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EFFECTS OF ALCOHOL-SOLUBLE COMPONENTS IN SOYMILK RESIDUE ON PLASMA LIPID LEVELS, LIPID DIGESTION AND BODY WEIGHT GAIN IN SWISS MICE

Dao Ngoc Anh[1], Nguyen Manh Duc[2] and Nguyen Phuc Hung[1,*]
[1]*Faculty of Biology, Hanoi National University of Education, Hanoi city, Vietnam*
[2]*HUS High School for Gifted Students, Vietnam National University, Hanoi city, Vietnam*
*Corresponding author: Nguyen Phuc Hung, e-mail: hungnp@hnue.edu.vn

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Abstract. The present study was conducted to examine the effects of alcohol-soluble components (alcohol extract, AE) in soymilk residue (SR) on body weight gain, plasma lipid levels, and lipid digestion in Swiss mice. Aqueous ethanol was used to extract the SR, afterwhich the residue and the supernatant were separated. The supernatant was then dried to produce the AE. Four experimental diets were formulated and designated as CD (control diet), CD+AE20 (CD supplemented with 20 g AE/kg), CD+AE40 (CD supplemented with 40 g AE/kg), and CD+AE60 (CD supplemented with 60 g AE/kg). Six mice (with an initial body weight of 24 g) were distributed into each of eight cages, with two replicate cages per dietary treatment. The experimental mice were fed with the test diets *ad libitum* for 4 weeks. The results showed that the final body weights of mice fed with AE-supplemented diets were lower than those fed with the control diet, while no differences in feed intake were found among the treatments. Moreover, the AE significantly reduced body weight gain with significant differences observed in mice receiving AE supplementation levels of 40 and 60 g/kg. The mice fed with AE-supplemented diets exhibited lower plasma concentrations of total cholesterol, low-density lipoprotein, and triglyceride as well as lower intestinal total bile acid level and lipase activity, but higher lipid content in feces, compared with those fed with the control diet. These findings indicated that the AE in SR negatively affected the lipid digestion process, therefore reducing plasma cholesterol levels and body weight gain in Swiss mice.

Keywords: alcohol-soluble components, soymilk residue, plasma cholesterol, lipid digestion, weight gain, mice.

1. Introduction

Dietary lipid and lipid digestion play important roles in lipid metabolism and body weight gain. A high intake of lipids and efficient absorption of dietary lipid promote elevated plasma lipid levels, adipose fat deposition, and increased body weight. These negative changes may induce dyslipidemia and obesity, which in turn increase the risk of diabetes, atherosclerosis, coronary artery disease, and stroke [1]. Scientific studies have revealed the roles of natural compounds in the treatment and prevention of dyslipidemia and obesity; however, the effects of those compounds on lipid metabolism disorders depend on many factors, such as extraction methods, compound sources, and component combinations [2].

Soybean has been known to contain several bioactive compounds. It has been reported that alcohol-soluble components in soybean negatively affect growth performance, feed utilization, and induce numerous physiological disorders in aquatic animals, including fish [3], [4]. Several studies have demonstrated that ethanol extracts from soybean reduce bile acid synthesis and secretion, and lower lipase activity, thereby decreasing lipid digestibility and growth performance in many fish species [5], [6]. Moreover, hypocholesterolemia is a prominent symptom in carnivorous fish fed with soybean meal-based diets [7], [8]. These findings in aquatic animals suggest that alcohol-soluble components in soybean may negatively affect lipid digestion process, decrease plasma cholesterol levels, and reduce body weight gain in mammals, including mice as well.

Soymilk residue (SR) is a solid by-product remaining after soybeans are extracted with water during the production of soymilk or soybean curd [9], [10]. On a dry matter basis, the SR contains relatively high levels of nutrients. The amounts of fiber, nitrogen-free extract, protein, lipid, and ash range from 9.1 to 18.6%, 27.5 to 46.3%, 15.2 to 32.2%, 6.9 to 10.9%, and 3.0 to 4.5%, respectively [10], [11]. Since water is used in the extraction process, SR may retain a high amount of alcohol-soluble components. To date, no studies have been conducted to evaluate the effects of alcohol-soluble components of the SR on lipid metabolism in mice. Therefore, the present study aimed to examine the effects of alcohol extract (AE) from SR on body weight gain, plasma lipid levels, and lipid digestion in Swiss mice.

