

Effects of Ethanol Extract from Soybean Meal on Plasma Lipid Level, Bile acid Concentration, Lipase Activity, Fecal Lipid Content and Weight Gain in Swiss Mice

Phuc Hung Nguyen¹, Ngoc Linh Nguyen², Thu Thao Lai¹, Van Hung Mai^{3*}

¹ Department of Human and Animal Physiology, Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam

² National College for Education, Vietnam

³ Department of Science Education, Faculty of Education, VNU University of Education, Vietnam National University, Hanoi, Vietnam

ABSTRACT

This study aimed to examine the effects of ethanol-soluble components (Es) in soybean meal (SBM) on plasma lipid level, bile acid concentration, lipase activity and fecal lipid content in mice. The SBM was extracted with aqueous ethanol, then the supernatant was separated from the residue and dried to produce the Es. Four experimental diets were prepared and denoted as CD (standard diet, control), CD+Es10 (CD plus Es 10 g/kg), CD+Es20 (CD plus Es 20 g/kg) and CD+Es30 (CD plus Es 30 g/kg). Nine mice with an initial body weight of 31 g were allocated to each of 8 cages, resulting in two replicate cages per dietary treatment. For 3 weeks, the mice were fed the experimental diets *ad libitum*. The results showed that the final body weight and weight gain tended to be lower in mice fed diets supplemented with the Es, while feed intake did not differ among the treatment. Mice fed diets supplemented with the Es had lower plasma total cholesterol and triglyceride levels, inferior bile acid concentration and lipase activity in the intestine, but higher lipid content in feces as compared to those fed CD. The findings of the present study suggested that the Es in SBM negatively affected lipid digestion process in Swiss mice and could be used to reduce dyslipidemia and obesity.

Keywords: Ethanol-soluble components, soybean meal, bile acid, lipase, lipid digestion, mice

Correspondence:

Van Hung Mai

Department of Science Education, Faculty of Education, VNU University of Education, Vietnam National University, Hanoi, Vietnam

E-mail: hungmv@vnu.edu.vn

INTRODUCTION

Dyslipidemia is known as a risk factor for many diseases such as atherosclerosis, coronary artery disease and stroke [1]. This disease is indicated by an increase in plasma triglyceride and low-density lipoprotein cholesterol and a decrease in high-density lipoprotein cholesterol. Several studies have demonstrated the roles of natural to treat and prevent against the dyslipidemia, however, the effects of natural compounds on lipid metabolism disorders depend on sources of compounds, extraction methods and the combination of the components. Defatted soybean meal (SBM), which is produced by defatting soybean with solvents, has been commonly used as an alternative ingredient to replace fish meal in aquatic animal feed production [2]. However, several studies have demonstrated that anti-nutritional factors (ANFs) in SBM, such as trypsin inhibitors, β -conglycinin, glycinin, stachyose, raffinose, lectins, saponins, and phytate, negatively affect growth performance, feed utilization, and physiological conditions of aquatic animals, including fish [3, 4]. Among the ANFs, ethanol-soluble components have been reported to decrease lipase activity and bile acid level in the intestine, thereby, reduce lipid digestibility and growth performance in carnivorous fish species [5, 6]. In addition to these digestive disorders, hypocholesterolemia is a prominent symptom in fish given SBM-based diets [7, 8]. These findings suggest that ethanol-soluble components in SBM may reduce plasma cholesterol level and interfere with lipid digestion process in terrestrial animals, hence, play an important role to decrease body weight gain. To date, there have been no studies on alcohol-soluble components of SBM mice. Therefore, this study aimed to examine the effects of ethanol extract (Es) in SBM on plasma lipid level, bile acid concentration, lipase activity, lipid digestion and body weight gain in Swiss mice.

