

LIPID DIGESTION IN POMPANO *Trachinotus blochii* FED WITH DIFFERENT SOYBEAN MEALS

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Abstract: A 3-week feeding trial was conducted to determine the effects of defatted soybean meal, ethanol-extracted soybean meal and fermented soybean meal on lipid digestion in pompano *Trachinotus blochii*. Four diets which were formulated and designated as FM (fish meal), SBM (defatted soybean meal), ExtSBM (ethanol-extracted SBM), and FSBM (defatted soybean meal fermented by *Bacillus subtilis*) were fed to juvenile pompano *Trachinotus blochii*. Results showed that lipids in plasma and in tissues, total bile acid and lipase activity in the anterior intestine of fish fed with SBM were significantly lower than those of fish fed with FM. However, these parameters of fish fed with ExtSBM and FSBM were comparable to those of fish fed with FM. Significantly higher lipid content was observed in SBM fed fish compared to FM fed fish while there were no statistical differences in posterior lipid contents recorded among fish fed with ExtSBM, FSBM and FM. These results indicated that SBM interfered with lipid digestion and absorption in pompano *Trachinotus blochii*. The reduction of bile acid level and lipase activity in the anterior intestine was responsible for poor dietary lipid utilization of pompano fed with SBM. The findings in the present study also suggest that ethanol extraction and fermentation could be effective and potential ways to improve nutritional quality of SBM for culturing this fish species.

Keywords: *Trachinotus blochii*, Ethanol extraction, fermentation, lipid digestion, pompano, soybean meal.

1. INTRODUCTION

Pompano fish *Trachinotus blochii* is an economically important aquaculture fish species in Asia with high quality product (Paking et al., 2011; Gopakumar et al., 2012; Jayakumar et al., 2014). It is a carnivorous species which require a high protein level in diets. Fish meal (FM) is an excellent protein source which has been commonly used as a main ingredient in aquaculture feeds, including pompano. However, its increased cost and projected problems with supply highlight the need for alternative protein sources. Among alternative protein sources available, defatted soybean meal (SBM) has been considered to be the most cost-effective alternative to FM because of its high protein content, high digestibility, relatively well-balanced amino acid profile, and reasonable price

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(Storebakken et al., 2000; Porter & Jones, 2003). It has been reported that when feeding diets containing above 40% of soybean protein, several carnivorous species such as Atlantic salmon *Salmo salar* (Refstie et al., 2005), rainbow trout *Oncorhynchus mykiss* (Romarheim et al., 2008), red sea bream *Pagrus major* (Takagi et al., 2002), and yellowtail *Seriola quinqueradiata* (Nguyen et al., 2013; 2017) resulted in inferior growth and feed performances. Moreover, feeding SBM has been known to induce numerous abnormalities in carnivorous fish such as low blood cholesterol level (hypocholesterolemia) and green liver syndrome in red sea bream (Goto et al., 2001), hypocholesterolemia and low lipid digestion in yellowtail (Nguyen et al., 2013) and damaged intestinal morphology in Atlantic salmon (Krogdahl et al., 2003) and in rainbow trout (Yamamoto et al., 2008). Among those abnormalities, hypocholesterolemia and low lipid digestion seems to be more severe in carnivorous species fed with SBM.

The efficiency of dietary lipid digestion and absorption affects the body lipid level and growth of fish. It has been reported in our previous studies that ethanol extraction and fermentation could improve lipid digestion in yellowtail - a carnivorous fish species. To date, there have been no studies investigating the effects of SBM and anti-nutritional factor eliminated SBMs through ethanol extraction or fermentation on lipid digestion in pompano. Therefore, to increase the feasibility of using SBM in pompano feeds, this study aimed to evaluate lipid digestion process of pompano fish fed with original SBM, ethanol-extracted SBM and fermented SBM.

