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Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*)

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ABSTRACT

Fermented soy pulp (FSP) is currently capturing attention worldwide because of the high price of fish meal and its inconsistent supply in recent years. FSP replaces fish meal as a source of protein and energy towards improved fish health and growth. The protein replacement was employed in this study to assess the growth and health status, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish, *Clarias gariepinus*. The duration of the feeding experiment was 70 days. Five isonitrogenous. (32% crude protein) diets were prepared with FSP replacing D1 (0% FSP), D2 (25% FSP), D3 (50% FSP), D4 (75% FSP) and D5 (100% FSP) to FM component of the diets. D1 diet with 0% FSP was considered as a control. The fermentation process of FSP was carried out for three weeks. This resulted in the experimental diets having significantly different ($p < 0.05$) growth parameters. The D3 diet showed the highest weight gain and SGR with a mean and standard deviation of 1552.41 ± 81.67% and 1.73 ± 0.03%, respectively. D3 diet had better relative protein digestibility (RPD) value of 92.33 ± 2.19 compared with fish fed with the control diet. Amylase and lipase activities were found to be significantly higher in the D3 diet. The muscle amino acid profiles (arginine, isoleucine, histidine, and leucine) and gene expression (TGF-β1, lyzg, NF-kβ, and hsp90a) were significantly highest ($p < 0.05$) in the D3 diet. Fresh insights have been demonstrated by the findings of the study into the production of FSP as a replacement product. These insights would efficiently enhance the generation of aquafeed, which are low in cost and healthy towards the production of African catfish and other freshwater species. In conclusion, a new theory on using FSP as a plant-based replacement material and a protein replacement for fish growth and health status booster may be achieved at 50% of FSP inclusion.

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1. Introduction

Aquaculture is rapidly becoming the quickest growing food-producing business in the world due to the increasing demands for fish and other seafood. Nowadays, aquaculture industries often suffer severe financial losses that threaten their growth and health, caused mainly by the outbreaks of various diseases (FAO, 2016). However, the consistent supply and the cost of acquiring feed are the main issues in aquaculture (Pati et al., 2016; Zeller et al., 2018). Feed also contributes the most to the cost of sustainable fish farming for all species, including African catfish. The cost of feed is crucial because it usually makes up 30–70% of the whole operational costs and influences profitability in aquaculture investments (Muzinic et al., 2006; Danial, 2018). This makes the research into other protein sources vital as they could replace the fish meal (FM), which gradually decreases production. Higher FM prices have affected the sustainable production of fish products. Overfishing is a huge global challenge impacting FM production, which is expected to be unable to supply global demand by 2050 as aquaculture expands (Jiang et al., 2018). The partial or complete replacement of FM with other protein sources has been accelerated by the rise in FM cost, reduced availability, irregular supply, and poor quality (Sharawy et al., 2016).

Plant proteins are the most promising option because of their widespread availability and low cost (Zulhisyam et al., 2020a). As a result, the selection and development of plant ingredients as an option to FM and its expensive cost has piqued the global aquaculture sector's attention (Cheng et al., 2013; Azarm and Lee, 2014; Goda et al., 2014). This will eventually decrease the use of FM. Due to its stable supply, price, and nutritional value, soy pulp (SP) is deemed a potential alternative protein source from the various plant-based feed being researched as an FM substitute (Rahman et al., 2014). SP is a primary soybean waste for tofu, milk, sauce, milk powder, dried tofu, and juice productions and is either thrown out or given to ruminants. The latter is due to its nutritional value and superior functional characteristics (Rahman et al., 2014; Harthan and Cherney, 2017; Zulhisyam et al., 2020b).

Different types of chemicals and drugs are used to control diseases in the aquaculture industry (Dawood, 2021). These chemicals and medicines are nearly prohibited by the European Union (EU) and other countries because of their adverse effect on humans and the environment (Adel and Dawood, 2021). As a result, farmers and scientists nowadays use probiotics and plant-based protein to replace the chemicals and drugs in the aquaculture industry. Probiotics are used instead of medicines and chemicals to boost the growth of valuable microbes within the fish gut for increased growth and health status (Ding et al., 2015; Batista et al., 2016; Hasan et al., 2018; Uczay et al., 2019). However, the delivery of probiotics to the fish gut requires an effective method and medium to ensure its success in an aquatic environment. 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, an example being phytate, which is phosphate (P) in its main storage form (NRC, 2001). Trypsin inhibitors and lectins are ANFs that interfere with the activity of digestive enzymes (Gemede and Ratta, 2014). 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 fermentation approach may be achieved by reducing ANFs and improving the nutritional benefits of plant-based protein sources such as soybean meal (SBM) (Shiu et al., 2013; Azarm and Lee, 2014). Several recent pieces of research have looked into fermented soybean meal usage in animal feed (Rahman et al., 2014) and aquaculture (Yamamoto et al., 2010; Azarm and Lee, 2014) to replace FM as soybean meal is viewed as a novel 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 the current study, fermented soy pulp (FSP) was designated as a model replacement material of FM to deliver probiotics to the fish gut. *Lactobacillus* spp. was employed as a model probiotic and model bacterium to demonstrate this research into African catfish production. To get the optimum growth and health performance, the plant protein inclusion level should also be at optimum. Therefore, this study was carried out to determine the impact of FSP replacement at different percentages (0%, 25%, 50%, 75%, and 100%) on the growth and health, digestive enzymes, amino acid content, and gene expression in African catfish (*Clarias gariepinus*) from the utilization of FSP as a consumable replacement substance.

2. Materials and methods

2.1. Ethics statements

This study was carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals and was approved by the Animal Ethical Committee of Universiti Malaysia Kelantan (Malaysia).

2.2. Experimental fish and husbandry conditions

Two thousand one hundred African catfish fry (Length: 7 cm; Weight: 7 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 then stocked in three indoor fibre tanks (1000*L*) at a 700 fish/tank density. The experimental fish were acclimatized for 10 days. When the experiment was started, the fish were then stocked in 15 indoor fibre tanks (360 L) at 70 fish/tank density. The duration of the feeding experiment was 70 days. The fish were fed to satiation thrice daily in the early morning, late afternoon, and night (i.e., 6.30 am, 7 pm, and 11 pm). Throughout the experiment, the pH (7.4 ± 0.7), morning temperature (26.6 ± 1.9 °C), dissolved oxygen (5.9 ± 0.61 mg/L), total ammonia (1.23 ± 0.32 mg/L), nitrite (0.14 ± 0.3 mg/L), alkalinity (62.0 ± 12.2 mg $CaCO_3$/L) and hardness (94.8 ± 1.7 mg $CaCO_3$/L) of the water were maintained during the study.

2.3. Experimental diets

2.3.1. Preparation of FSP and experimental diets

Experimental diets were prepared in various levels of fermented soy pulp (FSP) at D1 (0% FSP), D2 (25% FSP), D3 (50% FSP), D4 (75% FSP), and D5 (100% FSP) of proteins from FM were replaced with that from FSP. The D1 was used as a control diet and contain 0% FSP. In brief, FSP was made through fermentation and mixed with Sigma^R commercial *Lactobacillus acidophilus* powder with a concentration of 10^{10} CFU/g and 10% molasses and kept in an HDPE container for three weeks for every 100 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 level. 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 pellets. These were sundried to about 10% moisture content, 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 observed the contents of the diet of essential amino acids (Kabir et al., 2015) expressed as a percentage of protein.

