120.88 ± 2.88^{b}	126.76 ± 2.58^{a}
MCH (pg)	37.36 ± 0.76^{c}	43.43 ± 3.43^{a}	42.10 ± 0.80^{ab}	39.26 ± 3.05^{bc}	41.10 ± 0.26^{abc}
MCHC (g/dl)	29.83 ± 0.80^{b}	34.43 ± 3.30^{a}	32.66 ± 1.19^{ab}	32.96 ± 0.90^{ab}	32.43 ± 0.55^{ab}
RDW (%)	7.60 ± 0.51^{a}	5.90 ± 0.36^{b}	5.26 ± 0.38^{b}	6.96 ± 0.82^{a}	7.20 ± 0.55^{a}
PLT (10^3/µl)	41.33 ± 3.78^{b}	30.33 ± 4.16^{c}	37.66 ± 1.52^{bc}	40.33 ± 3.21^{b}	64.44 ± 6.65^{a}
MPV (µm^3)	6.93 ± 0.70^{a}	5.90 ± 0.26^{b}	6.30 ± 0.45^{ab}	6.36 ± 0.30^{ab}	5.56 ± 0.61^{b}
PCT (%)	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}
PDW (%)	7.71 ± 0.44^{c}	9.06 ± 0.51^{ab}	7.60 ± 0.40^{c}	9.86 ± 0.40^{a}	8.43 ± 0.89^{bc}

Note: Different superscripts in each row indicate significant differences ($p < 0.05$).
Abbreviations: WBC, White blood cell; LYM, Lymphocytosis; MON, Monocytes; GRA, Granulocytosis; RBC, Red blood cell; HGB, Haemoglobin; HCT, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; RDW, Red Cell Distribution Width; PLT, Platelet; MPV, Mean Platelet Volume; PCT, Procalcitonin; PDW, Platelet Distribution Width.

Table 8
Blood biochemical variables from fish fed with different dietary FSP protein supplement levels (n = 3). Data expressed as mean ± SD.

Item	Diets (g/kg)				
	0 % FSP (D1)	25 % FSP (D2)	50 % FSP (D3)	75 % FSP (D4)	100 % FSP (D5)
ALB (g/dl)	0.73 ± 0.15^{c}	0.76 ± 0.05^{c}	1.10 ± 0.10^{b}	1.16 ± 0.05^{b}	1.50 ± 0.10^{a}
GLOB (g/dl)	2.00 ± 0.17^{c}	2.33 ± 0.20^{b}	2.69 ± 0.08^{a}	2.68 ± 0.08^{a}	2.79 ± 0.04^{a}
TP (g/dl)	2.86 ± 0.20^{d}	3.23 ± 0.15^{c}	4.01 ± 0.27^{ab}	3.95 ± 0.05^{b}	4.03 ± 0.09^{a}
BUN/urea (mg/dl)	3.90 ± 0.26^{bc}	3.53 ± 0.35^{c}	3.56 ± 0.35^{c}	4.09 ± 0.11^{ab}	4.46 ± 0.15^{a}
CREA (mg/dl)	0.13 ± 0.05^{b}	0.13 ± 0.05^{b}	0.16 ± 0.05^{b}	0.20 ± 0.00^{ab}	0.27 ± 0.02^{a}
ALKP (u/l)	10.70 ± 0.51^{b}	11.66 ± 0.57^{ab}	12.00 ± 1.00^{ab}	11.00 ± 1.00^{b}	12.66 ± 0.57^{a}
ALT (u/l)	12.00 ± 1.00^{d}	21.00 ± 2.00^{c}	29.00 ± 2.00^{b}	33.33 ± 4.04^{b}	42.00 ± 3.60^{a}
AST (u/l)	65.00 ± 7.21^{d}	81.00 ± 5.00^{c}	78.00 ± 5.56^{c}	110.33 ± 8.08^{b}	147.33 ± 5.50^{a}
GGT (u/l)	0.96 ± 0.05^{b}	0.87 ± 0.12^{b}	0.95 ± 0.05^{b}	0.92 ± 0.10^{b}	1.20 ± 0.20^{a}
GLU (mg/dl)	58.00 ± 3.60^{d}	69.33 ± 2.30^{c}	73.33 ± 5.50^{bc}	77.66 ± 4.50^{ab}	81.00 ± 2.00^{a}
CHOL (g/dl)	12.00 ± 1.00^{a}	8.96 ± 1.05^{c}	7.86 ± 1.05^{c}	9.30 ± 1.53^{bc}	11.27 ± 0.92^{ab}
TBIL (mg/dl)	0.10 ± 0.00^{b}	0.13 ± 0.05^{ab}	0.13 ± 0.05^{ab}	0.16 ± 0.05^{ab}	0.23 ± 0.05^{a}