2. MATERIALS AND METHODS

2.1. Soybean meals

SBM (crude protein, CP 49%) was extracted with 70, 80 and 90% aqueous ethanol at a ratio of 1:3 (w/v), starting from lower to higher concentrations as described previously (Nguyen et al., 2017). At each extraction, SBM was blended manually with the aqueous solution for 2h, and left at room temperature for 24h. The supernatant was then removed by decanting. The remaining residue was extracted twice in the same way as described above. The residues from all three extracted times were then pooled together and dried in an oven at 100°C to produce ethanol-extracted SBM (ExtSBM, CP 58%). Fermented SBM (FSBM, CP 52%) was processed using *Bacillus subtilis* B3. The SBM fermentation conditions were referred to the description by Nguyen et al., (2018).

2.2. Diet preparation

Four diets were formulated and named FM, SBM, ExtSBM, and FSBM (Table 1). Taurine (1.5 g/100 g diet) and methionine (0.5 g/100 g diet) were supplemented to all soybean protein-containing diets for carnivorous fish species as suggested by Nguyen et al., (2013, 2017). After the powdered ingredients were thoroughly mixed with pollock liver oil, water was added to produce a stiff dough. The dough was then pelleted using a laboratory pellet mill and stored at -30°C until used.

Table 1. Formulation and composition of the experimental diets (%)

Ingredient	FM	SBM	ExtSBM	FSBM
Fish meal	64.0	32.0	32.0	32.0
Soybean meal	0.0	44.0	0.0	0.0
Ethanol-extracted soybean meal	0.0	0.0	37.0	0.0
Fermented soybean meal	0.0	0.0	0.0	42.0
Corn gluten meal	5.0	5.0	5.0	5.0
Wheat flour	12	3.0	3.0	3.0
Cellulose	8.5	0.5	7.5	2.5
Fish oil	6.5	9.5	9.5	9.5
Vitamin and mineral mixture ¹	2.0	2.0	2.0	2.0
Methionine	0.0	0.5	0.5	0.5
Guar gum	0.5	0.5	0.5	0.5
CMC-Na ²	2.0	2.0	2.0	2.0
Taurine	0.0	1.5	1.5	1.5
Proximate composition (dry matter basis)				
Crude protein	45.6	45.4	45.2	45.5
Crude lipid	12.7	12.3	12.4	12.6
Ash	12.2	10.3	10.4	10.7

¹ Vitamin and mineral mixture contains (mg or unit/kg): thiamine mononit HCl, 2.4; riboflavin, 4.4; pyridoxine HCl, 2.4; folic acid, 2.4; nicotinic acid, 7.2; calcium pantothenate, 14; biotin, 7.0; inositol, 169; choline chloride, 1168; calcium ascorbate, 178; cyanocobalamin, 1.6; vitamin A palmitate, 4.0; vitamin D₃, 0.0045; vitamin E (DL- α -tocopherol), 176; menadione-NaHSO₄, 5.1; KH₂PO₄, 412; Ca (H₂PO₄)₂·H₂O, 618; calcium lactate, 282; iron proteinate, 166; ZnSO₄·H₂O, 9.99; Mn SO₄·H₂O, 6.3; CuSO₄·H₂O, 2.0; CoSO₄·7H₂O, 0.05; KIO₃, 0.15.

² CMC-Na: Carboxymethyl cellulose - sodium.

2.3. Fish and rearing condition

A 3-week trial of pompano was carried out at The National Broodstock Center for Mariculture Species, Research Institute for Aquaculture No.1 located in Xuan Dam commune, Cat Ba island, Cat Hai district, Hai Phong city. Juvenile pompano was reared in 500 l holding capacity (mean body weight at 35 g, 2 tanks/diet, 15 fish/tank, duplication). Fish were hand-fed the experimental diets to apparent satiation twice a day (8:30 a.m. and 4:00 p.m.). Dissolved oxygen and water temperature were monitored daily and ranged from 5.1 to 6.7 mg/L and 25.3 °C to 29.2 °C, respectively.

2.4. Sampling

At the end of the trial, blood samples were taken for 5 fish/tank from the caudal vein at 6 h after feeding using 1 mL heparinized syringes. The blood samples were then centrifuged at 10,000 rpm for 10 minutes to separate plasma for lipid analysis. After blood sampling, fish were returned to their original tanks and further fed with the experimental diets. Then, 6 fish from in each tank were removed at 3h after feeding to dissect and collect anterior and posterior intestinal digesta for analyzing lipase activity, total bile acid level and lipid content. The division of the intestinal tract was referred to the description

of Murashita et al., (2008), and intestinal digesta were taken from the whole-straight anterior or posterior region. The digesta samples from 3 fish each tank were then pooled together. At 24 h after feeding, 5 fish of each tank were also dissected to collect liver, muscle samples for lipid analysis. All samples were stored at -30 °C until analyzed.

