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Effects of Dietary Replacement of Fish Meal by Raw and Fermented Soybean Residues on Growth Performance, Biological Parameters and Nutrient Digestibility in Red Tilapia Fish (*Oreochromis* sp.)

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HPN, DVT, NTTT and HTTM involved in conception and design of study, performed experimental work and drafted the manuscript. NTKH, TNTN, NPT, TTL and NTHM involved in sampling, analysis and interpretation of data.

Key words
Soybean residue, Fermentation, Growth, Digestion, Red tilapia

ABSTRACT

This study was conducted to examine the effects of dietary replacement of fish meal (FM) by raw soybean residue (SR) and fermented soybean residue (FSR) on growth performance, biological parameters and nutrient apparent digestibility coefficients (ADCs) of red tilapia (*Oreochromis* sp.). FSR was obtained by fermenting SR with *Bacillus subtilis* V37. Five isonitrogenous and isoenergetic diets were formulated to replace 35% or 50% of FM by SR or FSR. The diets were denoted as follows: FMD, SR35D, FSR35D, SR50D, and FSR50D. The FMD (the reference diet) contained FM as a main source of dietary protein. A total of 300 fingerling red tilapia with an initial body weight (BW) of 13.7 g were randomly distributed into 15 tanks (20 fish/tank, 3 tanks/dietary treatment) and fed the experimental diets twice daily, for 8 weeks. Results showed that fish fed SR35D and SR50D had significantly lower final BW, weight gain (WG) and specific growth rate (SGR), but higher feed conversion ratio (FCR), than fish fed FMD ($P < 0.05$). Meanwhile, no significant differences in growth performance and feed utilization between the FSR35D and FMD groups were detected. Fish fed FSR-included diets showed significantly better growth performance and FCR than those fed SR-included diets ($P < 0.05$). Feeding the fish with SR35D and SR50D reduced digestive enzyme activity, bile juice secretion, and nutrient ADCs. These parameters of the experimental fish were markedly improved by feeding with FSR35D and FSR50D, and no statistical differences were observed between FSR35D- and FMD-fed fish. The results of the current study indicated that SR interfered with digestive enzyme activity, bile juice secretion, nutrient ADCs, and growth and feed performances. The enhancements of these parameters in fish fed FSR-included diets suggested that fermentation of SR with *Bacillus subtilis* V37 benefited digestive physiology, growth performance and feed utilization of red tilapia fed soybean protein-based diets.

INTRODUCTION

Fish meal (FM) is commonly used as the main protein source in aquafeeds; however, the limited supply of this ingredient and the increase of aquaculture industry require alternatives for replacement of FM (Olsen and Hasan, 2012). Soybean residue (SR), so called okara, is a by-product left from ground soybean after extracting water-soluble components used to produce soymilk or soybean curd (Noguchi, 1987). SR is relatively rich in nutrients with large quantities produced every year (O'Toole, 1999),

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therefore, this meal can be a potential alternative to replace FM in aquafeeds. On dry matter basis, contents of crude protein, crude lipid, crude fiber, nitrogen free extract, and ash of SR range from 15.2 to 32.2%, 6.9 to 10.9%, 9.1 to 18.6%, 27.5 to 46.3%, and 3.0 to 4.5%, respectively (Bourne, 1976; Van der Riet *et al*., 1989; O'Toole, 1999; Li *et al*., 2012).

Soybean reportedly contains anti-nutritional factors, such as β-conglycinin, glycinin, trypsin inhibitors, stachyose, raffinose, saponins, lectins, and phytate, which negatively influence growth performance, feed utilization, and physiological conditions of aquatic animals, including fish species (Francis *et al*., 2001; Krogdahl *et al*., 2003; Iwashita *et al*., 2008). Since SR is the residue obtained after removal of water-soluble fractions from soybean, this ingredient may contain substances which induce detrimental effects on growth and feed performances and physiological conditions in fish. It has been suggested that fermentation is an effective method to decrease the amount of anti-nutritional factors and improve the nutritional quality of soybean meal (Hong *et al*., 2004; Feng *et al*., 2007). Some studies have demonstrated that feeding fish with fermented soybean meal enhances growth, FCR, nutrient ADCs, and digestive physiology as compared to unfermented soybean meal (Yamamoto *et al*., 2010; Nguyen *et al*., 2015; Wang *et al*., 2019; He *et al*., 2020). These findings suggest that fermentation of SR and inclusion of fermented SR (FSR) in aquafeeds may be beneficial to body growth, feed utilization and physiological conditions of fish.

Red tilapia (*Oreochromis* sp.) is one of the most preferred and produced fish in aquaculture in Asia due to its fast growth rate, good meat quality and color, and high market demand (Boerlage *et al*., 2017). To date, there have been no studies to replace FM by SR or FSR in diets for this fish. Therefore, the current study aimed to examine the effects of dietary replacement of FM by SR or FSR on growth and feed performances, biological parameters, and nutrient ADCs of red tilapia.

MATERIALS AND METHODS

Soybean residue fermentation

Commercially available SR (crude protein content 17%) was purchased from Vinasoy Corp (Tu Son, Bac Ninh, Vietnam). This meal was fermented with *Bacillus subtilis* V37 provided by Department of Biotechnology and Microbiology, Hanoi National University of Education, Hanoi, Vietnam (Thuoc and Hung, 2021) to produce FSR. Briefly, the SR was autoclaved at 121°C for 30 min. After cooling, the SR (moisture content, 70%) was inoculated with 7.5% (v/w) of a culture broth of *Bacillus subtilis* V37 containing 10^8 CFU/ml. After through mixing under sterile conditions, the inoculated SR was incubated in a chamber at 37°C for 18 h. The processed materials were then dried in an oven at 60°C for 48 h. Finally, the FSR was ground to below 400 µm mesh size and stored at -20°C until use. The SR was also dried, ground and stored at the same conditions as the FSR. The proximate composition and total spore count of the SR and FSR were analyzed and presented in Table I.

Table I. Proximate composition of the SR and FSR (g/kg, dry matter basis).

