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INFLUENCE OF *Aspergillus oryzae* FERMENTED SOYBEAN MEAL ON PLASMA CHOLESTEROL AND BILE ACID LEVELS IN HYBRID TILAPIA AND POMPANO FISH

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Abstract. Fermentation has been suggested as an economical and effective method to eliminate anti-nutritional factors and improve the nutritional value of Defatted soybean meal (SBM). The present study aimed to examine the effects of *Aspergillus oryzae* fermented SBM (FSBM) on plasma cholesterol and bile acid levels in hybrid tilapia and pompano. Two isonitrogenous and isoenergetic experimental diets for each fish species were formulated with SBM and FSBM as main ingredients. The diets were denoted as SBMD (SBM-based diet) and FSBMD (FSBM-based diet). Thirty juvenile hybrid tilapia (initial body weight 55 g) and eighteen juvenile pompanos (initial body weight 61 g) were allocated to each of the 4 concrete tanks (1500-L holding capacity) and each of the 4 circular polyvinyl chloride tanks (500-L holding capacity), respectively, resulting in two replicate tanks per dietary treatment for each fish species. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily. The results showed that feeding FSBMD tended to increase plasma total cholesterol level, gallbladdersomatic index, anterior intestinal total bile acid level, and posterior/anterior intestinal total bile acid reduction rate compared to SBMD in both hybrid tilapia and pompano. The differences in these parameters between FSBMD and SBMD dietary groups were significant in hybrid tilapia ($P < 0.05$), while no significant differences were found between pompano fed FSBMD and SBMD. These results indicated that fermentation of SBM with *A. oryzae* could effectively improve physiological conditions of hybrid tilapia fed soybean protein-based diet and suggested that inclusion of *A. oryzae* FSBM might enhance growth and feed performances of the fish.

Keywords: fermented soybean meal, *Aspergillus oryzae*, plasma cholesterol, bile acid, hybrid tilapia, pompano.

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1. Introduction

Fish meal, which contains a high protein level, has been a major ingredient in fish diets because of its good protein quality and palatability. However, increasing demand, high cost, and unstable supply of fish meal have resulted in nutritionists studying alternative sources, especially plant proteins to replace fish meal protein in the diet for freshwater and marine fish species [1, 2]. Among plant proteins, defatted soybean meal (SBM) produced by defatting soybean oil with solvents is a good candidate for replacement of fish meal in diets because of its high protein content, relatively well-balanced amino acid profile, and reasonable price [3, 4]. However, anti-nutritional factors (ANFs) in SBM, such as glycinin, β-conglycinin, trypsin inhibitors, raffinose and stachyose, saponins, lectins, and phytate, reportedly reduce growth performance associated with physiological abnormalities including hypocholesterolemia and inferior bile acid level in fish [5-8]. Therefore, to increase the feasibility of using SBM in fish feeds, it is necessary to improve such physiological conditions by the elimination of ANFs in SBM.

Fermentation has been suggested as an economical and effective method to eliminate ANFs and improve the nutritional value of SBM. Lactic acid fermentation can reduce the number of ANFs such as oligosaccharides, soy antigens, and trypsin inhibitors in SBM [9]. Fermentation using *Aspergillus oryzae* reportedly breaks down β-conglycinin, the major antigenic protein in soybean with high molecular weight, into smaller peptides [10, 11]. Some studies have demonstrated that feeding of fermented SBM (FSBM) can improve growth performance, feed utilization, and physiological conditions in some fish species, such as rainbow trout (*Oncorhynchus mykiss*) [12], black sea bream (*Acanthopagrus schlegeli*) [13], hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) [14], yellowtail (*Seriola quinqueradiata*) [15], Florida pompano (*Trachinotus carolinus*) [16], and yellow croaker (*Larimichthys crocea*) [17].

Hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) and pompano (*Trachinotus blochii*) are economically important fish species for aquaculture due to their fast growth rate, good meat quality, and high market demand. To date, there have been no studies on using SBM fermented by *A. oryzae* in these species. Therefore, the present study aimed to examine the effects of *A. oryzae* FSBM on plasma cholesterol and bile acid levels of hybrid tilapia and pompano in a short-term feeding period.

2. Content

2.1. Materials and methods

**** Fermented soybean meal***

Commercially available defatted SBM (crude protein [CP] 48%, dry matter basis) was fermented with *A. oryzae* provided by Research Institute for Aquaculture No. 2, Ho Chi Minh city, Vietnam. Briefly, SBM was soaked with distilled water to maintain moisture content of 350 g/kg for 60 min. Hydrated SBM was cooked in a steam tank at 60 - 70 ºC for 1 h. The cooked SBM was cooled to room temperature for 1 h, inoculated with 3 g *A. oryzae*/kg (10,000 counts/g of SBM), then mixed and fermented in a bed-

packed incubator for 48 h. After fermentation, fresh FSBM was dried at 50 - 60 ºC for 72 h. Finally, dried FSBM (CP 50%, dry matter basis) was ground to below 400 µm mesh size.