2.5. Analytical methods

Plasma triglyceride and total cholesterol were performed with an autoanalyzer (Type Architect C8000; Abbott Ltd., Abbott Park, IL, USA) in Medlatec Hospital of Hanoi City. Bile acids in intestinal digesta were extracted with 90% ethanol, then with chloroform:methanol (1:1, v/v) following a method described by Setchell et al., (1983). The extract was used for total bile acid level quantification. The total bile acid level was determined with a commercial assay kit (MAK309, Sigma-Aldrich, USA). Total lipid levels in liver and muscle were determined gravimetrically after extraction with chloroform:methanol (2:1, v/v) according to the method described by Folch et al. (1957). The measurement of lipase activity was described previously (Nguyen et al., 2013). The proximate composition of the experimental diets and crude lipid content in posterior intestinal digesta were analyzed according to the Association of Official Analytical Chemists standard methods (Association of Official Analytical Chemists, 1995).

2.6. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1. Lipids in plasma, liver and muscle

Plasma lipid levels of pompano at 6h after feeding are shown in Table 2. Total cholesterol and triglyceride were significantly lower in fish fed with SBM than those in fish fed with FM. There were no statistical differences in plasma lipid component levels among fish fed with FM, ExtSBM and FSBM. Figure 1 shows that crude lipid contents in both liver and muscle of fish fed with FM were significantly greater than those of fish fed with SBM while no significant differences in these parameters were observed among fish fed with FM, ExtSBM and FSBM.

Table 2. Plasma lipid components at 6h after pompano were fed with the experimental diets

Lipid components	Dietary groups			
	FM	SBM	ExtSBM	FSBM
Total cholesterol	224.8 ± 23.6 ^b	173.5 ± 17.4 ^a	209.4 ± 18.9 ^{ab}	218.5 ± 22.3 ^b
Triglyceride	129.4 ± 20.5 ^b	86.0 ± 19.3 ^a	114.6 ± 18.2 ^b	125.4 ± 21.7 ^b

Note: Values are means and standard deviations (n = 10). Values of each parameter in the same row with different superscripts are significantly different (P < 0.05). Total cholesterol and triglyceride are calculated as mg/dl.

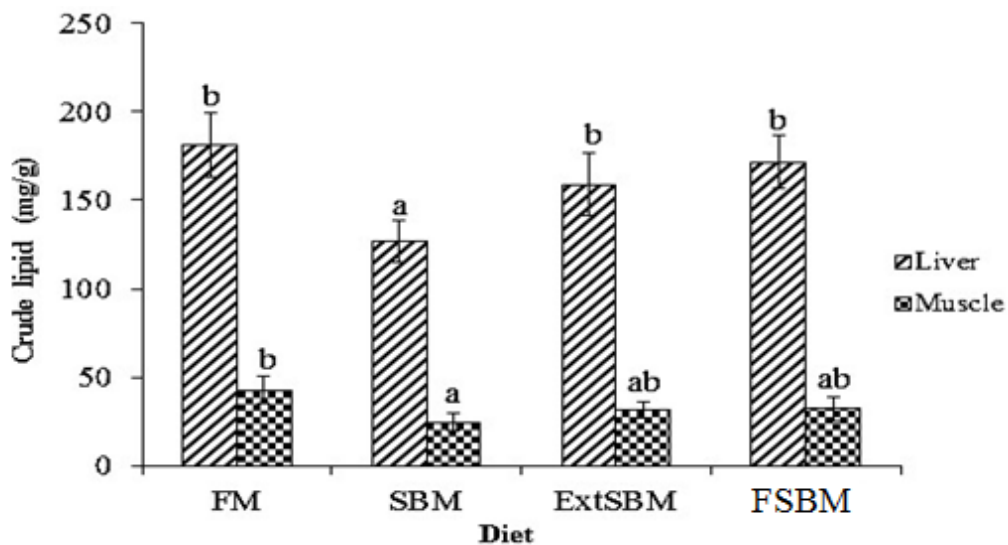


Figure 1. Lipid contents in liver and muscle at 24 h after pompano were fed with the experimental diets. Note: Values are mean and standard deviation ($n = 10$). Different letters within each organ denote significant differences ($P < 0.05$). Crude lipid is calculated as mg/g dried tissue.