2. Content

2.1. Materials and methods

2.1.1. Alcohol-soluble components of SR

Commercially available SR was purchased from Vinasoy Corp. (Tu Son, Bac Ninh, Vietnam). This meal was dried in an oven at 60 °C, then ground to a particle size below 400 μm. Three aqueous ethanol solutions (70, 80, and 90%) were used to extract the dried SR [5], [12]. At each extraction, the aqueous ethanol solution was mixed with SR at a ratio of 3:1 (v/w) for 2 h and then allowed to stand at room temperature for 24 h. After that, the residue and supernatant were separated by decantation. The supernatants

from the three independent extractions were pooled and then evaporated to produce the alcohol-soluble components (alcohol extract, AE; dry matter content, 10%).

2.1.2. Experimental diets

Four experimental diets were designated as CD (control diet), CD+AE20 (CD plus 20 g AE/kg), CD+AE40 (CD plus 40 g AE/kg), and CD+AE60 (CD plus 60 g AE/kg). The amount of AE supplemented into the experimental diets was based on studies on alcohol extracts from soybean meal in fish conducted by Nguyen *et al*. [5], [12]. The control diet consisted of standard feed pellets for Swiss mice containing 25% protein, 6% lipid, 40% carbohydrate (on a dry matter basis), with a gross energy content of 3800 kcal/kg. This diet was obtained from the National Institute of Hygiene and Epidemiology (Hanoi, Vietnam). The standard feed pellets were finely ground into powder and then supplemented with AE. The new feed pellets for experimental mice were produced using a laboratory pellet mill with a size similar to that of the standard feed. The moist pellets were then dried in an oven and stored at -20 °C until use.

2.1.3. Animal rearing conditions

Male Swiss mice (*Mus musculus,* 4-week old) obtained from the National Institute of Hygiene and Epidemiology were randomly distributed into experimental cages and fed the CD diet for one week to acclimate to experimental conditions. After that, six mice (with an initial body weight of 24 g) were distributed to each of eight cages (40 cm × 50 cm × 30 cm), resulting in two replicate cages per dietary treatment. For 4 weeks, the test diets were fed *ad libitum* to the experimental mice. Feed and water were renewed daily, and the room temperature was kept constantly at 25 °C.

2.1.4. Sample collection

At the end of the feeding trial (4 weeks), all the experimental mice were fasted for 24 h, and then individually weighed to determine final body weight and weight gain. After that, three mice were randomly selected from each cage for blood collection. Blood samples were taken from tail veins as described by Madetoja *et al.* (2009) [13], and then used for plasma lipid component analyses. The remaining experimental mice in each cage continued to be fed with the test diets to collect fecal samples for lipid analysis. Fecal samples were collected from the bottom of the cages. After a sufficient amount of feces for lipid analysis was collected, the mice were dissected 6 h after feeding to sample intestinal digesta. For this purpose, the mice were fed with the experimental diets for 30 min, and then dissected 6 h later. The intestinal digesta were collected from the same intestinal section in all mice (a 3-cm segment at the beginning of the small intestine) and then used for lipase activity and bile acid concentration analyses.

2.1.5. Analytical methods and calculation

A commercial automatic analyzer (Architect c16000, Abbott, Illinois, USA) at Medlatec (Hanoi, Vietnam) was used to analyze plasma triglyceride and cholesterol levels. The intestinal digesta samples were freeze-dried and used for bile acid extraction according to the method described by Setchell *et al.* (1983) [14], using 90% ethanol, followed by a methanol:chloroform (1:1, v/v) extraction. Finally, the total bile acid level was quantified employing a commercial assay kit (MAK309; Sigma-Aldrich Corp., St. Louis, MO, USA). Freeze-dried intestinal digesta was homogenized and extracted with

cold distilled water (1:4, w/v) as described by Nguyen *et al.* [5]. Lipase activity of the extract was measured according to Murashita *et al.* (2007) [15]. In summary, lipase activity was measured as follows: an aliquot of 150 µL of enzyme extract was incubated with a solution containing 0.4 mM *p*-nitrophenyl myristate (Sigma-Aldrich, St. Louis, MO, USA), 7.5 mM sodium deoxycholate, 24 mM ammonium bicarbonate, and 0.5% Triton X-100, pH 8.5 at optimal temperature (37 °C). After that, the absorbance of the product, *p*-nitrophenol (*p*NP), was recorded at 405 nm for 5 min at 1 minute-intervals. Reaction rates were then calculated in units (U), defined as µmol *p*NP/min. Body weight gain was calculated according to the following formula: weight gain (%) = 100 × (final mean body weight - initial mean body weight)/initial mean body weight.