MATERIALS AND METHODS

Ethanol extract from SBM

Commercially available defatted SBM was extracted with 70, 80, and 90% aqueous ethanol, respectively. At each extraction step, SBM was mixed manually with the aqueous ethanol solution at a ratio of 1:3 (w/v) for 2 h and then left at room temperature for 24 h. The supernatant and residue were separated by decanting. The supernatants from the three independent extractions were pooled and evaporated to produce ethanol extract (Es; dry matter content, 20%). The ES was stored at -20°C until use.

Experimental diets

Four experimental diets were prepared and denoted as CD (control diet), CD+Es10 (CD plus Es 10 g/kg), CD+Es20 (CD plus Es 20 g/kg), and CD+Es30 (CD plus Es 30 g/kg). The CD was the standard feed containing 25% protein, 5% lipid and 40% carbohydrate (dry matter basis) which was purchased from the National Institute of Hygiene and Epidemiology. The Es was supplemented into the CD as follows: the standard feed was grinded, then the powder was mixed well with the Es. Water was added to the mixture to produce a stiff dough. The dough was then pelleted with a laboratory pellet mill with a size similar to the standard feed. The moist pellets were then dried by an oven and stored at -20°C until use.

Animal rearing conditions

Male Swiss mice (*Mus musculus*, 6-week-old) provided by the National Institute of Hygiene and Epidemiology were acclimated to the experimental conditions for 2 weeks before the start of the feeding trial. Nine mice with an initial body weight of 31 g were allocated to each of 8 cages (40 cm × 50 cm × 30 cm), resulting in two replicate cages per dietary treatment. The mice were given the experimental diets and water *ad libitum*, for 3 weeks. The

room temperature was kept constantly at 25°C, and feed and water were renewed daily.

Sample collection

At the beginning of the feeding trial, three mice in each cage were randomly selected to collect blood samples from tail veins after starving for 24 h. The blood samples were used for total cholesterol and triglyceride analyses. The sampled mice were then removed from the cages. At the end of the feeding trial, all mice were also starved for 24 h, then weighed individually to determine the average final body weight. After weighing, blood samples were collected through tail veins of three random mice in each cage. These blood samples were then used for total cholesterol and triglyceride analyses. The remaining mice continued to be fed the experimental diets and feces were collected from bottom of the cage and used for fecal lipid analysis. After collecting an enough amount of feces, the mice were sampled for the intestinal digesta at 6 h after feeding. For this purpose, the mice were given the tested diets for 30 min, then the diets were removed, and the mice were dissected at 6 h after feeding. The intestinal digesta were collected at the same intestinal section (whole 3-cm section at the beginning of small intestine) for all the mice. The intestinal digesta samples were then used for bile acid concentration and lipase activity analyses.

Analytical methods and calculation formula

Plasma total cholesterol and triglyceride were 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 methanol:chloroform (1:1, v/v), according to the method described by Setchell *et al.* (1983) [9]. The

bile acid extract from the intestinal digesta was used for quantification of total bile acid level with a commercial assay kit (MAK309; Sigma-Aldrich Corp., St. Louis, MO, USA). Lipase in freeze-dried intestinal digesta was extracted by homogenization into four volumes (v/w) of cold distilled water as described previously [10]. Lipase activity in the extract was measured in accordance with the method described by Murashita *et al.* (2007) [11]. Briefly, lipase activity was measured as follows: a total of 150 µl of enzyme extract was incubated with 0.4 mM *p*-nitrophenyl myristate (Sigma-Aldrich, St. Louis, MO, USA) in 24 mM ammonium bicarbonate, 7.5 mM sodium deoxycholate, and 0.5% Triton X-100, pH 8.5 (total volume: 1.5 ml). Lipase catalytic activity was determined by measurement of the rate of *p*-nitrophenol (*p*NP) production at its optimal reaction temperature (37°C). The increase in absorbance at 405 nm was recorded every minute for 5 min. Reaction rates were calculated in units (U), such that 1 U was defined as 1 µmol of *p*NP released in 1 min. Weight gain was calculated using the following formula: weight gain (%) = 100 × (final mean body weight - initial mean body weight)/initial mean body weight.

Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA) using SPSS for Windows (version 16.0) statistical software (SPSS Inc., Chicago, IL, USA). The Tukey-Kramer test was performed to assess statistical differences between groups. A probability (P) value of < 0.05 was considered statistically significant.

RESULTS

Body weight, weight gain and feed intake

Table 1. Body weight, weight gain and feed intake of mice fed the experimental diets¹

Parameters	Dietary treatment			
	CD	CD+Es10	CD+Es20	CD+Es30
Initial body weight (g)	31.5 ± 1.7	31.3 ± 1.4	31.2 ± 1.9	31.3 ± 1.5
Final body weight (g)	42.0 ± 2.5	41.4 ± 2.1	40.5 ± 2.7	40.6 ± 2.4
Weight gain (%)	33.3 ± 3.1	31.3 ± 2.7	29.8 ± 3.4	29.7 ± 2.9
Feed intake (g/mouse/day)	3.9 ± 0.4	3.9 ± 0.3	3.8 ± 0.2	3.7 ± 0.3

¹Values are presented as the mean ± standard deviation of two replicates.

Body weight, weight gain and feed intake of mice fed the experimental diets are shown in Table 1. The final body weight and weight gain tended to decrease when mice were fed diets supplemented with Es as compared to those fed CD, though no significant differences were observed (P > 0.05). The tested diets did not alter the feed intake of the experimental mice.

Plasma total cholesterol and triglyceride levels

As presented in Table 2, there were no significant differences in plasma total cholesterol and triglyceride levels at the beginning of the trial (P > 0.05). However, at the end of the trial, mice fed diets supplemented with Es reduced both total cholesterol and triglyceride levels in plasma. The mice fed CD+Es20 and CD+Es30 resulted in significantly lower levels of plasma total cholesterol and triglyceride as compared to those fed CD (P < 0.05).

Table 2. Plasma total cholesterol and triglyceride level of mice fed the experimental diets¹.

Parameters		Dietary treatment			
		CD	CD+Es10	CD+Es20	CD+Es30
Total cholesterol (mmol/l)	Initial	1.45 ± 0.09	1.42 ± 0.10	1.47 ± 0.07	1.43 ± 0.08
	Final	1.55 ± 0.07 ^c	1.50 ± 0.08 ^{bc}	1.34 ± 0.06 ^{ab}	1.28 ± 0.05 ^a
Triglyceride (mmol/l)	Initial	0.60 ± 0.04	0.58 ± 0.05	0.62 ± 0.03	0.56 ± 0.04
	Final	0.62 ± 0.05 ^b	0.58 ± 0.04 ^b	0.43 ± 0.03 ^a	0.42 ± 0.03 ^a

¹Values are presented as the mean ± standard deviation (n = 6). The values of each parameter in the same row with different letters are significantly different (P < 0.05).

Total bile acid concentration and lipase activity in the intestinal digesta

Figure 1 shows that total bile acid concentrations in the intestinal digesta of mice fed CD+Es20 and CD+Es30 were similar and significantly lower than those in mice fed CD and CD+Es10 (P < 0.05). There was no significant

difference in total bile acid concentration in the intestinal digesta between the CD and CD+Es10 experimental groups (P > 0.05).

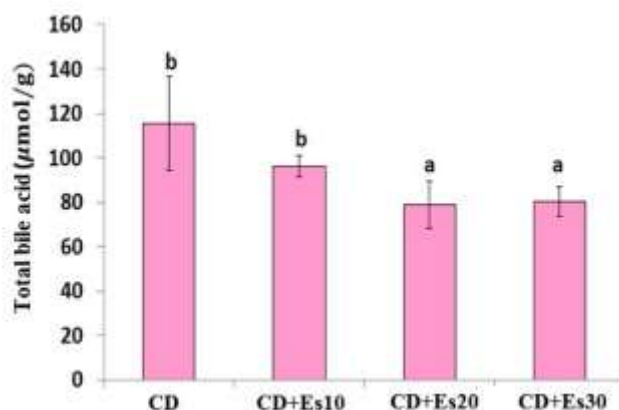


Figure 1. Total bile acid concentration in the intestinal digesta of mice fed the experimental diets. Values are presented as the mean ± standard deviation (n = 6). The values with different letters denote significant differences (P < 0.05).