**** Experimental diets***

Two isonitrogenous and isoenergetic experimental diets for each fish species were formulated with SBM and FSBM as main ingredients. The diets were denoted as SBMD (SBM-based diet) and FSBMD (FSBM-based diet) (Table 1). All of the powdered ingredients were manually mixed, then pollock liver oil was slowly added to the mixture. After the powdered ingredients were thoroughly mixed with pollock liver oil, water was added to produce a stiff dough. Finally, the dough was pelleted using a laboratory pellet mill and stored at -20°C until use.

Table 1. Formulation and proximate composition of the experimental diets

Ingredients (g/kg)	**Hybrid tilapia**		**Pompano**	
	SBMD	***FSBMD***	***SBMD***	***FSBMD***
Fish meal	150	150	300	300
Defatted soybean meal	350	0	440	0
Fermented soybean meal	0	340	0	430
Corn gluten meal	60	60	60	60
Wheat flour	200	200	65	65
Cellulose	145	155	20	15
Pollock liver oil	55	55	95	95
Vitamin and mineral mixture*	15	15	15	15
DL-Methionine	10	10	5	5
Sodium carboxymethyl cellulose	15	15	15	15
Proximate composition (g/kg, dry matter basis)				
Crude protein	325	328	453	451
Crude lipid	71	73	124	127
Ash	84	85	102	104

**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.*

**** Fish and rearing conditions***

The hybrid tilapia was reared at the Faculty of Biology, Hanoi National University of Education (Hanoi, Vietnam). Thirty juvenile hybrid tilapia with an initial body weight of 55 g were allocated to each of the 4 concrete tanks (1500-L holding capacity), resulting in two replicate tanks per dietary treatment. The tanks were aerated and supplied with fresh water at a rate of 4 L/min. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily (09:00 am and 4:00 pm). The pompano was reared at The National Broodstock Center for Mariculture Species, Research Institute for Aquaculture No.1 (Haiphong, Vietnam). Eighteen juvenile pompanos with an initial body weight of 61 g were allocated to each of the 4 circular polyvinyl chloride tanks (500-L holding capacity), resulting in two replicate tanks per dietary treatment. The tanks were aerated and supplied with filtered seawater at a rate of 3 L/min. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily (09:00 am and 4:00 pm).

**** Sampling, analytical methods and calculations***

At the end of the feeding trial, all fish of the two experiments fasted for 48 h before sampling. Five fish from each tank were anesthetized with 400 ppm 2-phenoxyethanol and weighed individually to determine the body weight, then blood samples were collected with heparinized syringes from the caudal vein and used for total cholesterol quantification. These fish were then dissected to collect gallbladders for gallbladdersomatic index determination and bile acid analysis. The remaining fish continued to be fed the experimental diets, and six fish in each tank were dissected at 4 h after feeding to collect anterior and posterior intestinal digesta for bile acid quantification. The dissected fish from each tank were divided into two groups (three fish each), and the anterior and posterior intestinal digesta from each group were pooled. The anterior and posterior intestinal sections were divided as the entire straight region from the beginning of the intestine to the first turn of the intestine and from the last turn of the intestine to the anus, respectively.

The plasma total cholesterol level was quantified using a commercial automatic analyzer (Architect c16000, Abbott, Illinois, USA). Bile acids were extracted from the freeze-dried intestinal digesta with 90% ethanol, followed by chloroform : methanol (1:1, v/v), according to the method described by Setchell *et al.* (1983) [18]. The bile acid extract from the digesta and bile juice diluted with distilled water at a ratio of 1:1200 were used for quantification of total bile acid concentration with a commercial assay kit (MAK309, Sigma-Aldrich Corp., St. Louis, MO, USA). The proximate compositions of the experimental diets were analyzed according to the Association of Official Analytical Chemists standard methods (AOAC, 2005) [19]. The gallbladdersomatic index and total bile acid reduction rate in posterior/anterior intestines were calculated using the following formulas:

Gallbladdersomatic index (%) = 100 × individual gallbladder weight/wet body weight.

Total bile acid reduction rate in posterior/anterior intestines (%) = 100 × (total bile acid level in anterior intestinal digesta - total bile acid level in posterior intestinal digesta)/total bile acid level in anterior intestinal digesta.