2.1.6. Statistical analysis

One-way analysis of variance (ANOVA) from SPSS statistical software for Windows (version 16.0) (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Statistical differences between groups were determined using the Tukey-Kramer test. A probability (*P*) value < 0.05 was considered statistically significant.

2.2. Results

2.2.1. Growth performance and feed intake

Initial and final body weight, weight gain, and feed intake of experimental mice are presented in Table 1. The final body weight tended to decrease in mice fed with AE-supplemented diets as compared to those fed with the CD diet, although no significant differences were observed ($P > 0.05$). Despite the lack of significant differences in final body weight, body weight gain was statistically higher in mice fed with CD+AE40 and CD+AE60 diets than those fed with the CD diet ($P < 0.05$). The tested diets did not affect the feed intake of the experimental mice.

Table 1. Growth performance and feed intake of the experimental mice

Parameters*	Dietary groups			
	CD	***CD+AE20***	***CD+AE40***	***CD+AE60***
Initial body weight (g)	24.2 ± 0.9	24.1 ± 0.5	24.2 ± 0.6	24.0 ± 0.7
Final body weight (g)	35.3 ± 1.8	34.4 ± 1.5	33.4 ± 1.4	32.9 ± 1.6
Weight gain (%)	45.9 ± 3.3^{b}	42.7 ± 3.2^{ab}	38.0 ± 3.1^{a}	37.1 ± 3.2^{a}
Feed intake (g/mouse/day)	3.9 ± 0.3	3.8 ± 0.4	3.8 ± 0.2	3.8 ± 0.3

**Values are presented as mean ± standard deviation of two replicates. The values with different superscripts in the same row are significantly different (P < 0.05).*

2.2.2. Plasma cholesterol and triglyceride concentrations

As presented in Table 2, the experimental mice fed with AE-supplemented diets exhibited lower total cholesterol and triglyceride levels compared with those fed with the CD diet, with statistically significant differences observed between CD+AE40, CD+AE60, and CD groups ($P < 0.05$). HDL-C concentration tended to increase in the mice fed with AE-supplemented diets as compared to those fed with the CD diet,

although there were no significant differences among the treatments. In contrast, LDL-C level was significantly lower in the mice fed with CD+AE20, CD+AE40, and CD+AE60 diets than those fed with the CD diet ($P < 0.05$).

Table 2. Plasma cholesterol and triglyceride concentrations of the experimental mice

Parameters*	Dietary groups			
	CD	CD+AE20	CD+AE40	CD+AE60
TC (mmol/L)	1.62 ± 0.08^{b}	1.53 ± 0.10^{ab}	1.39 ± 0.09^{a}	1.35 ± 0.07^{a}
HDL-C (mmol/L)	0.71 ± 0.03	0.76 ± 0.04	0.81 ± 0.03	0.79 ± 0.02
LDL-C (mmol/L)	0.91 ± 0.05^{c}	0.77 ± 0.02^{b}	0.58 ± 0.03^{a}	0.56 ± 0.02^{a}
TG (mmol/L)	0.68 ± 0.08^{b}	0.55 ± 0.09^{ab}	0.47 ± 0.06^{a}	0.42 ± 0.07^{a}

**Values are presented as mean ± standard deviation (n = 6). The values with different superscripts in the same row are significantly different (P < 0.05). Abbreviations: TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.*

2.2.3. Intestinal bile acid level and lipase activity

Figure 1 shows that total bile acid levels in the intestinal digesta of mice fed with CD+AE20, CD+AE40, and CD+AE60 diets were significantly lower than those in mice fed with the CD diet ($P < 0.05$). No significant differences were observed in total bile acid concentration in the intestinal digesta among dietary groups fed with AE ($P > 0.05$).