2.4. Measurement of fish growth parameters

The African catfish were made to fast for 24 h, and their total length

Table 1
Composition and proximate analysis (g/100 g dry weight) of the five experimental diets fed to African catfish (*Clarias gariepinus*).

Ingredients (%)	Diets (%)				
	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
Fish meal	36	27	18	9	0
Fermented soy pulp[1]	0	9	18	27	36
Soybean meal	36	36	36	36	36
Wheat	17	17	17	17	17
Vitamin-mineral premix	2	2	2	2	2
Fish oil	3	3	3	3	3
Vegetable oil	3	3	3	3	3
Binder[2]	3	3	3	3	3
Total	100	100	100	100	100
Proximate composition (%)					
Moisture	5.3	5.53	7.6	8.5	9.4
Protein	32.11	32.26	31.72	31.81	32.3
Lipid	5.42	4.13	4.39	4.61	4.85
Fibre	4.51	4.66	4.78	4.91	5.2
Ash	5.71	5.75	6.3	6.22	6.35
Carbohydrate	45.73	44.7	43.6	41.5	40.31

[1] FSP were added with 0.001 *Lactobacillus acidophilus* and 10% molasses for every 100 kg.
[2] Carboxymethyl Cellulose (CMC).

Table 2
The amino acid composition of the test diets is expressed as a percent of protein fed to *Clarias gariepinus* and their requirements. Data expressed as mean ± standard deviation (SD).

Amino acids	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)	*C. gariepinus* req*
Arginine	7.58 ± 0.05^{a}	5.91 ± 0.2^{b}	4.99 ± 1.2^{c}	4.20 ± 1.1^{d}	4.78 ± 0.7^{e}	1.20
Histidine	2.30 ± 2.07^{e}	6.69 ± 1.5^{a}	6.45 ± 0.0^{b}	5.93 ± 4.2^{c}	4.65 ± 2.0^{d}	0.42
Isoleucine	0.76 ± 1.98^{e}	0.86 ± 1.3^{d}	0.92 ± 0.0^{b}	0.9 ± 0.05^{c}	0.95 ± 1.1^{a}	0.73
Leucine	1.83 ± 0.05^{d}	1.80 ± 0.0^{e}	2.08 ± 0.0^{c}	2.70 ± 2.0^{a}	2.22 ± 4.3^{b}	0.98
Lysine	1.66 ± 0.05^{e}	1.45 ± 1.4^{b}	1.28 ± 0.0^{d}	1.35 ± 0.3^{c}	1.48 ± 0.0^{a}	1.43
Phenylalanine	1.15 ± 2.07^{e}	3.20 ± 0.0^{a}	2.40 ± 0.0^{c}	2.15 ± 0.2^{d}	2.95 ± 1.5^{b}	1.40
Threonine	0.90 ± 2.87^{c}	0.29 ± 0.2^{d}	0.96 ± 1.9^{b}	0.68 ± 0.3^{e}	1.06 ± 0.0^{a}	0.56
Methionine	1.02 ± 0.05^{a}	0.66 ± 3.3^{e}	0.76 ± 0.6^{c}	0.71 ± 0.6^{d}	0.98 ± 0.0^{b}	0.64

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).
* Amino acid requirement according to NRC (1993).

and weight measurements were taken under anesthetics 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. 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 weight - Initial weight) / Day of experiment x 100
Hepatosomatic index (%)	=	100*(Weight of liver / Total body weight)
Visceral somatic index (%)	=	(Viscera Weight / Fish Weight) x 100
Feed efficiency Rate (FER)	=	Live weight gain / Total feed intake
Protein efficiency rate (PER)	=	(Final Weight-Initial Weight) / Protein Intake
Intraperitoneal fat (IPF)	=	(IPF Weight / Fish Weight) x 100
Feed Conversion Rate (FCR)	=	(Total Feed Consumption / Weight Gain of Fish)

2.5. Determining relative protein digestibility (RPD)

An in vitro technique using the pH drop method was used to determine relative protein digestibility (RPD) before conducting the test for intestinal enzymes using a protein suspension mixture. The extraction of the crude intestinal enzyme was performed following the method proposed by Chisty (2005) with a few alterations and the Bradford (1976) method utilizing bovine serum albumin (BSA) as the standard was used to determine the protein concentration in the extracted crude intestinal enzyme. For the protein suspension, each diet was prepared using an equal amount of protein suspension. The mixture was kept at room temperature for 1 h while being occasionally stirred and then was placed in a vortex to mix it thoroughly. The supernatant was obtained by centrifuging the mixture at 10,000 x g, and the Bradford (1976) method with bovine serum albumin (BSA) as the standard was used to measure the protein concentration. In their work, Lazo et al. (1998) described this in vitro reactive protein digestibility method. The relative protein digestibility is determined by the crude enzymes isolated from the fish intestine and the protein suspension mixtures (Satterlee et al., 1979; Chisty, 2005; Sharifah et al., 2014). The following formula was employed in the calculation of relative protein digestibility (RPD):

$$\mathrm{Relative\,Protein\,Digestibility\,(RPD)} = \{(-\Delta\mathrm{pH feedstuff})/(-\Delta\mathrm{pH casein})\} \times 100$$

2.5.1. Total protein content in intestinal crude enzyme

The total protein content in intestinal crude enzyme was determined by the Bradford (1976) method using a Bio-Rad protein assay kit. Briefly, the prepared bio rad dye agent (200 μL in quantity) was added to all wells containing the extracted enzymes and BSA solution. The mixtures were incubated for 15 min at room temperature. The mixtures' absorbance was determined after incubation with a bio rad microplate reader (Model 680) at 595 nm. The absorbance of different BSA concentrations was used to prepare the standard curve.

2.6. Methodology for determining the activities of digestive enzymes

A random sample of five fish that had been starved for 4 h was chosen from each replicate tank at the end of the feeding trial. The fish samples were killed in distilled water at ice-cold temperature, and the whole intestinal tract (but not the stomach) was dissected on ice as approved by the Animal Ethics Committee of Universiti Malaysia Kelantan. The homogenate was placed in a centrifuge at 15,000 ×*g* at 4 °C for 15 min. The casein digestion method was used to measure protease activity as described by Walter (1984). One unit of specific protease activity is

defined as the enzyme quantity required to release one micromole tyrosine per mg protein of the enzyme extract (U/mg protein). The evaluation of amylase activity was conducted following the procedure described by Worthington (1988), which uses a substrate of soluble starch that reacts with dinitro salicylic acid. Maltose as the standard was used to measure amylase activity in the enzyme extract. One unit of amylase-specific activity is defined as the amount in micromoles of maltose released per mg protein (U/mg protein). The method proposed by Bier (1955) was used to measure lipase activity with a few alterations (Natalia et al., 2004). Lipase activity (U) is defined as the volume of 0.01 M NaOH needed to neutralize fatty acid release during the 4 h incubation period with the substrate and after correction by the appropriate blank.