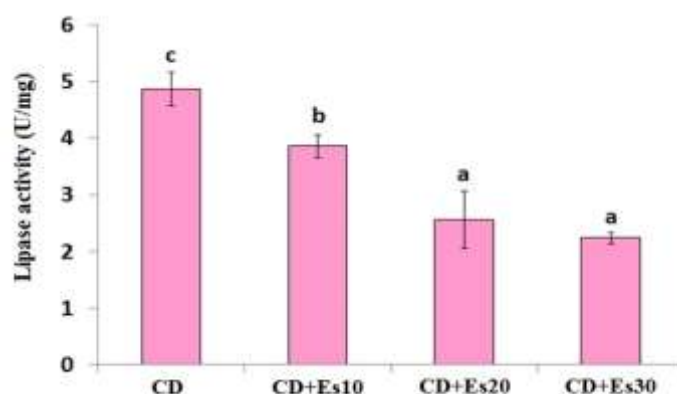


Figure 2. Lipase activity in the intestinal digesta of mice fed the experimental diets. Values are presented as the mean ± standard deviation (n = 6). The values with different letters denote significant differences (P < 0.05).

As shown in Figure 2, the Es significantly reduced lipase activity in the intestinal digesta of mice. This parameter in mice given CD+Es10 was significantly lower than that in the CD group ($P < 0.05$). In addition, the lipase activity was further decreased in mice fed CD+Es20 and CD+Es30, and significant differences were observed ($P < 0.05$).

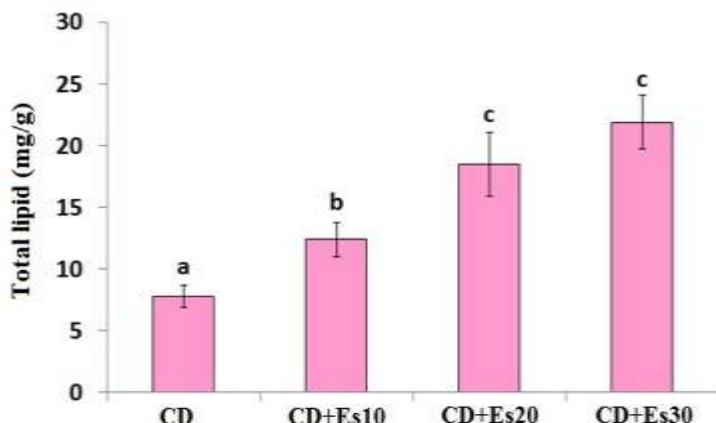


Figure 3. Total fecal lipid content of mice fed the experimental diets. Values are presented as the mean \pm standard deviation of two replicates. The values with different letters denote significant differences ($P < 0.05$).

DISCUSSION

In the present study, the final body and weight gain in the mice fed diets supplemented with the Es tended to be lower than those fed CD, though no significant differences were observed. Meanwhile, the feed intake was similar among the treatments. These results suggested that the Es in SBM might reduce weight gain in a long-term feeding. It has been reported that some fish species, such as Chinook salmon [12], rainbow trout [13], and yellowtail [5] resulted in poor growth performance when they were fed diets supplemented with alcohol extracts of SBM. In the present study, the feed intake did not differ among the treatments, indicating that the inferior growth performance of the experimental mice might be attributable to poor nutrient digestion and absorption.