Note: Different superscripts in each row indicate significant differences ($p < 0.05$).
Abbreviations: ALB, Albumin; GLOB, Globulin; TP, Total protein; BUN, Blood urea nitrogen; CREA, Creatine; ALKP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma-glutamyltransferase; GLU, Glucose; CHOL, Cholesterol; TBIL, Total bilirubin.

was higher than the liver and intestine protein. Consequently, the protein deposition in the muscle was not affected by the dietary protein intake for all FSP diets. The protein level was significantly higher in this group, although it coincided with a significantly lower lipid level. Dawood et al. (2016) asserted that decreasing lipid or fat might indicate better digestion of lipid due to improved lipase activity and intestinal morphology by the act of colonized probiotic bacteria in the fish gut. Moreover, a good food fish would have body muscles consisting of high protein and low lipid contents (Wee and Tacon, 1982). Atlantic salmon (Dimitroglou et al., 2011) and striped catfish (Akter et al., 2019) fed with probiotics usually had increased protein and reduced lipid content. When the fish has reached proper growth, the higher protein levels observed in the muscle than the liver and intestine indicate protein mobilization from the former to the latter organ. Generally, the muscles have more proteins compared to the intestine and liver. This study obtained results that are similar to the work by previous researchers (Abidin et al., 2006; Kabir et al., 2015; Akter et al., 2015). These outcomes could be explained by the FSP diet fractions that contributed to the differences within the growth parameter values between the groups. While the diet influenced the protein content within the tissues, the FSP's nutritional quality influenced the growth and immune system development of African catfish.

In this study, the gut of fish fed with D3 had an intact epithelial barrier with a very well-organized villus structure, tunica muscularis, and goblet cells arrangement compared with the other groups of feed treatments (Fig. 1). Previous studies have reported morphological changes in the liver and intestine of fish fed with soybean meal (SBM) or other plant materials (Heikkinen et al., 2006; Lilleeng et al., 2007; Yamamoto et al., 2010; Nagel et al., 2012). After 18 weeks of feeding juvenile rainbow trout with SBM, typical histological changes included round-shaped and irregularly arranged absorptive vacuoles of epithelial cells of mucosal folding, presence of goblet cells, totally non-vacuolated areas, and an extent of inflammatory cells (Heikkinen et al., 2006). The nutritional status of fish is indicated by histological analysis of the intestine and liver (Caballero et al., 2004; Shiu et al., 2013). Depending on the species and feed utilized in the experiments, the intestinal and liver histopathological changes may vary. The inclusion of FSP causes histological, morphological, and functional changes within African catfish intestine and liver tissues. Atlantic salmon were observed to exclusively experience pathomorphological changes in the distal intestine, including the infiltration of a mixed leukocyte population within the lamina propria and submucosa, loss of the supranuclear vacuolization within the absorptive cells of the intestinal epithelium, widening and shortening of the intestinal folds, widening of the central lamina propria within the intestinal folds, and increase in animal tissue quantity (Lilleeng et al., 2007). Refstie et al. (2005) observed similar intestinal pathomorphological changes in Atlantic salmon, but the changes caused by SBM within the intestines were less distinct in fish given a diet of lactic acid-fermented soybean white flakes. Usually, the presence of biologically active compounds or ANFs like b-conglycinin and glycinin protein could cause abnormal morphology in the intestine and impaired capacity for digesting and absorbing nutrients in animals (Feng et al., 2007). Moreover, Kiers et al. (2000) observed a close to complete breakdown of b-conglycinin subunits and polypeptides from glycinin