2.7. Amino acid analysis

A method proposed by Kabir et al. (2015) was used to analyze amino acids present in the experimental ingredients, diets, fish muscle, liver, and intestine. To summarise the method, muscle, liver, and intestine samples were collected from all experimental fish groups at the end of the feeding trial and frozen at −20 °C before analysis. The body tissue samples were freeze-dried after being measured (Labconco model Freezone 2.5, Labconco Corporation, Kansas City, England) and ground. Amino acid components in the diets and fish body tissues were measured using hydrolysis with 6 N HCL at 110 °C for 24 h and derivatized with an AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamite) before undergoing chromatographic separation using an AccQ TagTM reversed-phase (3.9 × 150 mm) analytical column. An HPLC (High-Performance Liquid Chromatography) system was used to quantify the amino acid analysis. This system consisted of a Waters 1525 Binary HPLC Pump, 717 Plus auto-sampler (Waters (R) and Waters 2475 Multi λ Fluorescence detector (wavelength excitation 250 nm, emission 395 nm). In the quantitative measurement, the α-aminobutyric acid (AABA) was used as an internal standard. The eluents were acetonitrile and AccQ.TagTM. BreezeTM software version 3.20 was used to integrate, identify, and quantify the chromatographic peak by comparing it to known standards (Amino acid standard H, Pierce, Rockford, Illinois, USA). Sulfur amino acids, methionine, and cysteine were not measured in this work, and all analyses were run in triplicate.

2.8. Gene expression of growth and immune regulatory gene

Samples of 3 fish each were taken randomly from each replicate tank for each treatment after being denied food for 24 h. The work area was sterilized using DNase/RNase WIPER (ITN-21131, Intron Biotechnology) with 70% alcohol before starting the operation activities. Ice blocks were used to perform all dissections at a room temperature of 20 °C in the laboratory. The total RNA was extracted from the head kidney, and distal intestine of the individual African catfish as these tissues are known to possess the very best mRNA for growth and immunologic responses. One hundred mg of head kidney and intestine were taken and mixed with 1 ml lysis buffer in a 2 ml collection tube.

This research investigated the effectiveness of five experimental diets towards growth and immune response gene expressions (Transforming Growth Factor beta 1, Nuclear Factor kappa-B gene, heat shock protein 90, and Lysozyme G expressed in African catfish (*Clarius gariepinus*). The reference gene was beta-actin (β-Actin). Online Primer3 software was used to design gene-specific primers, which supported the cDNA sequences in GenBank, and the NCBI BLAST software was used to confirm all sequences (http://blast.ncbi.nlm.nih.gov). The verification of the proper amplification for four target genes and one house-keeping gene (reference gene) was done by conventional PCR. For the total RNA extraction, the protocol of easy spinTM (DNA free) Total RNA Extraction Kit (Cat No. 17221. iNtRON Biotechnology, Inc) was employed to extract the total RNA from the head kidney and intestine of *Clarias gariepinus*. The RNA samples and cDNA templates were analyzed for their purity using a NanoPhotometer (Implen, USA) to check for other possible contaminants in total RNA.

2.8.1. Quantitative real time-PCR

Growth and immune regulatory gene expressions were measured by quantitative real time-PCR. The housekeeping gene was β-Actin. The primer sequences of the genes studied are shown in Table 3. The qPCR reaction was carried out in triplicate in a final volume of 10 μl. qPCR in light Cycler 480 (Roche) using SYBR green (Qiagen) was used to quantify mRNA levels following the manufacturer's instructions. The method can be summed up by the following. Ten μl reactions were prepared in 96-well plates and enclosed 4 μl of 25 × diluted cDNA template, 1 μl of each primer pair at 5 μM and 5 μl of QuantiTect SYBR green containing ROX as the reference dye (Qiagen). The samples were amplified (45 cycles) after being denatured (15 min at 95 °C), which is consistent with the subsequent thermal cycling profile: denaturation for 10 s at 95 °C, annealing for 20 s at 60 °C, and extension for 20 s at 72 °C. The proper amplification was verified using gel electrophoresis, and the specificity of the qPCR reactions was firm up by melting curve analysis. The β-Actin reference gene was used to normalise the expression. The expression was presented as a relative expression in comparison with the non-treated control group. The correct amplification was verified using gel electrophoresis. The spreadsheet format was used to export all the C_T values. Gene expression was measured by the relative quantification method, which was carried out using the $2^{-\Delta\Delta}$CT method as stated by Schmittgen and Livak (2008). The following steps are specified by this method to measure the gene expression:

Step 1: Normalise the ΔC_T values of target genes with the reference gene:

$$\Delta C_T\ (\text{Supplemented diet}) = \Delta C_T\ (\text{Target gene, Supplemented Diet}) - \Delta C_T\ (\text{Reference gene, Supplemented Diet})$$

$$\Delta C_T\ (\text{Control}) = \Delta C_T\ (\text{Target gene, Control}) - \Delta C_T\ (\text{Reference gene, Control})$$

Step 2: Normalise C_T values of bioactive feeding supplment with the control diet

$$\Delta\Delta C_T = \Delta C_T\ (\text{Supplemented diet}) - \Delta C_T(\text{Control})$$

Finally, calculate the expression ratio as $2^{-\Delta\Delta CT}$ = Normalised expression ratio.

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

Table 3
The primer sequence for the growth and immune target genes used in this study.

Primer pair	Gene sequence	Length	Tm	Amplicon
Lyzg1_F	GAAGACTGACAGTGAGAGAATG	22	60	110 bp
Lyzg1_R	TGGACTCTCTGGAGATGATG	20	60	
NF-KB_1F	CCTAAATATCGGGACCAGAAC	21	60	111 bp
NF-KB_1R	CTGTGGATGGTAGGTGAAAG	20	60	
TGF-b1_F	TCCAGCAAGCTCAGAATAAC	20	60	122 bp
TGF-b1_R	GGGATTCTTGATCCGAAGAC	20	60	
Hsp90a_1F	CATCACAGGTGAGACCAAAG	20	60	146 bp
Hsp90a_1R	CCAGGTTCTTGCCATCATAC	20	60	
β-Actin_F	GCGTGACATCAAGGAGAAG	19	60	196
β-Actin_R	CAAGACTCCATACCCAAGAAAG	19	60	

presented as mean ± SD.

3. Results

3.1. Fish growth performance

There were significant differences ($p < 0.05$) in the growth parameters in the experimental diet groups in terms of final weight (g), final length (cm), weight gain (%), specific growth rate (%), hepatosomatic index (%), feed conversion rate (FCR), protein efficiency rate (PER) and intraperitoneal fat (%) (Table 4). The highest weight gain and specific growth rate occurred in the D3 diet compared with other groups. The D3 diet had a significantly ($p < 0.05$) higher value for the final length than other diets. African catfish in the D3 diet group had a significantly lower ($p < 0.05$) food conservation rate (FCR) compared with the other experimental diets. Specific growth rate (%), intraperitoneal fat (%), and protein efficiency rate (PER) were significantly highest ($p < 0.05$) in the D3 diet compared with other groups. 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. Relative protein digestibility (RPD)

Relative protein digestibility (RPD) in *Clarias garipinus* was positively affected by the FSP supplemented diets (Fig. 1). RPD values for all feed treatments were significantly different ($p < 0.05$). However, the D3 diet had a better RPD compared with fish fed with the control diet. Similar to growth performance, RPD values in the diet treatments increased significantly at D2 and continued until the D3 diet.