Digestive disorders can interfere with nutrient digestion and absorption, resulting in poor weight gain. In the present study, the lipid digestion physiology of the experimental mice was investigated by measuring bile acid level and lipase activity in the small intestine. The results showed that both the total bile acid level and lipase activity were reduced by supplementing the Es into the diet. BAs are synthesized from cholesterol in the liver, stored in the gallbladder, then secreted into the small intestine [14]. In addition, the pancreas produces digestive enzymes including lipase, which are secreted into the intestine [15]. In the present study, the inferior bile acid level in the intestinal digesta in the mice fed diets supplemented with the Es might be due to its low secretion and/or synthesis. Similarly, poor synthesis and/or secretion of lipase could be the factor responsible for low lipase activity in the intestine of mice fed the Es-included diets. Cholecystokinin (CCK) is an important hormone that stimulates secretion of pancreatic digestive enzymes and releases bile juice from the gallbladder [16]. The CCK level was reportedly decreased in some animals fed SBM-based diets, causing low secretion of bile acids and pancreatic digestive enzymes into the intestine [5]. Further studies are necessary to identify the mechanism

3.4. Total lipid content in feces

Figure 3 shows that mice fed diets supplemented with Es significantly increased total lipid content in feces as compared to those fed CD ($P < 0.05$). The highest values of total fecal lipid content were found in mice fed CD+Es20 and CD+Es30, followed by those fed CD+Es10.

inducing low bile acid level and lipase activity in the intestine of mice fed the Es-included diets.

It has been reported that alcohol-soluble components in SBM reduced lipid digestion in aquatic animals [5, 6]. In the current study, total lipid content in feces of mice fed diets supplemented with the Es was higher than that in the CD group. This result indicated that the Es in SBM interfered with lipid digestion and absorption of mice. This finding, together with total bile acid level and enzyme activity in the intestine, suggested that the poor lipid digestion and absorption caused by the Es in SBM was due to the insufficiency of bile acids and lipase in the intestine. The poor dietary lipid digestion and absorption could be contributable to low plasma total cholesterol and triglyceride levels. The findings of the present study suggested that the Es in SBM negatively affected lipid digestion process in Swiss mice and could be used to reduce dyslipidemia and obesity.

Some alcohol-soluble components in SBM have been shown to cause digestive physiological disorders in aquatic animals. Soya saponins reportedly reduce bile salt level and aminopeptidase activity in the intestinal digesta and decrease lipid digestibility in Atlantic salmon [17]. Feeding Atlantic salmon with soybean molasses, which mainly contains stachyose, raffinose and sucrose, reduces lipid digestibility [6]. Moreover, stachyose and raffinose in SBM can also cause enteritis in the distal intestine of salmonids [3]. Therefore, these alcohol-soluble components in SBM might be the factors responsible for the digestive physiological inhibitions observed in mice in the current study.

CONCLUSION

In conclusion, the Es in SBM reduced bile acid level and lipase activity in the intestine of Swiss mice, resulting in poor lipid digestion and absorption. These negative effects of the Es on lipid digestion process contributed to decrease plasma total cholesterol and triglyceride levels and weight gain. The findings of the present study

suggested that the Es in SBM could be used to reduce dyslipidemia and obesity.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted in the author guidelines, have been adhered to. The authors also confirm that they have followed European Union standards for the protection of animals used for scientific purposes.