3.2. Total bile acid level and lipase activity in anterior intestinal digesta and crude lipid content in posterior intestinal digesta

Table 3 shows that fish fed with SBM had the lowest level of total bile acid in anterior intestinal digesta compared to those fed with FM ($P < 0.05$). In contrast, experimental fish showed the improvement of the total bile acid level in the anterior intestine when they were fed with ethanol-extracted SBM and fermented SBM. A similar tendency was found in lipase activity in the anterior intestine. There were no significant differences of lipase activity among fish fed with FM, ExtSBM and FSBM diets while the enzyme activity of fish fed with SBM was statistically lower than that of fish fed with FM. Crude lipid in posterior intestinal digesta of fish fed with FM was significantly lower compared to that of fish fed with SBM. There were no differences among fish fed with ExtSBM, FSBM and FM diets.

Table 3. Total bile acid and lipase activity in the anterior intestinal digesta and crude lipid in the posterior intestinal digesta of pompano at 3h after feeding¹

Parameters	Dietary groups			
	FM	SBM	ExtSBM	FSBM
Total bile acid	103.5 ± 9.7 ^b	69.8 ± 8.2 ^a	98.5 ± 10.0 ^b	93.7 ± 12.2 ^b
Lipase activity	4.45 ± 1.02 ^b	2.34 ± 0.87 ^a	4.12 ± 0.62 ^{ab}	4.32 ± 0.73 ^b
Crude lipid	12.4 ± 1.2 ^a	19.7 ± 1.6 ^b	13.4 ± 1.3 ^a	14.5 ± 1.4 ^a

Note: Values are mean and standard deviation ($n = 4$). Values of each parameter in the same row with different superscripts are significantly different ($P < 0.05$). Total bile acid is calculated as $\mu\text{mol/g}$ dried digesta. Lipase activity is calculated as U/mg dried digesta. Crude lipid is calculated as mg/g dried digesta.

Plasma lipid levels after feeding are considered as one of the most important criteria to reflect the efficiency of dietary lipid digestion and absorption. In the present study, fish fed with SBM exhibited the lowest values of all plasma lipid components at 6h after feeding. In addition, fish fed with ExtSBM and FSBM resulted in the highest plasma lipid levels among the soybean-based diets. A similar tendency was found in crude lipid contents in both liver and muscle tissues. Since body lipid level is influenced by the efficiency of lipid digestion and absorption from the diet (Lee et al., 2002; Romarheim et al., 2006), these results indicated that fish fed with SBM might have poor dietary lipid digestion and absorption, and that the ethanol extraction and fermentation improved lipid digestion and absorption of pompano fed with SBM-based diets.

The poor dietary lipid digestion and absorption when pompano fish were fed with SBM was proven by total bile acid level and lipase activity in the anterior intestine and crude lipid content in the posterior intestine. It has been well-known that bile acids play an important role in lipid digestion and absorption through lipid emulsification and activation of pancreatic lipases. Before being stored in the gallbladder, bile acids are synthesized in the liver from cholesterol and conjugated with taurine or glycine (Tuchweber et al., 1996). Conjugation of bile acids is exclusive to taurine and cholytaurine and chenodeoxycholytaurine are the main bile acids in carnivorous fish species (Goto et al., 1996). In the present study, taurine was supplemented at the same levels of soybean protein-based diets (SBM, ExtSBM, FSBM). Moreover, nutrients were comparable among these diets. Therefore, the lower bile acid level observed in the intestine of SBM fed fish compared to those in ExtSBM and FSBM fed fish might not be due to low synthesis of bile acids. Since bile acids are secreted from the gallbladder and liver into the intestine (Tuchweber et al., 1996), the low bile acid level in SBM fed fish might be due to its low secretion from production and store tissues. Similar to total bile acid level, lipase activity was inferior in fish fed with SBM compared fish fed with other soybean-based diets while there were no significant differences in lipase activity among fish fed with FM, ExtSBM and FSBM. The low secretion of lipase from pancreatic gland could be responsible for the low lipase activity in the intestine of fish fed with SBM. Lower levels of both bile acids and lipase activity in the anterior intestine could be the factor inducing the higher content of crude lipid in the posterior intestine of fish fed with SBM compared to other fish. Since lipid contents were similar among four experimental diets, higher lipid contents remaining in the posterior intestinal digesta of fish fed with SBM compared to those fed with FM, ExtSBM and FSBM indicated that SBM interfered with lipid digestion and absorption in pompano. The comparable levels of lipid in posterior intestinal digesta observed in FM, ExtSBM and FSBM also suggested that ethanol extraction and fermentation could improve lipid digestion and absorption in this fish species.