Table 4
Growth performance of African catfish fed with different FSP percentage for 70 days. Data expressed as mean ± standard deviation (SD).

Parameters	Diets (%)				
	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
IW(g)	23.73 ± 2.21	23.70 ± 2.16	24.10 ± 1.15	24.00 ± 2.18	23.70 ± 1.74
FW (g)	242.33 ± 2.07^{d}	306.33 ± 5.51^{b}	397.66 ± 3.52^{a}	277.66 ± 3.77^{c}	177.61 ± 1.5^{e}
IL (cm)	13.22 ± 2.31	13.02 ± 1.11	14.06 ± 0.17	13.92 ± 1.72	13.22 ± 1.52
FL (cm)	31.08 ± 1.86^{c}	32 ± 0.96^{b}	37.67 ± 1.86^{a}	32.68 ± 1.96^{b}	31.88 ± 2.22^{c}
WG (%)	992.97 ± 111.09^{d}	1200.27 ± 123.11^{b}	1552.41 ± 71.66^{a}	1064.03 ± 112.56^{c}	652.58 ± 61.53^{d}
SGR (%)	1.44 ± 0.16^{c}	1.59 ± 0.26^{b}	1.73 ± 0.13^{a}	1.52 ± 0.16^{d}	1.25 ± 0.07^{d}
VSI (%)	2.87 ± 0.35^{c}	3.70 ± 0.17^{ab}	3.55 ± 0.32^{b}	4.10 ± 0.45^{a}	4.20 ± 0.14^{a}
HSI (%)	1.39 ± 0.2^{e}	1.84 ± 0.4^{b}	1.58 ± 0.2^{d}	1.68 ± 0.3^{c}	2.01 ± 0.4^{a}
CF	0.76 ± 0.19^{ab}	0.84 ± 0.09^{a}	0.86 ± 0.16^{a}	0.82 ± 0.04^{a}	0.69 ± 0.03^{b}
SR (%)	90.95 ± 2.87^{e}	95.23 ± 3.49^{b}	96.57 ± 1.44^{a}	92.38 ± 2.87^{c}	91.90 ± 2.28^{d}
FCR (%)	1.62 ± 0.07^{a}	1.22 ± 0.04^{c}	1.01 ± 0.02^{d}	1.52 ± 0.23^{b}	2.19 ± 0.15^{a}
PER (%)	1.26 ± 0.26^{d}	1.42 ± 0.34^{b}	1.68 ± 0.92^{a}	1.39 ± 0.13^{c}	1.22 ± 0.14^{c}
IPF (%)	0.22 ± 0.23^{d}	0.49 ± 0.19^{c}	1.33 ± 0.50^{a}	0.61 ± 0.98^{b}	0.52 ± 0.38^{b}

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).
Abbreviation: IW, Initial weight; FW, Final weight; IL, Initial length; FL, Final length; WG, Wight gain; SGR, Specific growth rate; VSI, Visceral somatic index; HSI, Hepatosomatic index, CF, Condition factor; SR, Survival rate; FCR, Feed conversion rate; PER, Protein efficiency rate; IF, Intraperitoneal fat.

3.3. Blood plasma protease, lipase, and amylase

Digestive enzyme activities in the intestine are summarised in Table 5. Significantly higher amylase, protease, and lipase-specific activity levels were found in fish fed with the FSP supplemented diets. Amylase and lipase activities were significantly higher in the D3 diet than in the other treatments. However, protease enzyme activities were significantly higher in the D1 diet than in this study's experimental diets. The quantification of lipase activity was higher compared with amylase and protease enzyme activities in this study.

3.4. Muscle amino acids profile

Amino acid deposition in the muscle did not appear to follow a specific trend. Arginine content showed a significant increase ($p < 0.05$) in the muscle of fish fed with the D3 diet compared with other fish groups (Table 6). D1 and D3 diets showed isoleucine and histidine levels that were significantly higher ($p < 0.05$) than those fed the D4 and D5 diets. Arginine, leucine, lysine, isoleucine, threonine, glutamic acid, aspartic acid, glycine, and proline made up the main components of the muscle at all dietary FSP protein levels.

3.5. Liver amino acid profile

The fish liver amino acid profile varied and showed no discernible trend in response to the different FSP levels. For example, the D3 diet resulted in lysine and isoleucine levels that were significantly higher ($p < 0.05$) with mean and standard deviations of 4.63 ± 0.04% and 3.01 ± 0.07%, respectively, compared with other diets (Table 7). Arginine, leucine, histidine, glutamic acid, aspartic acid, glycine, and alanine were the main components of liver tissue amino acids at all FSP protein levels. The levels of aspartic acid and glutamic acid were significantly higher ($p < 0.05$) among the study's FSP protein levels.

3.6. Intestinal amino acid profile

The intestinal amino acid profile of African catfish fed with different FSP levels is shown in Table 8. Histidine, threonine, and valine contents were significantly higher ($p < 0.05$) in the intestine of fish fed with D2, D3, and D4 diets. The D5 diet showed a significantly higher amount of arginine compared with the other test diets. Arginine, lysine, valine, glutamic acid, alanine, and aspartic acid were the major amino acids in the intestine at all four FSP protein levels.

3.7. Growth, immune and stress gene expression in distal intestine and head kidney

Growth and immune-regulatory gene expressions were significantly up-regulated ($p < 0.05$) in fish given different levels of FSP diets (Figs. 2 and 3). The expression levels of TGF-β1 and lyzg mRNA in the fish intestinal tissue were significantly higher ($p < 0.05$) at the D3 diet compared with the other diets. In contrast, D2 and D3 diets resulted in levels of NF-kβ and hsp90a mRNA that were significantly higher ($p < 0.05$) than the other diets. The expression levels of fish kidney tissue TGF-β1 and lyzg mRNA were also significantly higher ($p < 0.05$) as the FSP levels increased from D2 to D5 diets compared with the control group. The D3 diet resulted in NF-kβ gene expression in the fish kidney tissue that was significantly higher ($p < 0.05$) than other diets, but there were no significant differences at the D3 and D4 diets for the Hsp90a kidney gene expression compared with the other FSP levels.

4. Discussion

4.1. Growth performances

In this study, FSP was found to be a high-quality protein, particularly

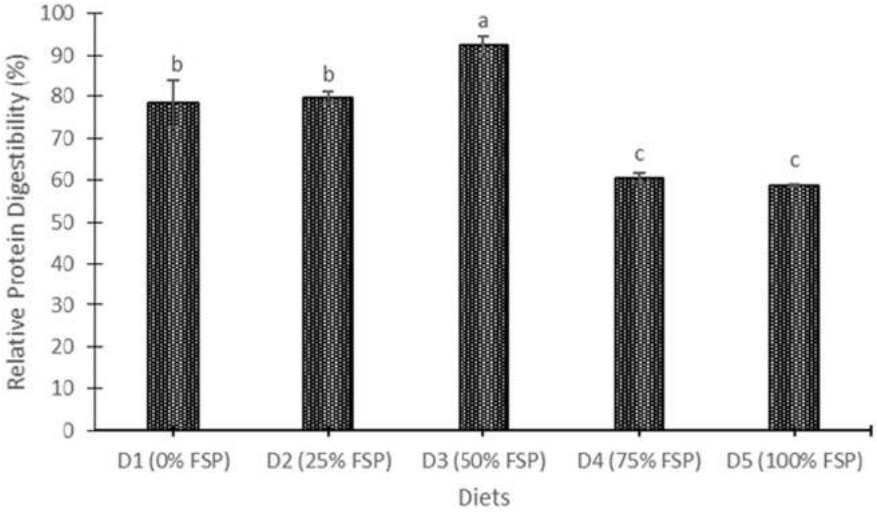

Fig. 1. Relative protein digestibility (RPD).