	SR[1]	FSR[2]
Crude protein	169	161
Crude lipid	49	61
Ash	41	43
Crude fiber	206	128
Nitrogen free extract[3]	535	607
Total spore count (10^6 CFU/g)	0	55.5

[1]Soybean residue; [2]Fermented soybean residue; [3]Nitrogen free extract (NFE)=100 - [crude protein (%) + crude lipid (%) + ash (%) + crude fiber (%)].

Experimental diets

Five isonitrogenous and isolipitic diets were formulated to replace 35% or 50% of fish meal by SR or FSR (Table II). The diets were named as follows: FMD (FM diet, the reference), SR35D (35% SR diet), FSR35D (35% FSR diet), SR50D (50% SR diet), and FSR50D (50% FSR diet). Because the content of methionine in SR was relatively low (Li *et al*., 2012), this amino acid was added in the SR35D and FSR35D at the level of 7 g/kg diet and in the SR50D and FSR50D at the level of 10 g/kg diet in order to equalize its content to FMD. All of the experimental diets were supplemented with chromium oxide (5 g/kg diet) as an inert marker for determinations of nutrient ADCs. All of the ingredients were thoroughly mixed, then water was added to produce a dough. Finally, diets were pelleted with a laboratory mincer and stored at -20°C until use.

Fish rearing conditions

The feeding trial was carried out at the Faculty of Biology, Hanoi National University of Education (Hanoi, Vietnam). Fingerling red tilapia were acclimated to the experimental conditions for 10 days by feeding with FMD. A total of 300 fingerling red tilapia (mean BW 13.7 g) were randomly distributed into 15 tanks (20 fish/tank, 3 tanks/dietary treatment). For 8 weeks, the fish were fed the test diets to apparent satiation twice daily (09:00 am and 16:00 pm).

Table II. Formulation and proximate composition of the experimental diets.

	FMD	SR35D	FSR35D	SR50D	FSR50D
Ingredients (g/kg)					
FM[1]	250	162.5	162.5	125	125
SR	0	350	0	500	0
FSR	0	0	350	0	500
Soybean meal[2]	200	200	200	200	200
Corn gluten meal[3]	100	100	100	100	100
Pollock liver oil[4]	50	40	40	35	35
Cellulose[5]	80	40	40	0	0
Corn starch[6]	293	73.5	73.5	3	3
Vitamin and mineral mixture[7]	12	12	12	12	12
DL-Methionine[8]	0	7	7	10	10
CMC-Na[9]	10	10	10	10	10
Chromium oxide[10]	5	5	5	5	5
Proximate composition (g/kg, dry matter basis)					
Crude protein	308	307	306	305	303
Crude lipid	76	72	77	74	79
Ash	74	66	67	61	64
Crude fiber	97	124	101	162	121
Nitrogen free extract	445	431	449	398	433

[1]Minh Tam Co. Ltd., Rachgia, Kiengiang, Vietnam; [2]Bresur S.A., Corrientes, Buenos Aires, Argentina; [3]Pangoo Biotech Hebei Co.,Ltd., Cangzhou, Hebei, China; [4]Pesquera pacific star S.A., Calle Ruta, Puerto Montt, Chile; [5,6]Shanghai Richem Internation Co., Ltd., Shanghai, China; [7]Hinter Biotechnology Group Co., Ltd., Zhuhai, Guangdong, China. Vitamin and mineral mixture (IU or mg/kg mixture): thiamine HNO_3, 1030; riboflavin, 3070; pyridoxine HCl, 1390; cyanocobalamin, 8.1; vitamin C (L-ascorbate-2-monophosphate), 18100; vitamin A acetate, 485000; vitamin D_3 (cholecalciferol), 172000; vitamin E (DL-α-tocopherol acetate, 7010; vitamin K_3 (menadione sodium bisulfite), 1850; folic acid, 550; nicotinamide, 5200; D-calcium pantothenate, 4250; D-biotin, 16.5; inositol, 15400; $ZnSO_4$, 2700; $MnSO_4$, 1730; $CuSO_4$, 1310; $FeSO_4$, 6250; $CoSO_4$, 156; potassium iodide, 175; sodium selenate, 38.1; [8]TRInternational, Inc., Seattle, WA, USA; [9]Sodium carboxymethyl cellulose, SINOCMC Chemical Co. Ltd., Qingdao, China; [10]Sigma-Aldrich Corp., St. Louis, MO, USA.

Abbreviations: FM, fish meal; FMD, fish meal diet; SR, soybean residue; SR35D, 35% soybean residue diet; SR50D , 50% soybean residue diet; FSR, fermented soybean residue; FSR35D, 35% fermented soybean residue; FSR50D, 50% fermented soybean residue.

Sample collection

At the end of the experiment, all fish were fasted for 24 h, then anesthetized with 400 ppm 2-phenoxyethanol. The fish were individually determined the final BW and measured the total body length. Three fish from each tank were randomly selected for whole body composition analysis. Blood samples were collected with heparinized syringes from the caudal veins of five fish in each tank for determinations of hematological parameters. These fish were then dissected to collect gallbladders to measure gallbladder somatic index (GBSI). The remaining fish were further fed the experimental diets for fecal collection through the method described by Kaushik *et al*. (2004). The fecal samples were used for measurements of nitrogen free extract (NFE), protein and lipid ADCs. Finally, six fish in each tank were dissected at 4 h after feeding to collect gallbladders and anterior intestinal digesta for determinations of GBSI and digestive enzyme activity, respectively. In each tank, the intestinal digesta samples from three fish were pooled. The intestinal tract was sectioned as described by Tengjaroenkul *et al*. (2000), and the anterior intestinal digesta were taken from the whole straight region. All samples were maintained at -20°C until analysis.