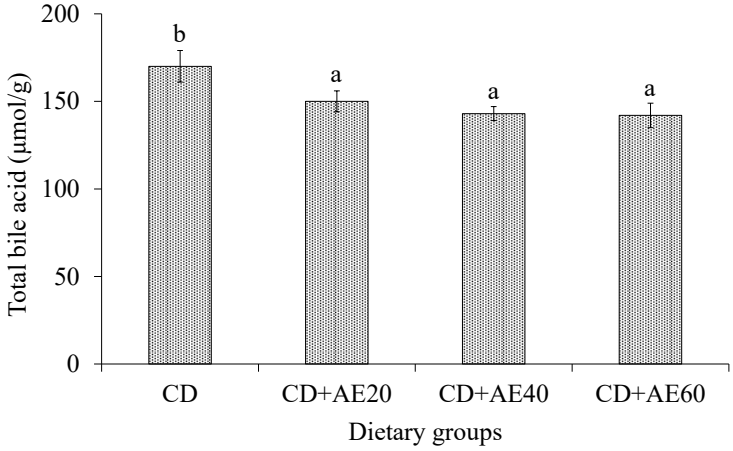

Figure 1. Intestinal total bile acid level of the experimental mice

Values are presented as mean ± standard deviation (n = 6).

Bars assigned with different letters denote significant differences (P < 0.05)

Figure 2 shows the intestinal lipase activity of the experimental mice. Similar to the total bile acid concentration, lipase activity in the intestinal digesta of mice was reduced by the presence of AE in the diets. Lipase activity in mice fed CD+AE40 and CD+AE60 diets was significantly lower than that in the CD group ($P < 0.05$). However, there was no significant difference in lipase activity between the CD and CD+AE20 groups ($P > 0.05$).

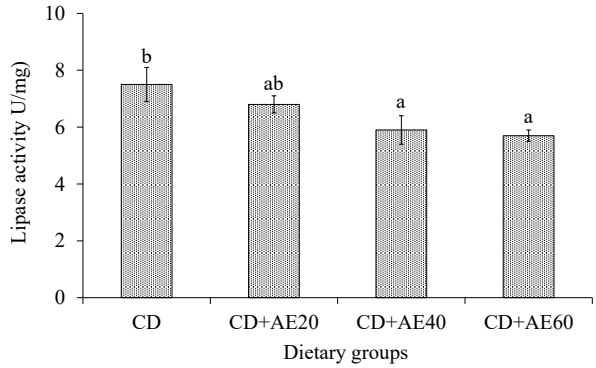

Figure 2. Intestinal lipase activity of the experimental mice

Values are presented as mean ± standard deviation (n = 6).

Bars assigned with different letters denote significant differences (P < 0.05)

2.2.4. Fecal lipid content

Figure 3 shows that all three dietary AE-supplemented levels significantly increased fecal lipid contents in the experimental mice ($P < 0.05$). The highest values of lipid content in feces were observed in mice fed with CD+AE40 and CD+AE60, followed by those fed with CD+AE20.

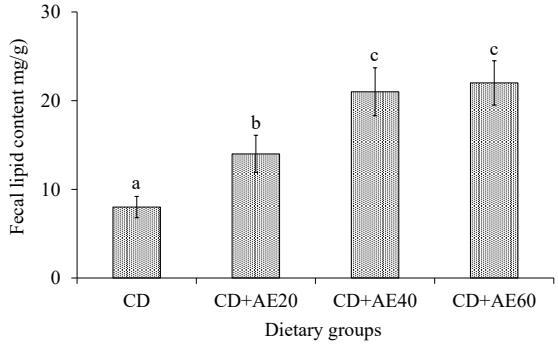

Figure 3. Fecal lipid content of the experimental mice

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

Bars assigned with different letters denote significant differences (P < 0.05)

2.3. Discussion

In this study, the experimental mice fed with AE-supplemented diets had lower body weight gain than those fed with the CD diet, with significant differences observed in the CD+AE40 and CD+AE60 groups as compared to the CD group. In contrast, the feed intake of the mice was similar among the treatments. These results indicate that the alcohol-soluble components in the SR might reduce body weight gain during long-term feeding, and that the dietary supplementation level of these compounds required to elicit negative effects in mice should be at least 40 g/kg diet. It has been reported that aquatic animals, such as Chinook salmon [16], rainbow trout [17], yellowtail [5], and pompano [12], showed inferior growth performance when fed with diets supplemented with alcohol extracts of soybean meal. In the current study, since there were no significant

differences in feed intake among the dietary groups, the lower body weight gain of the mice fed with AE-supplemented diets might be due to poor nutrient digestion and absorption.