**** Statistical analysis***

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

2.2. Results

2.2.1. Plasma total cholesterol level

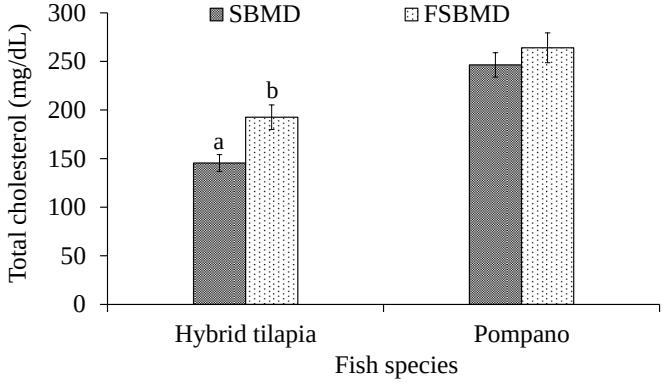

Figure 1. Plasma total cholesterol level of hybrid tilapia and pompano fed the experimental diets

Values are presented as the means ± standard deviations (n = 10). Bars assigned with different superscripts within each species denote significant differences (P < 0.05).

The plasma total cholesterol levels of the experimental fish are presented in Figure 1. Feeding FSBMD tended to increase plasma total cholesterol level compared to SBMD in both hybrid tilapia and pompano. However, this parameter in pompano did not significantly differ between the SBMD and FSBMD groups. Meanwhile, hybrid tilapia fed FSBM resulted in significantly higher total cholesterol level than those fed SBMD ($P < 0.05$).

2.2.2. Gallbladdersomatic index and gallbladder total bile acid level

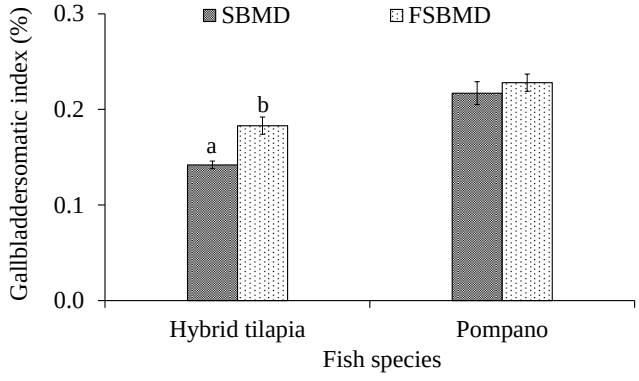

Figure 2. Gallbladdersomatic index of hybrid tilapia and pompano fed the experimental diets

Values are presented as the means ± standard deviations (n = 10). Bars assigned with different superscripts within each species denote significant differences (P < 0.05).

The gallbladdersomatic index and gallbladder total bile acid level of the experimental fish is shown in Figure 2 and Figure 3, respectively. The tested diets did not significantly affect the gallbladdersomatic index of pompano though fish fed FSBMD tended to have a higher value than those fed SBMD. In hybrid tilapia, a significantly higher gallbladdersomatic index was recorded in the FSBMD dietary group compared to the SBMD dietary group ($P < 0.05$).

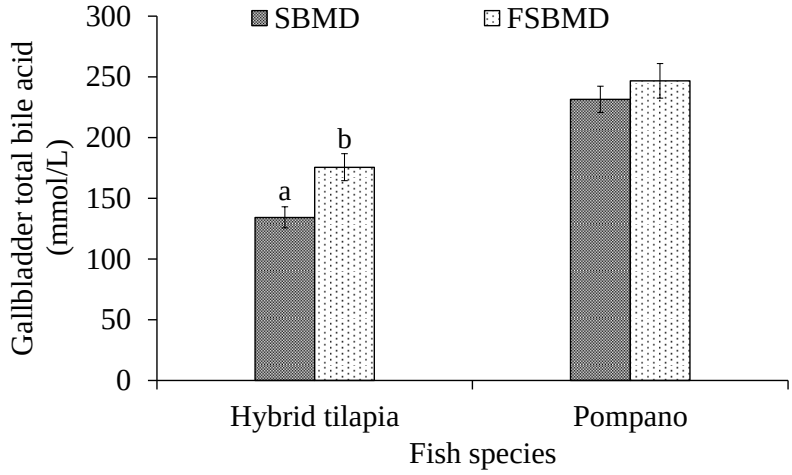

Figure 3. Gallbladder total bile acid level of hybrid tilapia and pompano fed the experimental diets

Values are presented as the means ± standard deviations (n = 10). Bars assigned with different superscripts within each species denote significant differences (P < 0.05).

2.2.3. Total bile acid level and total bile acid reduction rate in the intestine

The total bile acid level and total bile acid reduction rate in the intestine are presented in Table 2.