after fermentation with *B. subtilis*. Similarly, Shiu et al. (2013) showed degradation of the antigenic components in SBM after static fermentation with B. *subtilis* E20, resulting in abnormal histopathological changes in the liver and intestine tissues of grouper. Additionally, reared rainbow trout given a FSBM diet produced using several bacterial taxa, of which mainly were *Bacillus* spp., showed enhanced histopathological changes in the livers and intestines, including having lamina propria of mucosal folds within the distal intestine, dark-staining nuclei, and cytoplasm of hepatocytes, fewer vacuoles of cytoplasm in livers and disordered hepatic cell cords (Yamamoto et al., 2010).

The liver cells of the fish fed with the D3 diet had better nuclei and cytoplasm structure compared with other groups. However, in the D4 and D5 diets, the cytoplasm and nuclei were atrophied, and hepatic cell cords were disordered compared with other groups (Fig. 2). The inclusion of FSP improved the pathomorphological changes in intestines, and the D3 diet improved them in the liver. The fish liver had atrophied in this study, and the diet of above D4 resulted in disordered hepatic cell cords and dark-staining nuclei and cytoplasm of hepatocytes. Additionally, fish fed D2 and D4 diets showed reduced and little goblet cells in their distal intestines, lamina propria infiltration, lamina propria widths, fold fusions, and abnormal mucosal fold. The histopathological changes described above were caused by D5 diets; these were improved by the FSP 50 % diet because the results were similar to the control group. Consequently, some ANFs in soybean by-products cause pathomorphological changes that cannot be entirely removed by fermentation. Therefore, more studies using fermentation and different species to ferment soybean by-products are required to evaluate the removal of more antinutritional factors.

The positive effects within the fish gut morphology following the FSP inclusion might cause an increase in growth response and digestive enzyme activity. Dimitroglou et al. (2009) reported that gut morphology improvements have a positive impact on feed utilization and boost the mucosal epithelium health status and increase its capacity to stop opportunistic bacterial infections. The microscopic analysis showed a significant increase in microvillus length within the posterior gut and villus length in both the anterior and posterior gut of fish at D3 and D4 diets. Nevertheless, no significant changes were observed in other gut structures, including crypt depth and villus width, with increasing FSP concentrations. The results often show better nutrient utilization and retention due to the better growth and FSP's apparent protein digestibility (APD) (Spring and Privulescu, 1998; Zhou et al., 2011). The same observations occurred in the gilthead sea bream (Dimitroglou et al., 2010), rainbow trout (Yilmaz et al., 2006; Dimitroglou et al., 2009), and early larvae and post-larvae of the ECU lobster (*Homarus gammarus*), which showed greater microvillus and villus length when supplemented with mannan oligosaccharide (MOS) diets (Daniels et al., 2010). In the study, since the intake of nutrients is linked to microvillus length, the data indicated that up to 75 % of FSP inclusion increased the alimentary canal's absorptive area in African catfish, consequently impacting villus structure with varying microbiota present within the species, species assessed, and concentration, rearing conditions. Probiotics, including *L. acidophilus* used in soybean by-products, have been reported to prevent pathogenic bacteria attachment and colonization in the alimentary canal (Rodriguez-Estrada et al., 2013). FSP may positively influence fish health and promote beneficial microbes such as bifidobacterial and LAB to selectively colonize the gut and reduce mortality rate (Wu et al., 2014; Mohammadi et al., 2012). This colonization has been connected to enhanced digestive enzyme activities within the gut (Burr et al., 2005). Corroborating past studies, this research showed a simultaneous growth in LAB and TB levels due to FSP in the gut even at the lowest level of 25 % but decreasing slowly in 75 % and 100 % of inclusion level. This finding strongly shows that FSP indirectly influences the digestibility of nutrients by providing a supportive environment for LAB proliferation and manipulating the gut microbiota to establish a beneficial microbial community.