Analytical methods and calculations

Hematocrit and plasma constituents were measured using an automatic analyzer (Architect c16000, Abbott, IL). Activities of digestive enzymes in the intestinal digesta samples were determined according to the method described by Murashita *et al*. (2007). Viable spore count was evaluated by colony count after drying SR and FSR at 60°C for 48 h. The samples were serially diluted with 0.9% NaCl solution, and then 100 µl of the diluted samples were spread on LB (Luria Bertani) plates. After 48 h of cultivation at 35°C, the colonies were counted, statistically analyzed, and expressed as colony-forming unit per gram (CFU/g). The proximate compositions of SR, FSR, experimental diets, feces, whole body, and the inner marker were analyzed in accordance with the Association of Official Analytical Chemists standard methods (AOAC, 2005).

The WG, SGR, feed intake (FI), FCR, condition factor, GBSI, and nutrient ADC were calculated using the following formulas:

WG (%) = 100 × [final average BW (g) - initial average BW (g)]/initial average BW (g)

SGR (%BW/day) = 100 × [ln final average BW (g) - ln initial average BW (g)]/feeding days

FI (%BW/day)= 100 × total dry FI (g)/[total initial BW (g) + total final BW (g)]/2/ feeding days

FCR = total dry FI (g)/ [total final BW (g) - total initial BW (g)]

GBSI = 100 × individual gallbladder weight (g)/wet individual BW (g)

Condition factor (CF) = 100 × individual BW (g)/ individual total body length^3 (cm)

Nutrient ADC (%) = 100 × [100 - Cr_2O_3 in diet (%)/ Cr_2O_3 in feces (%) × nutrient in feces(%)/nutrient in diet(%)]

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). Statistical differences between groups were assessed with the Tukey-Kramer test, and the significance was based on a 5% level of probability.

RESULTS

Growth and feed performances

The growth and feed performances of red tilapia fed the test diets are presented in Table III. The experimental diets did not affect survival rate and FI of the experimental fish. The final BW, WG, and SGR of fish fed SR35D and SR50D were significantly lower than those fed FMD ($P < 0.05$). Meanwhile, there were no significant differences in growth performance between the FSR35D and FMD groups. FCR values of SR35D- and SR50D-fed fish were similar and significantly higher than those of FMD- and FSR35D-fed fish ($P < 0.05$). Feeding with FSR-included diets resulted in significantly better growth performance and FCR as compared to SR-included diets ($P < 0.05$).

Hematological parameters

As presented in Table IV, total cholesterol levels of fish fed SR35D and SR50D were the lowest among the treatments, and the significant differences were found among these groups and the FMD and FSR35D groups ($P < 0.05$). Hematocrit, total protein, glucose, and triglyceride levels did not differ among the treatment groups.

Condition factor, whole body composition and GBSI

The condition factor and whole-body composition of red tilapia fed the test diets are shown in Table V. The experimental diets did not alter condition factor and whole body composition of the fish. Before feeding (starving), no significant differences in the GBSI were observed among the treatments (Fig. 1). However, at 4 h after feeding, the FSR35D and FSR50D groups had significantly lower GBSI values than the SR35D and SR50D groups, respectively ($P < 0.05$).

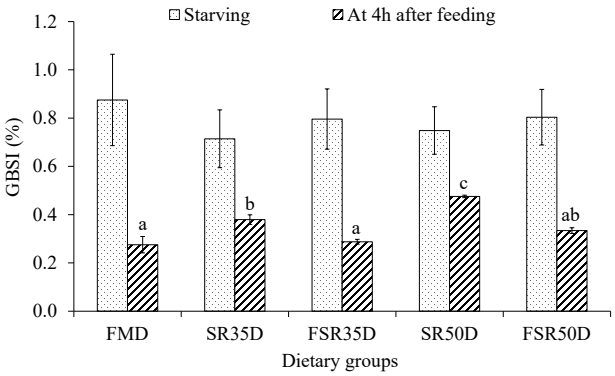

Fig. 1. GBSI of red tilapia fed the experimental diets. Values are presented as means and standard deviations (starving, n = 15; at 4h after feeding, n = 18). Bars assigned with different letters within each sampling time denote significant differences ($P < 0.05$).
For details of groups, see Table II.

Table III. Growth performance and feed utilization of red tilapia fed the experimental diets[1].

Dietary groups	Initial BW (g)	Final BW (g)	WG (%)	SGR (%BW/day)	FI (%BW/day)	FCR	Survival (%)
FMD	13.7 ± 0.2	90.1 ± 3.5^{d}	555.9 ± 16.9^{d}	4.09 ± 0.06^{d}	4.75 ± 0.19	1.42 ± 0.02^{ab}	97.5 ± 3.5
SR35D	13.6 ± 0.1	65.9 ± 1.6^{b}	383.7 ± 9.0^{b}	3.43 ± 0.03^{b}	4.94 ± 0.34	1.74 ± 0.09^{c}	100.0 ± 0.0
FSR35D	13.8 ± 0.2	94.5 ± 2.1^{d}	583.7 ± 27.3^{d}	4.18 ± 0.09^{d}	4.28 ± 0.25	1.30 ± 0.06^{a}	97.5 ± 3.5
SR50D	13.7 ± 0.1	55.4 ± 2.8^{a}	304.6 ± 23.9^{a}	3.04 ± 0.13^{a}	4.76 ± 0.15	1.82 ± 0.11^{c}	100.0 ± 0.0
FSR50D	13.7 ± 0.2	76.6 ± 2.3^{c}	458.5 ± 14.8^{c}	3.77 ± 0.04^{c}	4.32 ± 0.21	1.51 ± 0.07^{b}	100.0 ± 0.0

[1]Values are presented as means ± standard deviations of three replicates. The values in the same column with different superscripts are significantly different ($P < 0.05$).
For details of groups, see Table II.

Table IV. Hematological parameters of red tilapia fed the experimental diet[1].