Table 5
The activities of digestive enzymes (U/ mg protein) in the intestine of African catfish fed with experimental diets ($n = 3$). Data expressed as mean ± SD.

Diets (%)					
Enzyme (U/mg protein)	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
Protease	3.49 ± 0.12^{a}	3.19 ± 0.10^{b}	3.08 ± 0.10^{bc}	2.96 ± 0.09^{cd}	2.79 ± 0.04^{d}
Lipase	8.09 ± 0.15^{c}	8.38 ± 0.09^{b}	8.96 ± 0.04^{a}	8.04 ± 0.17^{c}	7.28 ± 0.07^{d}
Amylase	6.07 ± 0.16^{c}	6.36 ± 0.08^{b}	6.99 ± 0.11^{a}	6.02 ± 0.18^{c}	5.24 ± 0.06^{d}

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

in terms of crude protein and essential amino acids. The crude protein percentage of diets tested in this study (32%) is close to the range of the optimum protein requirement of African catfishes (Monebi and Ugwumba, 2013). It is clear that FSP diets have abundant higher concentrations of free amino acids that increase the flavor and promote the changes in the biochemical properties of the feed or diets (Dajanta et al., 2011). The study showed that FSP could be used as a replacement material with FM up to 50% of the diet without growth impairment compared with other treatments. The bioactive FSP as a protein supplement acts as a growth promoter in the African catfish diet because they improved protein metabolic utilization from the diet and delivered to the fish gut. Furthermore, the plant ingredients' quality used in aquafeeds is also evaluated by a series of indicators like growth performance, biochemical composition, and by evaluating the effects on disease and histopathology (Poleksic et al., 2006; Poleksic et al., 2010; Raskovic et al., 2011). Many researchers have shown that the nutritional value of soybean by-product is improved by lactic acid fermentation through partial elimination of feed allergens and anti-nutritional factors (ANFs) that increases fish growth performance, nutrient digestibility, and fish physiological conditions like bile status and intestinal microbiota (Zulhisyam et al., 2021). In the present study, the D3 diet resulted in the highest weight gain (%) and specific growth rate (SGR) compared with other experimental diets. An optimal essential amino acid profile is very crucial for fish growth (Peres and Oliva-Teles, 2009). All the experimental diets in the current trial are of high protein quality (Oyelese, 2007) because each of them contains all the EAA with values in the ranges of the requirement of *Clarias gariepinus* (NRC, 1993; Djissou et al., 2016). In this study, the weight gain (%) and the specific growth rate (SGR) were reduced at D4, and D5 diets may be due to the ANFs in the

Table 6
Amino acid composition (% of dry matter basis) of muscle from fish fed different dietary soy pulp protein supplement levels (n = 3). Data expressed as mean ± SD.

	Diets (%)				
	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
Essential amino acid					
Arg	7.81 ± 0.78^{c}	9.23 ± 0.19^{b}	10.07 ± 0.04^{a}	9.06 ± 0.04^{a}	8.95 ± 0.06^{b}
His	1.58 ± 0.21^{ab}	1.81 ± 0.02^{a}	1.75 ± 0.09^{a}	1.50 ± 0.10^{b}	1.20 ± 0.08^{c}
Ile	3.29 ± 0.36^{b}	3.62 ± 0.26^{a}	3.46 ± 0.08^{ab}	3.34 ± 0.10^{b}	2.43 ± 0.10^{c}
Leu	4.65 ± 0.20^{a}	4.26 ± 0.07^{b}	3.85 ± 0.07^{c}	3.63 ± 0.20^{c}	4.83 ± 0.03^{a}
Lys	2.57 ± 0.76^{c}	5.21 ± 0.06^{b}	5.23 ± 0.11^{b}	6.12 ± 0.09^{a}	5.04 ± 0.04^{b}
Phe	3.01 ± 0.16^{c}	4.25 ± 0.14^{a}	3.07 ± 0.11^{c}	3.07 ± 0.05^{c}	3.55 ± 0.11^{b}
The	3.73 ± 0.17^{a}	3.58 ± 0.26^{a}	3.15 ± 0.15^{b}	2.99 ± 0.03^{bc}	2.84 ± 0.07^{c}
Val	2.53 ± 0.22^{d}	4.34 ± 0.11^{a}	4.09 ± 0.11^{b}	3.72 ± 0.08^{c}	3.99 ± 0.08^{b}
Met	2.39 ± 0.15^{a}	1.68 ± 0.09^{b}	1.47 ± 0.06^{c}	1.44 ± 0.05^{c}	1.25 ± 0.07^{d}
Non-essential amino acid					
Ala	5.05 ± 0.11^{b}	5.88 ± 0.12^{a}	5.63 ± 0.07^{a}	5.12 ± 0.08^{b}	4.61 ± 0.09^{c}
Asp	6.29 ± 0.39^{d}	6.84 ± 0.14^{c}	8.73 ± 0.18^{a}	8.04 ± 0.07^{b}	6.18 ± 0.04^{d}
Glu	11.39 ± 0.69^{b}	12.84 ± 0.67^{a}	13.45 ± 0.34^{a}	12.70 ± 0.25^{a}	10.84 ± 0.14^{b}
Gly	5.37 ± 0.50^{b}	5.64 ± 0.17^{b}	4.76 ± 0.08^{c}	4.48 ± 0.17^{c}	7.72 ± 0.14^{a}
Pro	5.61 ± 0.19^{a}	4.73 ± 0.09^{b}	4.15 ± 0.06^{c}	3.72 ± 0.11^{d}	3.76 ± 0.08^{d}
Ser	2.81 ± 0.11^{a}	2.66 ± 0.10^{a}	2.22 ± 0.10^{b}	2.14 ± 0.06^{b}	1.83 ± 0.07^{c}
Tyr	3.81 ± 0.16^{a}	2.99 ± 0.11^{b}	2.34 ± 0.22^{c}	2.31 ± 0.09^{c}	2.31 ± 0.08^{c}

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

Abbreviation: Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, leucine; Lys, Lysine; Phe, Phenylalanine; Thr, Threonine; Val, Valine; Met, Methionine; Ala, Alanine; Asp; Aspartic acid; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine.

Table 7
Amino acid composition (% of dry matter basis) of liver from fish fed different dietary soy pulp protein supplement levels (n = 3). Data expressed as mean ± SD.