REFERENCES

- Franssen, R., Monajemi, H., Stroes, E. S. G., Kastelein, J. J. P., 2011. Obesity and Dyslipidemia. Medical Clinics of North America, 95, 893-902. <https://doi.org/10.1016/j.mcna.2011.06.003>
- Porter, M. A., Jones, A. M., 2003. Variability in soy flour composition. Journal of the American Oil Chemists' Society, 80, 557-562. <https://doi.org/10.1007/s11746-003-0737-6>
- Van den Ingh, T. S. G. A. M., Olli, J. J., Kroghdahl, A., 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases, 19, 47-53. <https://doi.org/10.1111/j.1365-2761.1996.tb00119.x>
- Francis, G., H. P. Makkar, K. Becker., 2001. Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227. doi:10.1016/S0044-8486(01)00526-9
- Nguyen, H. P., Khaoian, P., Furutani, T., Nagano, J., Fukada, H., Masumoto, T., 2017. Effects of alcohol extract of defatted soybean meal on growth performance and digestive physiology of yellowtail *Seriola quinqueradiata*. Fisheries Science, 83, 99-106. <https://doi.org/10.1007/s12562-016-1049-x>
- Olli, J. J., Kroghdahl, A., 1995. Alcohol soluble components of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar* L. Aquaculture Research, 26, 831-835. <https://doi.org/10.1111/j.1365-2109.1995.tb00876.x>
- Sugano M, Goto S, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, Kimoto M, 1990 Cholesterol-lowering activity of various undigested fractions of soy protein in rats. J Nutr 120, 977-985. <https://doi.org/10.1093/jn/120.9.977>
- Higaki N, Sato K, Suda H, Suzuka T, Komori T, Saeki T, Nakamura Y, Ohtsuki K, Iwami K, Kanamoto R, 2006. Evidence for the existence of a soybean resistant protein that captures bile acid and stimulates its fecal excretion. Biosci Biotechnol Biochem 70, 2844-2852. <https://doi.org/10.1271/bbb.60237>
- Setchell, K. D. R., Lawson, A. M., Tanida, N., Sjovall, J., 1983. General methods for the analysis of metabolic profiles of bile acids and related compounds in feces. Journal of Lipid Research, 24, 1085-1100.
- Nguyen, H. P., Khaoian, P., Nagano, J., Fukada, H., Suzuki, N., Masumoto, T., 2013. Feeding fermented soybean meal diet supplemented with taurine to yellowtail *Seriola quinqueradiata* affects growth performance and lipid digestion. Aquaculture Research, 46, 1101-1110. <https://doi.org/10.1111/are.12267>
- Murashita, K., Fukada, H., Hosokawa, H., Masumoto, T., 2007. Changes in cholecystokinin and peptide Y gene expression with feeding in yellowtail (*Seriola quinqueradiata*): Relation to pancreatic exocrine regulation. Comparative Biochemistry and Physiology Part B, 146, 318-325. <https://doi.org/10.1016/j.cbpb.2006.11.009>
- Bureau, D. P., Harris, A. M., Cho, C. Y., 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 161, 27-43. [https://doi.org/10.1016/S0044-8486\(97\)00254-8](https://doi.org/10.1016/S0044-8486(97)00254-8)
- Yamamoto, T., Goto, T., Kine, Y., Endo, Y., Kitaoka, Y., Sugita, T., Furuita, H., Iwashita, Y., Suzuki, N., 2008. Effect of an alcohol extract from a defatted soybean meal supplemented with a casein-based semi-purified diet on the biliary bile status and intestinal conditions in rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquaculture Research, 39, 986-994. <https://doi.org/10.1111/j.1365-2109.2008.01969.x>
- Tuchweber, B., Yousef, I. M., Ferland, G., Perea, A., 1996. Nutrition and bile formation. Nutrition Research, 16, 1041-1080. [https://doi.org/10.1016/0271-5317\(96\)00104-2](https://doi.org/10.1016/0271-5317(96)00104-2)
- Fange, R., Grove, D., 1979. Digestion. In: W. S. Hoar, D. J. Randall, J. R. Brett (Eds.), Fish Physiology. Academic Press, New York, NY, USA, pp. 161-260.
- Shiratori, K., Watanabe, S., Chey, W. Y., Lee, K. Y., Chang, T. M., 1986. Endogenous cholecystokinin drives gallbladder emptying in dogs. American Journal of Physiology, 251, G553-G558. <https://doi.org/10.1152/ajpgi.1986.251.4.G553>
- Chikwati, E. M., Venold, F. F., Penn, M. H., Rohloff, J., Refstie, S., Guttvik, A., Hillestad, M., Kroghdahl, A., 2012. Interaction of soya saponins with plant ingredients in diets for Atlantic salmon, *Salmo salar* L. British Journal of Nutrition, 107, 1570-1590. <https://doi.org/10.1017/S0007114511004892>