Dietary groups	Hematocrit (%)	Total protein (g/100ml)	Glucose (mg/100ml)	Total cholesterol (mg/100ml)	Triglyceride (mg/100ml)
FMD	33.7 ± 2.3	2.8 ± 0.5	114.8 ± 6.3	170.4 ± 10.8^{b}	52.6 ± 3.6
SR35D	32.2 ± 1.9	2.7 ± 0.4	102.5 ± 8.6	132.8 ± 6.5^{a}	46.8 ± 4.0
FSR35D	34.4 ± 2.8	2.9 ± 0.5	108.1 ± 9.2	168.6 ± 8.2^{b}	48.5 ± 3.8
SR50D	31.6 ± 2.1	2.6 ± 0.7	110.4 ± 11.8	118.3 ± 9.6^{a}	45.3 ± 2.4
FSR50D	32.9 ± 2.7	2.7 ± 0.3	107.5 ± 7.3	144.7 ± 7.2^{ab}	50.6 ± 2.9

[1]Values are presented as means ± standard deviations (n = 15). The values in the same column with different superscripts are significantly different ($P < 0.05$).
For details of groups, see Table II.

Table V. Condition factor and whole body composition of red tilapia fed the experimental diets[1].

Parameters	Dietary groups				
	FMD	SR35D	FSR35D	SR50D	FSR50D
Condition factor	2.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.1	1.7 ± 0.2	1.8 ± 0.1
Whole body composition (g/100g, wet matter basis)					
Moisture	73.8 ± 0.6	74.9 ± 0.9	73.7 ± 1.5	74.8 ± 2.1	76.9 ± 2.3
Protein	15.6 ± 0.4	15.2 ± 0.6	15.8 ± 0.3	15.5 ± 0.8	15.0 ± 0.5
Lipid	7.1 ± 0.5	6.8 ± 0.6	7.2 ± 0.4	6.7 ± 0.5	6.8 ± 0.3
Ash	4.3 ± 0.3	4.0 ± 0.3	4.2 ± 0.1	3.9 ± 0.2	4.1 ± 0.4

[1]Values are presented as means ± standard deviations (condition factor, n = 60; whole body composition, n = 9). The values in the same raw with different superscripts are significantly different ($P < 0.05$).
For details of groups, see Table II.

Digestive enzyme activity in anterior intestinal digesta

As shown in Figure 2, the SR35D and SR50D groups showed lower digestive enzyme activities in the anterior intestine than the FMD group, and significant differences were recorded for amylase and trypsin activities ($P < 0.05$). Meanwhile, fish fed FSR35D had similar digestive enzyme activities as compared to those fed FMD. At each dietary replacement level of FM, feeding the experimental fish with FSR-included diets resulted in higher intestinal amylase and trypsin activities as compared SR-included diets ($P < 0.05$).

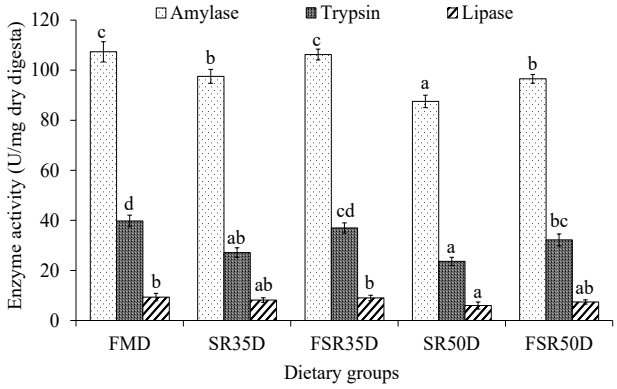

Fig. 2. Digestive enzyme activities in anterior intestinal digesta of red tilapia fed the experimental diets. Values are presented as means and standard deviations (n = 6). Bars assigned with different letters within each enzyme activity denote significant differences ($P < 0.05$).
For details of groups, see Table II.

Nutrient ADC

The nutrient ADCs of red tilapia fed the experimental diets are shown Figure 3. The SR35D and SR50D groups had significantly lower protein, lipid and NFE ADCs than the FSR35D and FSR50D groups, respectively ($P < 0.05$). No statistical differences in NFE, protein and lipid ADCs were detected between fish fed FMD and FSR35D.

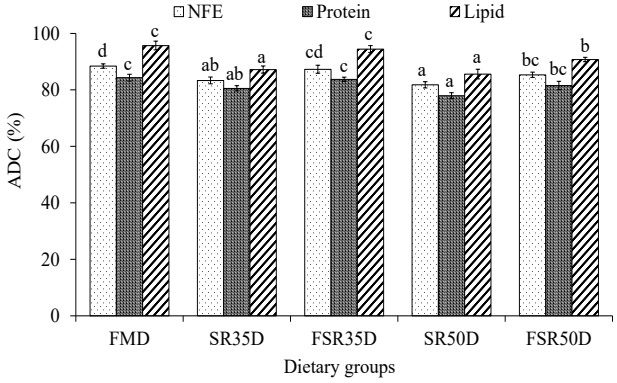

Fig. 3. Nutrient ADCs of red tilapia fed the experimental diets. Values are presented as means and standard deviations of triplicates. Bars assigned with different letters within each nutrient ADC denote significant differences ($P < 0.05$).
For details of groups, see Table II.

DISCUSSION

In the current study, fish fed SR35D and SR50D showed significantly lower final BW, WG, and SGR, but higher FCR, than those fed FMD. These results indicated that the replacement of FM by SR at the rate of 35% in the diet impaired growth and feed performances of red tilapia. Fish fed FSR35D and FSR50D showed significantly better growth performance and FCR than those fed SR35D and SR50D. Moreover, fish fed FSR35D had similar final BW, WG and FCR as compared to those fed FMD. These observations suggested that SR fermentation benefited growth performance and feed utilization of red tilapia, and FSR could replace 35% of FM in red tilapia diets. Dietary FSR inclusion promoted growth and FCR of the

fish, however, these parameters of the FSR50D group were inferior to those of the FSR35D and FMD groups. Some studies have demonstrated that growth and feed performances of fish decrease as dietary soybean protein proportion increases (Refstie *et al*., 2005; Wang *et al*., 2016; He *et al*., 2020). Thus, the excessive inclusion level of FSR in the diet could be the reason causing the poor growth and feed performances of fish fed FSR50D in the current study.