	Diets (%)				
	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
Essential amino acid					
Arg	3.57 ± 0.03^{d}	5.59 ± 0.06^{c}	7.69 ± 0.02^{b}	8.60 ± 0.04^{a}	8.61 ± 0.07^{a}
His	3.37 ± 0.04^{a}	2.83 ± 0.07^{b}	2.02 ± 0.06^{c}	1.56 ± 0.05^{d}	1.35 ± 0.03^{e}
Ile	2.54 ± 0.06^{c}	2.47 ± 0.05^{c}	3.01 ± 0.07^{a}	2.70 ± 0.04^{b}	2.77 ± 0.04^{b}
Leu	5.31 ± 0.09^{a}	4.52 ± 0.03^{b}	3.12 ± 0.03^{c}	3.09 ± 0.02^{c}	2.95 ± 0.03^{d}
Lys	3.09 ± 0.05^{d}	4.41 ± 0.01^{c}	4.63 ± 0.04^{a}	4.54 ± 0.04^{b}	4.50 ± 0.01^{b}
Phe	2.63 ± 0.06^{d}	2.80 ± 0.06^{c}	3.11 ± 0.02^{ab}	3.02 ± 0.04^{b}	3.13 ± 0.04^{a}
The	2.72 ± 0.04^{a}	2.72 ± 0.02^{a}	2.31 ± 0.03^{b}	2.29 ± 0.04^{b}	2.19 ± 0.04^{c}
Val	1.62 ± 0.04^{e}	2.80 ± 0.03^{d}	3.56 ± 0.04^{c}	3.63 ± 0.01^{b}	3.71 ± 0.04^{a}
Met	1.46 ± 0.08^{d}	1.93 ± 0.02^{a}	1.84 ± 0.02^{b}	1.56 ± 0.03^{c}	1.43 ± 0.02^{d}
Non-essential amino acid					
Ala	6.45 ± 0.07^{a}	4.86 ± 0.07^{b}	3.23 ± 0.06^{c}	3.09 ± 0.02^{d}	3.10 ± 0.01^{d}
Asp	2.75 ± 0.07^{e}	3.70 ± 0.02^{d}	5.33 ± 0.06^{a}	4.72 ± 0.03^{c}	5.01 ± 0.08^{b}
Glu	4.07 ± 0.02^{e}	5.44 ± 0.03^{d}	6.75 ± 0.03^{a}	6.56 ± 0.03^{b}	6.36 ± 0.06^{c}
Gly	4.24 ± 0.05^{a}	3.39 ± 0.05^{b}	3.17 ± 0.02^{c}	3.01 ± 0.07^{d}	3.07 ± 0.05^{cd}
Pro	3.20 ± 0.10^{a}	2.51 ± 0.01^{d}	2.88 ± 0.03^{c}	3.09 ± 0.03^{b}	3.06 ± 0.01^{b}
Ser	2.16 ± 0.02^{a}	2.09 ± 0.01^{a}	1.97 ± 0.03^{b}	1.84 ± 0.04^{c}	1.77 ± 0.02^{d}
Tyr	2.23 ± 0.07^{a}	1.72 ± 0.08^{b}	1.08 ± 0.03^{c}	0.81 ± 0.03^{d}	0.80 ± 0.02^{d}

Note: Different superscripts in each row indicate significant difference ($p < 0.05$)
Abbreviation: Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, leucine; Lys, Lysine; Phe, Phenylalanine; Thr, Threonine; Val, Valine; Met, Methionine; Ala, Alanine; Asp; Aspartic acid; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine.

Table 8
Intestinal amino acid composition (% of dry matter basis) of fish fed different dietary soy pulp protein supplement levels (n = 3). Data expressed as mean ± SD.

	Diets (%)				
	D1 (0% FSP)	D2 (25% FSP)	D3 (50% FSP)	D4 (75% FSP)	D5 (100% FSP)
Essential amino acid					
Arg	9.29 ± 0.14^{e}	9.97 ± 0.07^{d}	10.33 ± 0.10^{c}	13.89 ± 0.09^{b}	16.12 ± 0.20^{a}
His	1.23 ± 0.04^{b}	1.29 ± 0.03^{ab}	1.37 ± 0.06^{a}	1.34 ± 0.06^{a}	1.20 ± 0.04^{b}
Ile	2.74 ± 0.08^{a}	2.50 ± 0.06^{b}	2.47 ± 0.05^{b}	1.76 ± 0.06^{c}	1.72 ± 0.06^{c}
Leu	2.63 ± 0.08^{e}	2.90 ± 0.12^{d}	3.19 ± 0.09^{c}	4.91 ± 0.04^{a}	4.52 ± 0.15^{b}
Lys	4.40 ± 0.08^{b}	4.80 ± 0.06^{a}	4.40 ± 0.12^{b}	4.20 ± 0.11^{c}	3.52 ± 0.09^{d}
Phe	2.70 ± 0.06^{b}	3.06 ± 0.03^{a}	2.63 ± 0.05^{b}	2.99 ± 0.10^{a}	2.40 ± 0.11^{c}
The	2.32 ± 0.10^{b}	2.59 ± 0.07^{a}	2.55 ± 0.12^{a}	2.61 ± 0.07^{a}	2.22 ± 0.09^{b}
Val	3.17 ± 0.05^{b}	3.49 ± 0.06^{a}	3.59 ± 0.06^{a}	3.60 ± 0.09^{a}	3.54 ± 0.05^{a}
Met	0.31 ± 0.09^{ab}	0.40 ± 0.10^{a}	0.40 ± 0.06^{a}	0.37 ± 0.07^{a}	0.19 ± 0.02^{b}
Non-essential amino acid					
Ala	3.41 ± 0.16^{d}	3.94 ± 0.06^{b}	4.15 ± 0.06^{a}	3.73 ± 0.09^{c}	3.39 ± 0.11^{d}
Asp	4.27 ± 0.13^{d}	5.17 ± 0.08^{c}	5.96 ± 0.07^{a}	5.52 ± 0.11^{b}	5.00 ± 0.08^{c}
Glu	6.84 ± 0.09^{d}	8.06 ± 0.07^{b}	8.76 ± 0.11^{a}	6.80 ± 0.07^{d}	7.28 ± 0.15^{c}
Gly	4.36 ± 0.17^{b}	4.57 ± 0.14^{b}	4.83 ± 0.06^{a}	4.43 ± 0.09^{b}	3.88 ± 0.04^{c}
Pro	4.44 ± 0.05^{a}	3.93 ± 0.05^{b}	4.41 ± 0.07^{a}	3.76 ± 0.09^{c}	3.42 ± 0.09^{d}
Ser	2.08 ± 0.09^{a}	2.10 ± 0.09^{a}	1.97 ± 0.08^{a}	2.08 ± 0.02^{a}	1.54 ± 0.04^{b}
Tyr	1.33 ± 0.04^{d}	2.04 ± 0.06^{c}	2.29 ± 0.07^{b}	2.43 ± 0.04^{a}	2.09 ± 0.02^{c}

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).
Abbreviation: Arg, Arginine; His, Histidine; lle, Isoleucine; Leu, leucine; Lys, Lysine; Phe, Phenylalanine; Thr, Threonine; Val, Valine; Met, Methionine; Ala, Alanine; Asp; Aspartic acid; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine.

diet were higher compared with others percentage since more FSP used in the diet preparation and automatically effected the growth performances of African catfish. However, further research was needed to support the statement above. Probiotics like *Lactobacillus acidophilus* have been reported to cause the production of bioactive materials, which in turn have intrinsic biogenic effects (Mohammadi et al., 2021). According to Stanton et al. (2005) who revealed that fermentation could 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; Mohammadi et al., 2020).