Table 2. Total bile acid level and total bile acid reduction rate in the intestines of hybrid tilapia and pompano fed the experimental diets

Parameters*	**Hybrid tilapia**		**Pompano**	
	SBMD	***FSBMD***	***SBMD***	***FSBMD***
Total bile acid level in anterior intestinal digesta (µmol/g dry digesta)	52.1 ± 2.9^{a}	71.2 ± 3.8^{b}	82.4 ± 4.2	85.6 ± 4.6
Total bile acid level in posterior intestinal digesta (µmol/g dry digesta)	21.8 ± 2.1	24.8 ± 2.7	41.7 ± 3.3	40.1 ± 3.5
Total bile acid reduction rate in posterior/anterior intestines (%)	54.3 ± 2.4^{a}	65.2 ± 3.1^{b}	49.4 ± 2.0	53.2 ± 2.2

**Values are presented as mean ± standard deviation (n = 4). The values with different superscripts in the same row within each species are significantly different (P < 0.05).*

In pompano, there were no significant differences in both the intestinal total bile acid level and total bile acid reduction rate between the SBMD group and FSBMD group, though the anterior intestinal bile acid level and bile acid reduction rate tended to be higher in fish fed FSBMD compared to those fed SBMD. In hybrid tilapia, significantly higher total bile acid level in anterior intestinal digesta and total bile acid reduction rate were found in fish fed FSBMD than in fish fed SBMD ($P < 0.05$). The bile acid level in the posterior intestinal digesta did not significantly differ between hybrid tilapia fed SBMD and FSBMD.

2.3. Discussion

In this study, feeding SBM fermented by *A. oryzae* significantly improved plasma total cholesterol level, anterior intestinal total bile acid level, and posterior/anterior total bile acid reduction rate in hybrid tilapia. Feeding FSBM tended to increase these parameters in pompano, though no significant differences were observed between the FSBMD and SBMD groups. Hypocholesterolemia is a prominent symptom in animals given SBM-based feeds, including fish. It has been postulated that the undigested high molecular fraction of soybean protein is the determining factor of low plasma cholesterol levels [8, 20 - 24]. On the other hand, fermentation of SBM using *A. oryzae* reportedly breaks down β-conglycinin, the major protein in soybean with high molecular weight, into smaller peptides [10, 11]. Therefore, the increase of plasma total cholesterol level in hybrid tilapia fed FSBMD compared to those fed SBMD in the present study could be due to the reduction in molecular weight fraction of soybean protein caused by the fermentation. Feeding pompano with FSBMD did not significantly elevate plasma total cholesterol level. Since growth and feed performances of fish decrease as the dietary proportion of soybean protein increases [9, 17, 25], thus, the low level of plasma total cholesterol in pompano fed FSBMD might be due to an excessive inclusion level of FSBM in the diet.

Reductions in bile acid levels have been recorded in several fish species fed soybean protein-based diets, including yellowtail (*S. quinqueradiata*) [8], rainbow trout (*O. mykiss*) [12], and red sea bream (*Pagrus major*) [26]. In a previous study, fermentation of SBM was found to improve bile acid levels in yellowtail [15]. Similarly, in the current study, hybrid tilapia fed FSBMD tended to have a higher gallbalddersomatic index, gallbladder total bile acid level, and anterior intestinal bile acid level compared to those fed SBMD. In addition, the FSBMD-fed tilapia resulted in a greater intestinal bile acid reduction rate than SBMD-fed fish, suggesting that feeding FSBM enhanced bile acid reabsorption from the intestine of the fish. A similar tendency to hybrid tilapia was also found in pompano, however, no significant differences were detected between the two experimental groups. It has been reported that undigested high molecular weight fractions of soybean protein can bind bile acids in the intestine, consequently, interfere with enterohepatic circulation of bile acids and reduce their reabsorption [21-24]. Thus, the reduction of high molecular weight fractions of soybean protein due to the fermentation might be the factor responsible for high bile acid level in

the gallbladder, anterior intestine, and posterior/anterior bile acid reduction rate observed in fish fed FSBMD. Poor lipid digestion has been reported in fish fed SBM-based diets [8, 9, 15, 27]. It has been known that bile acids play an important role in lipid digestion and absorption through lipid emulsification and activation of pancreatic lipases [28]. Therefore, the improvement of bile acid level in fish fed FSBMD compared to those fed SBMD in the present study suggested that inclusion of FSBM in the diet might effectively enhance lipid digestion and growth performance of the fish.

3. Conclusions

Fermentation of SBM with *A. oryzae* tented to increase plasma total cholesterol level, gallbladder and anterior intestinal total bile acid concentrations, and bile acid reabsorption in hybrid tilapia and pompano. These improvements were clearly observed in hybrid tilapia. The positive effects of FSBM on bile acid level and bile acid reabsorption in the present study suggested that the inclusion of FSBM might effectively enhance lipid digestion and growth performance of fish fed SBM-based diets.

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