Feeding fish with soybean protein-based diets reportedly reduces intestinal bile acid level and digestive enzyme activity in fish (Nguyen *et al*., 2011b, 2015; Murashita *et al*., 2008, 2018). Low secretions of bile acids from the gallbladder and digestive enzymes from the pancreas are suggested to be responsible for the inferior bile acid concentration and low digestive enzyme activity in the intestine (Nguyen *et al*., 2017, 2021; Murashita *et al*., 2019). In the present study, there were no significant differences in GBSI before feeding (24 h fasted fish) among the treatment groups, however, fish fed SR35D and SR50D showed significantly higher GBSI values than those fed FMD at 4 h after feeding (Fig. 1). These results implied that the secretion of bile juice was inhibited in fish fed SR35D and SR50D. Bile acids are synthesized in the liver, then stored in the gallbladder. After meal, bile acids are secreted from the gallbladder into the intestine for functioning in the lipid digestion process, such as lipid emulsification and lipase activation (Tuchweber *et al*., 1996). Thus, the impairment of bile secretion from the gallbladder in fish fed SR-included diets might lead to the insufficiency of bile acid level in the intestine, thereby reducing dietary lipid digestion and absorption. In addition to the inferior bile secretion from the gallbladder, trypsin, lipase and amylase activities in the intestinal digesta of SR35D- and SR50D-fed fish were significantly lower than FMD-fed fish (Fig. 2). The reduced intestinal bile level and digestive enzyme activity of fish fed SR35D and SR50D suggested that feeding of SR negatively affected digestive physiology of red tilapia, and these adverse effects might interfere with dietary nutrient digestion and absorption. In the current study, bile juice secretion and intestinal digestive enzyme activity were markedly improved by feeding with FSR35D and FSR50D as compared to SR35D and SR50D. Moreover, FSR35D-fed fish showed similar bile juice release and intestinal digestive enzyme activity as compared to FMD-fed fish. These results indicated that fermentation of SR was beneficial to digestive physiology of red tilapia, and FSR could be included at a rate of 35% in the low FM diet without any negative effects on bile juice release and digestive enzyme activity. The positive effects of FSR on digestive physiology in the present study supported earlier findings in rainbow trout (Yamamoto *et al*., 2010), yellowtail (Nguyen *et al*., 2015, 2017), and pompano (Nguyen *et al*., 2021; Mai *et al*., 2021), where biliary bile acid secretion and intestinal digestive enzyme activity of the fish were elevated by including fermented soybean meals in diets. Cholecystokinin (CCK) plays important roles in stimulating the release of bile juice from the gallbladder (Shiratori *et al*., 1986; Einarsson *et al*., 1997) and secretion of digestive enzymes from the pancreas (Ramirez and Farrar, 1970). In this study, feeding of SR inhibited bile secretion from the gallbladder and lowered pancreatic digestive enzyme activity in the intestine. Thus, these adverse effects might have been caused by low CCK production and/or secretion. In agreement with this suggestion, feeding yellowtail with soybean protein-based diets reportedly decreases CCK mRNA levels (Furutani *et al*., 2012; Nguyen *et al*., 2017).

Feeding fish with soybean protein-based diets has been known to reduce nutrient ADCs (Biswas *et al*., 2007; Nguyen *et al*., 2015, 2017, 2020; Mai *et al*., 2021). In the current study, fish fed SR35D and SR50D resulted in lower ADCs of NFE, protein and lipid than fish fed FMD, indicating that SR decreased dietary nutrient digestion and absorption in red tilapia. In contrast, the FSR35D and FSR50D groups presented significantly higher NFE, protein and lipid ADCs than the SR35D and SR50D groups, respectively. Moreover, similar nutrient ADCs were found in the FSR35D group as compared to the FMD group. These observations, together with bile release and digestive enzyme activity in the anterior intestine, suggested that fermentation of SR enhanced dietary nutrient digestion and absorption through the improvements of bile secretion and intestinal digestive enzyme activity of red tilapia. The positive effects of FSR on nutrient digestion and absorption in the present study are in agreement with previous findings, where diets containing fermented soybean meals enhance nutrient ADCs of fish (Refstie *et al*., 2005; Yamamoto *et al*., 2010; Nguyen *et al*., 2015, 2020; Yuan *et al*., 2017).

Soybean reportedly contains several components which negatively influence growth performance, feed utilization, and physiological conditions of fish (Francis *et al*., 2001; Krogdahl *et al*., 2003; Iwashita *et al*., 2008). High molecular weight soybean proteins, such as β-conglycinin and glycinin (Fukushima, 2011), have been reported to decrease intestinal digestive enzyme activity, nutrient ADCs, and growth performance (Nguyen *et al*., 2011a; Li *et al*., 2017a, b; Han *et al*., 2019). Saponins, lectins, and oligosaccharides in soybean have been shown to inhibit the secretions of biliary bile acids and pancreatic digestive enzymes, lower nutrient ADCs, cause intestinal morphological changes, and interfere with growth and feed performances (Olli and Krogdahl, 1995; Refstie *et al*., 1998;

Iwashita *et al*., 2009; Chikwati *et al*., 2012; Nguyen *et al*., 2011b, 2017, 2021). Some studies have demonstrated that soybean fiber also decreases growth and feed performances in fish (Dioundick and Stom, 1990; Amirkolaie *et al*., 2005; Lekva *et al*., 2010; Zhonga *et al*., 2020). Fermentation has been suggested to be an effective method to improve the nutritional quality of soybean meal by reducing the amount of oligosaccharides, fiber, soy antigens, trypsin inhibitors, phytate, and breaking down β-conglycinin into smaller peptides (Hong *et al*., 2004; Feng *et al*., 2007; Li *et al*., 2012; Nguyen *et al*., 2020). It has been reported that growth performance, feed utilization, and physiological conditions of fish are elevated by feeding with fermented soybean meal-included diets as compared to unfermented soybean meal-included diets (Azarm and Lee, 2014; Nguyen *et al*., 2020; Mai *et al*., 2021). Moreover, dietary supplementation of bacteria, such as *Lactobacillus* sp. and *Bacillus* sp., promotes growth performance, feed utilization and digestive physiological conditions in fish (Al-Dohail *et al*., 2009; Apun-Molian *et al*., 2009; Truong Thy *et al*., 2017; Liu *et al*., 2018; Abarike *et al*., 2018). In the present study, FSR was obtained by fermenting SR with *Bacillus subtilis* V37, which resulted in a lower content of crude fiber, but a higher content of NFE, in comparison with SR (Table I). On the other hand, FSR contained a relatively high amount of bacterial spores. Therefore, the improvement of nutritional quality and the presence of *Bacillus subtilis* V37 spores in FSR could be the factors contributing the enhancements of nutrient digestion and absorption, thereby promoting growth and feed performances of the fish fed FSR-included diets. Apart from positive effects on digestive physiology, growth performance and feed utilization, fermentation reportedly improves the intestinal histology and microbiota in fish fed soybean protein-based diets (Refstie *et al*., 2005; Yamamoto *et al*., 2010; Wang *et al*., 2019; He *et al*., 2020). In the present study, morphology and microorganism population in the intestine were not examined. For this reason, further studies are required to evaluate whether FSR benefit such parameters in red tilapia fish.