4. CONCLUSIONS

Feeding SBM induced low lipid digestion and absorption in pompano *Trachinotus blochii*. The reduction of bile acid level and lipase activity in the anterior intestine was responsible for poor dietary lipid utilization. Ethanol extraction and fermentation with *Bacillus subtilis* could improve bile acid level and lipase activity, hence, increased lipid

digestion and absorption in pompano. These findings indicated that ethanol extraction and fermentation could be effective and potential ways to improve nutritional quality of SBM for culturing this fish species.

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TIÊU HÓA LIPID Ở CÁ CHIM VÂY VÀNG *Trachinotus blochii* ĐƯỢC NUÔI BẰNG CÁC LOẠI KHÔ ĐẬU TƯƠNG KHÁC NHAU

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Tóm tắt: Nghiên cứu được tiến hành nhằm tìm hiểu ảnh hưởng của khô đậu tương thường, khô đậu tương được chiết xuất bằng ethanol và khô đậu tương được lên men đến tiêu hóa lipid của cá chim vây vàng *Trachinotus blochii*. Bốn khẩu phần thức ăn được thiết lập và đặt tên dựa trên nguyên liệu chính, lần lượt là bột cá (FM), khô đậu tương thường (SBM), khô đậu tương được chiết xuất bằng ethanol (ExtSBM) và khô đậu tương được lên men bằng vi khuẩn *Bacillus subtilis* (FSBM). Cá chim vây vàng được nuôi bằng bốn khẩu phần thí nghiệm trên trong vòng 3 tuần. Kết quả cho thấy hàm lượng lipid trong máu và các mô, hàm lượng acid mật tổng số và hoạt tính của lipase trong vật chất tiêu hóa ở ruột trước của cá ăn khẩu phần SBM thấp hơn rõ rệt so với cá ăn khẩu phần FM. Tuy nhiên, những chỉ số này ở cá ăn khẩu phần ExtSBM và FSBM lại tương đương với cá ăn khẩu phần FM. Lượng lipid còn lại trong vật chất tiêu hóa ở ruột sau của cá ăn khẩu phần SBM cao hơn đáng kể so với cá ăn khẩu phần FM, trong khi đó, không phát hiện thấy khác biệt đáng kể về chỉ tiêu này ở các nhóm cá ăn khẩu phần ExtSBM, FSBM và FM. Các kết quả này chỉ ra rằng, khô đậu tương thường có ảnh hưởng tiêu cực đến quá trình tiêu hóa và hấp thu lipid khẩu phần của cá chim vây vàng *Trachinotus blochii*. Ảnh hưởng tiêu cực này thông qua việc làm giảm hàm lượng acid mật tổng số và hoạt tính của lipase trong ruột trước. Phát hiện trong nghiên cứu này cũng mở ra triển vọng có thể cải thiện giá trị dinh dưỡng của SBM và sử dụng làm thức ăn cho cá chim vây vàng thông qua việc lên men bằng vi khuẩn *Bacillus subtilis* hoặc chiết xuất bằng ethanol.

Từ khóa: *Trachinotus blochii*, Cá chim vây vàng, chiết xuất bằng ethanol, khô đậu tương, lên men, tiêu hóa lipid.

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