The significant difference in white blood cell (WBC) and red blood cell (RBC) levels between the experimental diets indicated that the physiological properties were different, resulting in the increased amount of antigens within the circulation system (Taufek et al., 2016), increased capacity for oxygen transportation, and better anti-infection properties and immunostimulatory effects, all of which leading to healthier fish. Red blood corpuscle, haematocrit (HCT), and white blood corpuscle are indicators for fish health feed and anti-nutritional toxicity (Ozovehe, 2013). Increasing HCT and RBC with no additional FSP level allows regulating protein quantity in the red blood corpuscle, consequently sustaining the fish physiology. Moreover, differences in haemoglobin (HGB), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular haemoglobin (MCH) values between the groups of FSP levels show the health status.

Albumin level increased significantly along with supplementation level. However, the highest albumin level was achieved at the D3 diet, and it reduced drastically at the D4 diet. Albumin and globulins ensure a healthy system and function as plasma carriers (Nya and Austin, 2009). In this study, the albumin level and total protein increase for fish fed with FSP support the research by Yeganeh et al. (2015). The decreasing albumin level at the D3 diet could be caused by the decline in certain essential plasma levels, as Andrews et al. (2011) observed. Albumin and globulin are needed when assessing various liver and kidney diseases. Besides that, blood sugar is a sensitive indicator of pollutants that produce environmental stress in fish. The results of this study were supported by Shalaby (2007) and Mekkawy et al. (2011) on Nile tilapia's blood sugar levels. Changes in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities in the blood can also be used to indicate stress, including tissue impairment (James et al., 1991). The enzymes mobilization from the liver into the blood increases the AST and ALT values (Yang and Chen, 2003; Perez-Rostro et al., 2004). This study reported significant differences in ALT and AST activities in the plasma of African catfish fed with D4 and D5 diet compared with the control group. Increased ALT and AST levels in plasma show liver impairment and cellular or hepatocellular damage in the liver, heart, or muscle from anti-nutritional factors (ANFs) in the plant-based ingredients (Yamawaki et al., 1986). The innate immune reaction, which includes macrophages and neutrophils, is the first line of defense in the adaptive immune response (Zhang and Huang, 2006). The non-specific immune response is imperative because several pathogens can infect fish (Staykov et al., 2007). In the aquaculture industries, fish is fed with appropriate diets to improve the immune reaction (Momeni--Moghaddam et al., 2015). Adding FSP in the diet did not cause similar trend changes in the blood parameters and showed highly similar results to previous studies in silver catfish (Saccol et al., 2013; Gressler et al., 2015). This is an already reliable finding regarding the 50 % FSP inclusion level. Studies show that the inclusion of FSP can enhance the blood parameters of African catfish. While adding 30 % soybean flour within the *Takifugu rubripes* diet reduced haemoglobin and haematocrit (Lim et al., 2011), incorporating 34 % soybean flour in *S. hasta* juveniles diet resulted in anaemia due to the decline in haematocrit and red blood corpuscle counts (Yaghoubi et al., 2016). The FSP underwent microbial hydrolysis in this study, affecting the African catfish red blood cells. The higher peptide metabolic utilization of FSP could be contributing to the upkeep of erythropoiesis in fish (Biswas et al., 2017).

5. Conclusion

In conclusion, the replacement of FM with FSP resulted in significant improvement in the growth and health status of *Clarias gariepinus*. The results showed that 50 % of FSP inclusion level in fish feed for delivering probiotics to the fish gut could be used in the aquafeed industry for better growth and health status of African catfish and other freshwater fish production. This study will help researchers uncover the critical areas of probiotic delivery techniques and viability of probiotic bacteria like *Lactobacillus* spp. in aqua fish feed pellets that many researchers could not explore previously by using the soybean-by product.

Author contributions

ZAK: Writing-Original draft preparation, Methodology. **MAK**: Supervision, validation, writing-review& editing. **KM:** Software, validation. **NDR**: Software, validation. **MKAAR**: Funding acquisition, writing-review & editing. **NSNAA:** Software, validation. **HAE**: Data curation, formal analysis. **MZAR**: Software, validation. **SP**: Data curation, formal analysis. **MAOD:** Supervision, validation, writing-review& editing. **LSW**: Administration, funding acquisition, supervision.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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