CONCLUSION

In conclusion, SR reduced bile juice secretion, intestinal digestive enzyme activity, nutrient digestion, and growth performance of red tilapia. Fermentation of SR with *Bacillus subtilis* V37 was beneficial to digestive physiology and growth and feed performances of fish fed soybean protein-based diets. The findings of the current study suggested that FSR could replace 35% of FM in the diet without any adverse effects on digestive physiological conditions, growth performance and feed utilization in red tilapia fish.

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Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

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

Al-Dohail, M.A., Hashim, R., and Aliyu-Paiko, M., 2009. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquacult. Res.*, **40**: 1642–1652. https://doi.org/10.1111/j.1365-2109.2009.02265.x

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J., and Schrama, J.W., 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). *Aquacult. Res.*, **36**: 1157–1166. https://doi.org/10.1111/j.1365-2109.2005.01330.x

AOAC, 2005. *Official methods of analysis:* 18th edn. Association of Official Analytical Chemists (AOAC). Gaithersburg, MD, USA.

Apun-Molina, J.P., Santamaria-Miranda, A., Luna-Gonzalez, A., Martinez-Diaz, S.F., and Rojas-Contreras, M., 2009. Effect of potential probiotic bacteria on growth and survival of tilapia *Oreochromis niloticus* L., cultured in the laboratory under high density and suboptimum temperature. *Aquacult. Res.*, **40**: 887–894. https://doi.org/10.1111/j.1365-2109.2009.02172.x

Azarm, H.M., and Lee, S.M., 2014. Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquacult. Res.*,

45: 994–1003. https://doi.org/10.1111/are.12040

Biswas, A.K., Kaku, H., Ji, S.C., Seoka, M., and Takii, K., 2007. Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. *Aquaculture*, **267**: 284–291. https://doi.org/10.1016/j.aquaculture.2007.01.014

Boerlage1, A.S., Tu, T.D., Tran, T.T.H., Davidson, J., Stryhn, H., and Hammell, K.L., 2017. Production of red tilapia (*Oreochromis* spp.) in floating cages in the Mekong Delta, Vietnam: Mortality and health management. *Dis. Aquat. Org.*, **124**: 131–144. https://doi.org/10.3354/dao03115

Bourne, M.C., 1976. Survey of the suitability of thirty cultivars of soybeans for soymilk manufacture. *J. Fd. Sci.*, **41**: 1204–1208. https://doi.org/10.1111/j.1365-2621.1976.tb14418.x

Chikwati, E.M., Venold, F.F., Penn, M.H., Rohloff, J., Refstie, S., Guttvik, A., Hillestad, M. and Krogdahl, A., 2012. Interaction of soya saponins with plant ingredients in diets for Atlantic salmon, *Salmo salar* L. *Br. J. Nutr.*, **107**: 1570–1590. https://doi.org/10.1017/S0007114511004892

Dioundick, O.B. and Stom, D.I., 1990. Effects of dietary cellulose levels on the juvenile tilapia, *Oreochromis mossambicus* (Peters). *Aquaculture*, **91**: 311–315. https://doi.org/10.1016/0044-8486(90)90196-T

Einarsson, S., Davies, P.S., and Talbot, C., 1997. Effect of exogenous cholecystokinin on the discharge of the gallbladder and the secretion of trypsin and chymotrypsin from the pancreas of the Atlantic salmon, *Salmo salar* L. *Comp. Biochem. Physiol. C*, **117**: 63-67. https://doi.org/10.1016/S0742-8413(96)00226-5

Feng, J., Liu, X., Xu, Z., Lu, Y., and Liu, Y., 2007. The effect of *Aspergillus oryzae* fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. *Anim. Feed Sci. Technol.*, **134**: 295–303. https://doi.org/10.1016/j.anifeedsci.2006.10.004

Francis, G., Makkar, H.P., and Becker, K., 2001. Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**: 197–227. https://doi.org/10.1016/S0044-8486(01)00526-9

Fukushima, D., 2011. Soy proteins. In: *Handbook of food proteins* (eds. G.O. Phillips and P.A., Williams). Woodhead Publishing Limited, Sawston, Cambridge, UK, pp. 210–232. https://doi.org/10.1533/9780857093639.210

Furutani, T., Masumoto, T. and Fukada, H., 2012. Response of cholecystokinin and digestive enzyme mRNA levels to various feed ingredients in yellowtail *Seriola quinqueradiata*. *Fish. Sci.,* **78**: 1075-1082. https://doi.org/10.1007/s12562-012-0537-x

Han, F., Wang, X., Guo, J., Qi, C., Xu, C., Luo, Y., Li, E., Qin. J.G., and Chen, L., 2019. Effects of glycinin and β-conglycinin on growth performance and intestinal health in juvenile Chinese mitten crabs (*Eriocheir sinensis*). *Fish Shellfish Immunol.*, **84**: 269–279. https://doi.org/10.1016/j.fsi.2018.10.013