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 it increased significantly at D4 and D5 diets. The lowest FCR was observed at the D3 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). In addition, the inclusion of probiotics in animal feed increased the protein efficiency rate (PER), which is a good indicator for growth performances and helped reduce the feed conversion rate. In this study, the performance trend of PER was similar to the weight gain (%) and specific growth rate (%), which is increasing from control feed to D3 diets but decreasing after the D4 diet. The inclusion of *Lactobacillus acidophilus* and other probiotics in diets resulted in more activity in the gastrointestinal tract (Marteau et al., 1993; Mohammadi et al., 2021) and automatically increased the PER and reduced FCR. The overall fish survival rate in this study was above 90%, and the highest is at the D3 diet. This result indicates that fish can learn and perceive tested diet conditions in the culture environment at the commercial stage.

The fundamental biological ratios in the present study such as the visceral somatic index (VSI) and intraperitoneal fat (IPF) were evaluated as it is essential parameters of assessing the feed value (Keri et al., 2014). The results showed that VSI was higher in fish fed with FSP than in the control diet. Ahmad et al. (2012) reported that VSI increased with increases in carbohydrate levels. However, in this study, the carbohydrate level was almost the same at a range of 40% to 45% in the experimental feed. Besides that, the D3 diet gave the highest value of IPF and, according to Chaiyapechara et al. (2003), indicated that lower IPF value could affect the growth and health status of fish and the nutritional qualities of fish fillets.

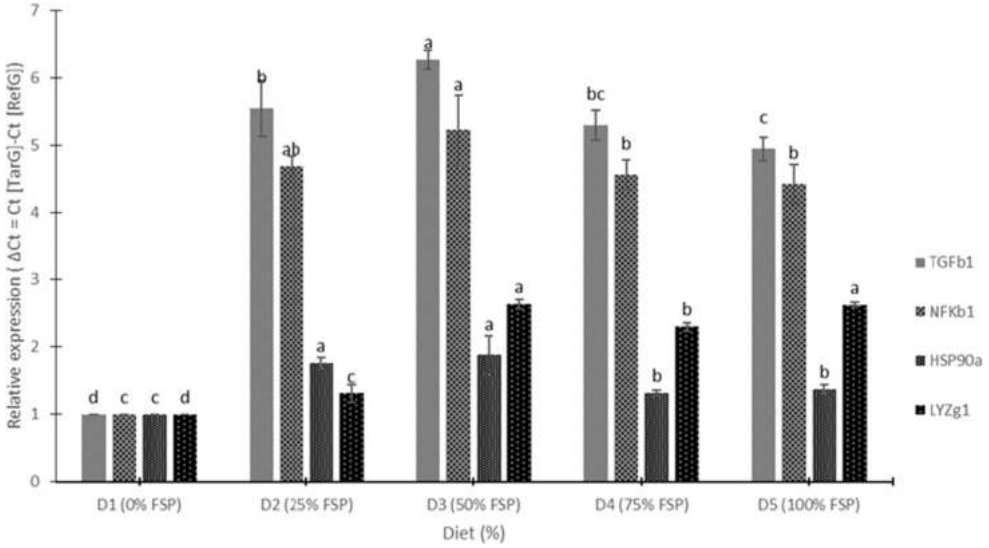

Fig. 2. Gene expression of intestine for all experimental diets.

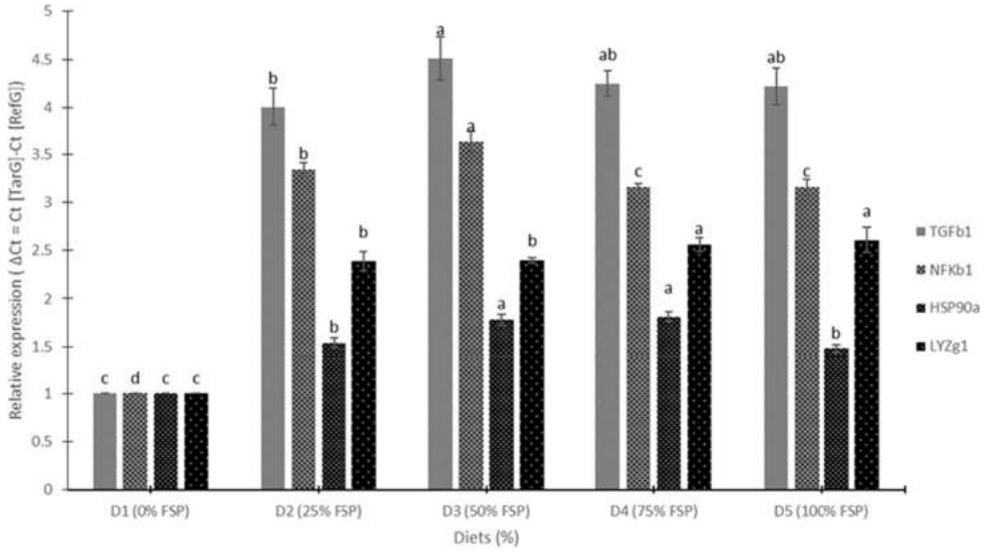

Fig. 3. Gene expression of kidney for all experimental diets.

4.2. Relative protein digestibility and plasma protease, lipase, and amylase

The consumption of soy pulp (SP) results in the assembly of bioactive microbial metabolites like vitamins, bioactive peptides, organic acids, or fatty acids during fermentation. These metabolites enhance overall nutrient intake in the gut, which enhances growth rates. Research into freshwater fish like *Clarias gariepinus* has generally demonstrated that FSP directly impacts the live microorganisms within the probiotics of LAB on the gastrointestinal wall, thus causing a superior rate of fermentation in the lumen as opposed to FSP's indirect action mechanism. In *Lactobacillus acidophilus* supplementation, a direct rise in its population in the gut could have replaced not only the pathogenic bacteria but also produce nutrients and stimulate digestive enzyme discharge (Cüneyt et al., 2008; Dawood et al., 2020). This activity is often complemented by the upper gut digestive enzyme activities in fish fed with FSP supplemented diets and results in better relative protein digestibility (Fig. 1) in fish fed with the FSP diet. The differences in FSP percentages among the experimental diets may have resulted in the various responses observed among the fish feed with FSP feed. In the upper amylase and lipase specific activity, amylase appeared to be more easily digested within the gut compared with protease and subsequently leads to a significantly better relative protein digestibility in the FSP diet as gut fermentation matures. As such, this research proposes that up to 12 weeks of prolonged FSP feeding positively affected nutrient digestibility and growth performance, though this benefit is neutralized when FSP addition crosses the 50% threshold in the tested diet.