Disorders in digestive physiology can negatively affect nutrient digestion and absorption, leading to reduced body weight gain. In the current experiment, we examined lipid digestion physiology by measuring intestinal lipase activity and bile acid levels, which play central roles in the lipid digestion process. The results showed that both lipase activity and total bile acid level were decreased by the addition of AE to the diets. It is well known that bile acids are synthesized from cholesterol in the liver and then stored in the gallbladder. When digesta enter the intestine, bile acids are secreted from these organs into the small intestine to emulsify lipids, thereby promoting the lipase digestive effectiveness [16]. Therefore, the low bile acid levels in the intestinal digesta of the mice fed with AE-supplemented diets in the current study might be attributable to reduced secretion and/or synthesis. Moreover, lipase is synthesized in the pancreas, then secreted into the intestine, and this enzyme plays a crucial role in lipid digestion [17]. Hence, impaired synthesis and/or secretion of lipase could be responsible for the reduced intestinal lipase activity observed in mice fed with the AE-containing diets, which could decrease lipid digestion and absorption. An important hormone stimulating the secretion of pancreatic digestive enzymes and the release of bile juice from the gallbladder is cholecystokinin (CCK) [18]. It has been reported that the CCK level was reduced in some animals fed with high soybean meal-based diets, resulting in decreased pancreatic digestive enzyme and bile acid secretions into the intestine [5], [12]. Therefore, further studies are necessary to identify the mechanism underlying the reduced lipase activity and bile acid levels in the intestine of mice fed with the AE-supplemented diets.

Alcohol-soluble components in soybean meal have been reported to reduce lipid digestion and absorption in some animals, including fish [5], [6], [12]. In the present study, the fecal lipid content of the mice fed with AE-supplemented diets was higher than that of the mice fed with the CD diet. Since dietary lipid content was similar among all tested diets, the higher fecal lipid levels observed in the mice fed with AE-supplemented diets indicate that the AE in SR interfered with lipid digestion and absorption in mice. This finding, together with the observed reduction in lipase activity and total bile acid level in the intestine, suggests that impaired dietary lipid digestion and absorption caused by AE from SR was attributable to the insufficient intestinal bile acid and lipase. Reduced dietary lipid digestion and absorption could be responsible for the lower plasma triglyceride and cholesterol concentrations. These findings suggest that AE in SR interferes with the lipid digestion process in the experimental mice.

Soybeans contain various alcohol-soluble components, and the negative effects of these components on digestive physiology have been reported in aquatic animals. Soya saponins have been known to reduce intestinal bile acid concentration, thereby decreasing lipid digestibility in Atlantic salmon [19]. Feeding diets containing soybean molasses, which mainly consist of raffinose, stachyose, and sucrose, has been shown to reduce lipid digestibility in Atlantic salmon [6]. In addition, soybean raffinose and stachyose can also induce enteritis in the distal intestine of salmonids [3], [20]. Since water is used in the extraction process to produce soymilk or soybean curd [9], SR is

likely to retain a high amount of alcohol-soluble components, including saponins, lectins, raffinose, stachyose, and sucrose. Therefore, these compounds in SR might be the factors responsible for the digestive physiological disorders and reduced lipid digestion observed in mice in the present study.

3. Conclusions

In conclusion, AE from SR reduced lipase activity and bile acid level in the intestine of Swiss mice, leading to impaired lipid digestion and absorption. These adverse effects of AE on lipid digestion were responsible for the reduction in plasma cholesterol and triglyceride levels and body weight gain in the experimental mice. The findings of the current study suggest that further studies are necessary to examine the potential of AE from SR in the prevention and treatment of dyslipidemia and obesity.

Author contribution. Dao Ngoc Anh contributed to data acquisition, analysis, and interpretation, and to drafting the manuscript. Nguyen Manh Duc contributed to data acquisition, analysis, and interpretation, to the drafting and revision of the manuscript. Nguyen Phuc Hung was involved in the conception and design of the study, revision, and approval of the final version of the manuscript.

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