He, M., Li, X., Poolsawat, L., Guo, Z., Yao, W., Zhang, C., and Leng, X., 2020. Effects of fish meal replaced by fermented soybean meal on growth performance, intestinal histology and microbiota of largemouth bass (*Micropterus salmoides*). *Aquacult. Nutr.*, **26**: 1058–1071. https://doi.org/10.1111/anu.13064

Hong, K.J., Lee, C.H., and Kim, S.W., 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Fd.*, **7**: 430–434. https://doi.org/10.1089/jmf.2004.7.430

Iwashita, Y., Suzuki N., Matsunari H., Sugita T., and Yamamoto T., 2009. Influence of soya saponin, soya lectin, and cholyltaurine supplemented to a casein-based semipurified diet on intestinal morphology and biliary bile status in fingerling rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.*, **75**: 1307-1315. https://doi.org/10.1007/s12562-009-0158-1

Iwashita, Y., Yamamoto T., Furuita H., Sugita T., and Suzuki N., 2008. Influence of certain soybean antinutritional factors supplemented to a casein-based semipurified diet on intestinal and liver morphology in fingerling rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.*, **74**: 1075–1082. https://doi.org/10.1111/j.1444-2906.2008.01627.x

Kaushik, S.J., Coves, D., Dutto, G., and Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*, **230**: 391–404. https://doi.org/10.1016/S0044-8486(03)00422-8

Krogdahl, A., Bakke-McKellep, A.M. and Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.*, **9**: 361–371. https://doi.org/10.1046/j.1365-2095.2003.00264.x

Lekva, A., Hansen, A.C., Rosenlund, G., Karlsen, O. and Hemre, G.I., 2010. Energy dilution with alpha-cellulose in diets for Atlantic cod (*Gadus morhua* L.) juveniles- effects on growth, feed intake, liver

size and digestibility of nutrients. *Aquaculture*, **300**: 169–175. https://doi.org/10.1016/j.aquaculture.2010.01.001

Li, B., Qiao, M. and Lu, F., 2012. Composition, nutrition, and utilization of okara (soybean residue). *Fd. Rev. Int.,* **28**: 231–252. https://doi.org/10.1080/87559129.2011.595023

Li, Y., Hu, H., Liu, J., Yang, P., Zhang, Y., Ai, Q., Xu, W., Zhang, W. and Mai, K., 2017a. Dietary soya allergen β-conglycinin induces intestinal inflammatory reactions, serum-specific antibody response and growth reduction in a carnivorous fish species, turbot *Scophthalmus maximus* L. *Aquacult. Res.*, **48**: 4022–4037. https://doi.org/10.1111/are.13224

Li, Y., Yang, P., Zhang, Y., Ai, Q., Xu, W., Zhang, W., Zhang, Y., Hu, H., Liu, J., and Mai, K., 2017b. Effects of dietary glycinin on the growth performance, digestion, intestinal morphology and bacterial community of juvenile turbot, *Scophthalmus maximus* L. *Aquaculture*, **479**: 125–133. https://doi.org/10.1016/j.aquaculture.2017.05.008

Liu, C.H., Wu, K., Chu, T.W., and We, T.M., 2018. Dietary supplementation of probiotic, *Bacillus subtilis* E20, enhances the growth performance and disease resistance against *Vibrio alginolyticus* in parrot fish (*Oplegnathus fasciatus*). *Aquacult. Int.*, **26**: 63–74. https://doi.org/10.1007/s10499-017-0189-z

Mai, H.V., Nguyen, T.D., Tran, N.T.T., and Nguyen, H.P., 2021. Effects of dietary fermented soybean meals on tissue lipid level, bile acid concentration, lipase activity and lipid digestibility in pompano fish (*Trachinotus blochii). Pakistan J. Zool.*, **00**: 1-9. https://doi.org/10.17582/journal.pjz/20201223071249

Murashita, K., Fukada, H., Hosokawa, H., and Masumoto, T., 2007. Changes in cholecystokinin and peptide Y gene expression with feeding in yellowtail (*Seriola quinqueradiata*): Relation to pancreatic exocrine regulation. *Comp. Biochem. Physiol. B*, **146**: 318–325. https://doi.org/10.1016/j.cbpb.2006.11.009

Murashita, K., Fukada, H., Ronnestad, I., Kurokawa, T., and Masumoto, T., 2008. Nutrient control of release of pancreatic enzymes in yellowtail (*Seriola quinqueradiata*): Involvement of CCK and PY in the regulatory loop. *Comp. Biochem. Physiol. A*, **150**: 438–443. https://doi.org/10.1016/j.cbpa.2008.05.003

Murashita, K., Matsunari, H., Fukada, H., Suzuki, N., Furuita, H., Oku, H., Ronnestad, I., Yoshinaga, H., and Yamamoto, T., 2019. Effect of a plant-based low-fishmeal diet on digestive physiology in yellowtail *Seriola quinqueradiata. Aquaculture,* **506**: 168-180. https://doi.org/10.1016/j.aquaculture.2019.03.040

Murashita, K., Matsunari, H., Furuita, H., Ronnestad, I., Oku, H., and Yamamoto, T., 2018. Effects of dietary soybean meal on the digestive physiology of red seabream *Pagrus major. Aquaculture,* **493**: 219-228. https://doi.org/10.1016/j.aquaculture.2018.05.005

Nguyen, H.P., Do, T.V., Tran, N.T.T., and Trieu, A.T., 2021. Ethanol-soluble components in soybean meal influence the digestive physiology, hepatic and intestinal morphologies, and growth performance of the marine fish pompano (*Trachinotus blochii*). *J. Anim. Physiol. Anim. Nutr.*, **105**: 766–776. https://doi.org/10.1111/jpn.13490

Nguyen, H.P., Do, T.V. and Tran, H.D., 2020. Dietary replacement of fish meal by defatted and fermented soybean meals with taurine supplementation for pompano fish: effects on growth performance, nutrient digestibility, and biological parameters in a long-term feeding period. *J. Anim. Sci.*, **98**: 1–9. https://doi.org/10.1093/jas/skaa367

Nguyen, H.P., Khaoian, P., Fukada, H., Nakamori, T., Furuta, H., and Masumoto, T., 2011a. Effects of different soybean proteins on lipid digestion and growth of yellowtail *Seriola quinqueradiata. Fish. Sci.*, **77**: 357–365. https://doi.org/10.1007/s12562-011-0338-7

Nguyen, H.P., Khaoian, P., Furutani, T., Nagano, J., Fukada, H., and Masumoto, T., 2011b. Effects of alcohol extract from soybean meal on pancreatic digestive enzyme and bile acid secretion in yellowtail *Seriola quinqueradiata*. *Aquacult. Sci.*, **59**: 465–472.