4.3. Amino acid composition of muscle, liver, and intestine

The nutritional quality of dietary FSP protein, i.e., amino acids, could affect the growth performance and health status of African catfish fingerlings. It is not clear if the effects of amino acids are independent or associated with each other. An adequate quantity of amino acids is essential for successful growth in animals; lack of protein might halt or retard growth later. Amino alkanoic acid profiles of the diets generally reflect the rise in protein levels in fish body tissues of the tested diets. Within the present study, amino acid level appeared not to be influenced by dietary intake, and amino acid deposition differed among the body tissues tested at different FSP protein level diets. The muscle amino acid

profiles of arginine, isoleucine, histidine, and leucine were significantly higher at the D3 dietary compared with the others, giving rise to speculation that the prime quantity of those amino acids may reflect a positive role within the fish weight gain. Kabir et al. (2015) found that a lack of one or more amino acids would likely limit protein synthesis, growth, or both in African catfish. Moreover, amino acid profiles like arginine, leucine, lysine, isoleucine, threonine, glutaminic acid, amino acid, lysine, and proline were significant components in the lowest protein level diet in the muscle of African catfish (*Clarias gariepinus*) fingerlings.

The fish liver amino acids in this study showed variable and inconsistent significant differences in all the FSP protein levels. This inconsistency could be caused by the liver's role in the uptake and output of nutrient requirements and the fact that the liver is the main producer of nutrients through metabolic activities. In the liver of African catfish (*C. garipinus*) fingerlings, amino acids like arginine, leucine, histidine, glutaminic acid, amino acid, glycine, and alanine were found at the highest concentration in the lowest dietary FSP protein levels. Gunasekera et al. (1997) discovered the highest levels of proline and histidine in the serum at various dietary protein levels in Nile tilapia, *Oreochromis niloticus*. The liver and intestinal amino acids are likely to be connected because essential nutrients are passed to the liver from the intestine through the blood during nutrient metabolism. In this regard, various similarities were observed. For example, equivalent amino acids of arginine, leucine, and glutaminic acid were observed at the highest concentrations in the intestine and liver. It is speculated that these amino acids could have a vital function in the synthesis of protein and, as such, could influence the fish growth parameters and health status. Rønnestad et al. (1999) suggested that the free amino acid pool is used as an energy substrate and for protein synthesis. Harpaz (2005) suggested that lysine could be used as a substrate in carnitine synthesis, which enhances fish growth performance. Essential amino acids including leucine, lysine, valine, and isoleucine and non-essential amino alkanoic acids including glutaminic acid, amino acid, alanine, and serine have been reported to be more abundant within the *Pangasianodon hypophthalmus* or shark catfish muscle, liver, and egg (Kabir et al., 2015). Knowledge concerning amino acids in the muscle and other body tissues could also be used to improve the quality of feed for African catfish (*C. gariepinus*) fingerling aquaculture. African catfish (*C. gariepinus*) dietary FSP protein level and the distribution of amino acids in its tissues differ from the species reported in the present study. Omnivorous and herbivorous fish normally require less dietary protein compared to carnivorous fish. The dietary FSP protein level and amino acid differences could be attributed to their species-specific role, diet, and experimental methodology, FCR, protein quality, digestibility, amino alkanoic acid composition of dietary protein, energy state in diet, and variations in lipid level (Colabella et al., 2011; Lanes et al., 2012; Kabir et al., 2015).

4.4. Growth, immune and stress gene expression in distal intestine and head kidney

The findings of this study, as well as the subsequent discussion, have mostly supported the growth, immune, and stress-related gene expressions in the fish head kidney and intestine segments, which are regarded immunologically important sites in fish (Rombout et al., 2011) where the FSP supplemented diets may affect the elicited responses immediately. The data from the study showed that the gene expressions in fish fed the experimental diets containing FSP were significantly modified compared with the control group fish. These modifications almost mirror the growth performance trend described earlier. These results have provided further evidence supporting the nutrigenomic principle that nutrients in a feed formula are dietary signals which may be detected by a cellular sensor system, affecting the expression of genes and, therefore the protein expression, and subsequently produce metabolites (Müller and Kersten, 2003) which improve the standard of the growth mRNA and immune regulatory genes like transforming growth factor (TGF) β1, interleukin (IL)-1, interleukin (IL)-8, interleukin (IL)-10, nuclear factor (NF) -β (Miyazaki et al., 1997; Letterio and Roberts, 1998; Gilmore, 2006). The resulting metabolites have been reported to act as biological response modifiers (Bhon and BeMiller, 1995; Dallard et al., 2007), in immunomodulation (Chanpul et al., 2012; Novak and Vetvicka, 2009), and immune stimulant roles (Meena et al., 2013) which enhance cytokine assembly to stimulate the NK cells, B-cells and T-cells to prepare for pathogenic infection (Bunselmeyer and Buddendick, 2010). The induction of heat shock proteins (hsp90a) in fish could be a cellular stress response against various stressors like osmotic stress, heat shock, or infections (Basu et al., 2002). Hsp90b1 (also referred to as gp96 or grp94) acts as the primary chaperone of the endoplasmic reticulum and has crucial immunological functions, functioning as a natural adjuvant for priming innate and adaptive immunity (Strabo and Polack, 2008). Distal intestine hsp90a and head-kidney hsp90a mRNA levels significantly suffered in the FSP protein supplement diets, with lower expression in fish fed with higher FSP levels during this study. Similar results have been found by Batista et al. (2016), who considered the effects of probiotics and found that *Senegalese sole* given a diet with yeast supplementation had higher hsp90b expression within the distal intestine, while fish fed multispecies probiotics displayed higher hsp90b1 and hsp90a transcript levels in the distal intestine and rectum, respectively. However, Hansen et al. (2006) found that hepatic expression of hsp70 and hsp90 was unchanged in Atlantic cod fed high dietary plant protein levels. It is presumed that this difference is attributable to species-specific variability and/or suboptimal diet differences. However, the relatively high hsp90a expression level in African catfish that were given FSP may also be connected to hsp90a characteristics. Hsp90a could be a constitutive heat shock cognate protein expressed under normal growth conditions such as development and cellular differentiation.

The analysis of distal intestine and head kidney tissues in the study presented treatment-related differences for lyzg transcript levels among the FSP diets. Kim and Austin (2006) showed that some nutrients or immunostimulants, which include probiotics, are often supplemented in the feed to modulate serum lysozyme activity in fish. Nutritional imbalances and diet composition might have roles in the natural system of fish oxidation processes and antioxidation defense mechanisms (Rueda-Jasso et al., 2004; Nayak, 2010). In African catfish, dietary FSP plant ingredients were connected to a decrease in the transcript levels of four genes in both head kidney and distal intestine, compared with the control group. This indicates that FSP protein supplement ingredients may stimulate growth, immune and stress defense mechanisms. These results suggest a significant variance in the expansion and immunostimulatory effect of FSP on fish species, diet composition, and probiotic strains and agree with previous studies into *Senegalese sole* (Batista et al., 2016) and rainbow trout (Merrifield et al., 2010).

5. Conclusion

In conclusion, replacing FM with FSP diets resulted in significant improvement in the growth and health status of *Clarias gariepinus*. The results showed that the replacement of 50% FM with FSP could be used in the aquafeed industry for better growth and health status of African catfish and other freshwater fish production. This study will aid researchers in identifying crucial aspects of probiotic delivery methodologies and viability of probiotic bacteria such as *Lactobacillus* spp. in aquafeed pellets that many researchers were previously unable to investigate due to the use of FSP as a substitute material.

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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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