Nguyen, H.P., Khaoian, P., Furutani, T., Nagano, J., Fukada, H., and Masumoto, T., 2017. Effects of alcohol extract of defatted soybean meal on growth performance and digestive physiology of yellowtail *Seriola quinqueradiata. Fish. Sci.*, **83**: 99–106. https://doi.org/10.1007/s12562-016-1049-x

Nguyen, H.P., Khaoian, P., Nagano, J., Fukada, H., Suzuki, N., and Masumoto, T., 2015. Feeding fermented soybean meal diet supplemented with taurine to yellowtail *Seriola quinqueradiata* affects growth performance and lipid digestion. *Aquacult. Res.*, **46**: 1101–1110. https://doi.org/10.1111/are.12267

Noguchi, A., 1987. Method for the preparation of textured soybean draff. U.S. Patent US 4 642 241. *Fd. Sci. Technol. Abstr.*, 87-08-V0076.

O'Toole, D.K., 1999. Characteristics and use of okara,

the soybean residue from soy milk production. A review. *J. Agric. Fd. Chem.,* **47**: 363–371. https://doi.org/10.1021/jf9807541

Olli, J.J., and Krogdahl, A., 1995. Alcohol soluble components of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar* L. *Aquacult. Res.*, **26**: 831–835. https://doi.org/10.1111/j.1365-2109.1995.tb00876.x

Olsen, R.L., and Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends Fd. Sci. Technol.,* **27**: 120–128. https://doi.org/10.1016/j.tifs.2012.06.003

Ramirez, M., and Farrar, J.T., 1970. The effect of secretin and cholecystokinin-pancreozymin on the intraluminal pressure of the jejunum in the unanesthetized dog. *Dig. Dis. Sci.,* **15**: 539-544. https://doi.org/10.1007/BF02238114

Refstie, S., Sahlstrom, S., Brathen, E., Baeverfjord, G., and Krogedal, P., 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture*, **246**: 331–345. https://doi.org/10.1016/j.aquaculture.2005.01.001

Refstie, S., Storebakken, T., and Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture*, **162**: 301–312. https://doi.org/10.1016/S0044-8486(98)00222-1

Shiratori, K., Watanabe, S., Chey, W.Y., Lee, K.Y., and Chang, T.M., 1986. Endogenous cholecystokinin drives gallbladder emptying in dogs. *Am. J. Physiol.*, **251**: G553-G558. https://doi.org/10.1152/ajpgi.1986.251.4.G553

Tengjaroenkul, B., Smith, B.J., Caceci, T., and Smith, S.A., 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. *Aquaculture*, **182**: 317–327. https://doi.org/10.1016/S0044-8486(99)00270-7

Thuoc, D.V., and Hung, N.P., 2021. Effect of temperature and pH on the production, activity, and stability of α-amylase from *Bacillus subtilis* V37 (in Vietnamese with English abstract). *Hnue. J. Sci.*, **66**: 72–79. https://doi.org/10.18173/2354-1059.2021-0009

Truong Thy, H.T., Tri, N.N., Quy, O.M., Kannika, K., Unajak S., and Areechon, N., 2017. Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfish (*Pangasianodon hypophthalmus*). *Fish Shellfish Immunol.*, **60**: 391–399. https://doi.org/10.1016/j.fsi.2016.11.016

Tuchweber, B., Yousef, I.M., Ferland, G., and Perea, A., 1996. Nutrition and bile formation. *Nutr. Res.*, **16**: 1041–1080. https://doi.org/10.1016/0271-5317(96)00104-2

Van der Riet, W.B., Wight, A.W., Cilliers, J.J.L., and Datel, J.M., 1989. Food chemical investigation of tofu and its byproduct Okara. *Fd. Chem.*, **34**: 193–202. https://doi.org/10.1016/0308-8146(89)90140-4

Wang, L., Zhou, H., He, R., Xu, W., Mai, K., and He, G., 2016. Effects of soybean meal fermentation by *Lactobacillus plantarum* P8 on growth, immune responses, and intestinal morphology in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, **464**: 87–94. https://doi.org/10.1016/j.aquaculture.2016.06.026

Wang, P., Zhou, Q., Feng, J., He, J.J., Lou, Y., and Zhu, J., 2019. Effect of dietary fermented soybean meal on growth, intestinal morphology and microbiota in juvenile large yellow croaker, *Larimichthys crocea*. *Aquacult. Res*., **50**: 748–757. https://doi.org/10.1111/are.13929

Yamamoto, T., Iwashita, Y., Matsunari, H., Sugita, T., Furuita, H., Akimoto, A., Okamatsu, K., and Suzuki, N., 2010. Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, **309**: 173–180. https://doi.org/10.1016/j.aquaculture.2010.09.021

Yuan, L., Chang, J., Yin, Q., Lu, M., Di, Y., Wang, P., Wang, Z., Wang, E., and Lu. F., 2017. Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets. *Anim. Nutr.*, **3**: 19–24. https://doi.org/10.1016/j.aninu.2016.11.003

Zhonga, Y.F., Shia, C.M., Zhoua, Y.L., Chena, J.Y., Lina, S.M., and Tang, R.J., 2020. Optimum dietary fiber level could improve growth, plasma biochemical indexes and liver function of largemouth bass, *Micropterus salmoides. Aquaculture,* **518**: 734661. https://doi.org/10.1016/j.aquaculture.2019.734661