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Effects of Germinated Soybean on Serum Lipids in Rats

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Abstract: The purpose of this research was to evaluate the effects of germinated soybean on serum lipids by feeding male Sprague-Dawley rats with 6 experimental diets containing 10% protein-casein (control), 10% protein- soy protein isolate (10% SPI), 10% protein-ungerminated soybean (10% UGS), 10% protein-germinated soybean (10% GS), 20% protein-germinated soybean (20% GS) and 28% protein-germinated soybean (28% GS). The diets were prepared from 6 formulas by AOAC, with the following composition:10%, 20% and 28% test protein, 8% oil, 5% water, 5% AIN-mineral, 1% AIN-vitamin, 1% cellulose, 35% sucrose and 35% corn starch. The results showed that serum cholesterol, triglyceride, HDL-c and LDL-c levels were not significantly different among the rats fed with 10% UGS and 10% GS diets. However, serum cholesterol, triglyceride and LDL-c level in rats fed with 20% GS and 28% GS diets were significantly lower than those in rats fed with 10% UGS and 10% GS diets. Serum cholesterol, triglyceride and LDL-c levels in rats fed with 28% GS diet (57.40±6.25, 30.30±5.25,11.80±2.69 mg/dL) were significantly lower than those in rats fed with SPI diet (73.10±13.32, 58.20±13.13, 23.40±5.01 mg/dL). The data indicated that the experimental diet with 28% protein-germinated soybean could reduce serum cholesterol, triglyceride and LDL-c in the experimental rats.

Key words: Germinated soybean, lipid, serum, experimental rat

INTRODUCTION

Soybean is consumed by Asian populations and is today advocated for Western diets because of its nutritional benefits (Rimal *et al*., 2008). The use of soybean in human foods has been limited by the presence of several anti-nutritional factors. The majority of processed soybean products have been derived from dry mature soybeans. However, the development of products from germinated soybean presents another option to further increase the versatility and utilization of soybeans. Germination is defined as the process by which , under favorable conditions (Carvalho and Nakagawa, 1988), the embryonic axis returns to its development, which had been interrupted at physiological maturity. Absorption of water by the seed is the first event of germination (Neto, 2004). Sprouting also removes antinutrients such as enzyme inhibitors in the seeds, thus making sprouts safe for human consumption (Mwikya *et al*., 2001).

Germination of soybeans is a simple, low-cost and effective process for achieving desirable changes in nutritional and sensory characteristics (Cho *et al*., 2009; Mostafa *et al*., 1987). Germination causes significant changes in biochemical and nutritional characteristics, such as secondary metabolite distribution, breakdown of seed-storage compounds and increase in digestibility of proteins and starches (Bau *et al*., 2000; Mora-Escobedo *et al*., 2009). In addition, soybean germination overcomes the disadvantages associated with soybean seeds, such as undesirable flavour and texture, and antinutritional factors (Bau *et al*., 1997).

Soybean is an interesting plant because it is the source of plant protein and fiber. The seeds are high in protein but also contain a number of minor constituents traditionally considered to be antinutritional factors including trypsin inhibitors, phytic acid, saponins and isoflavones. These compounds are now known to have beneficial biological effects in the diet, such as lowering of blood cholesterol or preventing cancer. Although the effects of soybean on serum lipids are well documented no such studies have been reported for germinated soybean. So the objective of this study was to evaluate the effects of germinated soybean on serum lipids in experimental rats.

MATERIALS AND METHODS

Preparation of germinated and ungerminated soybean flour: Soybean samples (2x5kg) were washed and soaked in water (7,500 ml) for 10-12 h. After 12 h, the

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soybeans were washed with clean water and put in 7 punching tins with flow-through water. By pouring water through the punching tin 2 times every 2 h to keep the temperature not exceeding 25°C, the soybean started to germinate in 2 days. Two days germinated soybean were collected , washed, steamed at 100°C for 30 min and dried at 65°C for 4 h 15 min followed by 70°C for 2 h. Dry germinated soybean were powdered by using a pin mill. Ungerminated soybean flour was prepared by the same method without germination process.

Preparation of experimental diets: Germinated soybean flour, ungerminated soybean flour , soy protein isolate and casein were prepared for the 6 experimental diets by the AOAC method (Horwitz and latimer, 2006) with the following composition: 10 %, 20% and 28% test protein, 8% soy oil, 5% water, 5% AIN-mineral, 1% AIN vitamin, 1% cellulose, 35% sucrose and 35% corn starch. The test protein in the six experimental diets were as follow:

1) 10% protein - casein diet (10% control diet)
2) 10% protein-soy protein isolate diet (10% SPI diet)
3) 10% protein - ungerminated soybean diet (10% UGS diet)
4) 10% protein - germinated soybean diet (10% GS diet)
5) 20% protein - germinated soybean diet (20% GS diet)
6) 28% protein - germinated soybean diet (28% GS diet) (Table 1).

Animals: Three-week-old weanling male Sprague -Dawley rats with a mean initial weight of 50-60 g, mean body weight difference within group not more than 10 g and between groups not more than 5 g, were obtained from the National Laboratory Animal Centre, Mahidol University, Thailand. The animals were divided into six groups, one control group and five test groups. All rats were housed in individual stainless steel metabolic cages in an experimentally controlled environment at 20-22°C, 60% relative humidity with a 12-h light - dark cycle. Rats were assigned by selection randomization to the six groups, with ten rats per group. The animals were given free access to diet and water during the 28 - day feeding period. Daily food intake and weekly body weight were recorded. The experimental protocol was developed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

Sample collection: After 28 days, the rats were anaesthetized with zoletil. Blood was drawn into a test tube using cardiac puncture and allowed to clot at room temperature for 30 min. The clotted blood was centrifuged at 3000 rpm for 10 min to obtain serum.

Experimental chemical laboratory: Soybean seeds (cultivar Chiang Mai 60 : CM 60) were obtained from Sukhothai Agricultural Research and Development Center, Department of Agriculture. Raw soybean, ungerminated soybean (soaked), germinated soybean and experimental diets were analyzed for γ-aminobutyric acid (GABA) by using HPLC (high-performance liquid chromatography) (Abe *et al*., 1998). Serum samples were analyzed using enzymatic calorimetric procedures to determine the lipid profile, such as cholesterol (TC) (NCEP, 2001), triglyceride (TG) (Bucalo and David, 1973; Fossali and Prencipe, 1982), low density lipoprotein - cholesterol (LDL-c) (Nuack *et al*., 2002) and high density lipoprotein-cholesterol (HDL-c).

Statistical analysis: Data were statistically analyzed using analysis of variance (ANOVA) and Duncan's new multiple range test. A value of $p<0.05$ was considered significant

RESULTS AND DISCUSSION

Content of GABA: The content of GABA in raw soybean, ungerminated soybean, germinated soybean and in experimental diets are shown in Table 2 and 3. The highest GABA content was obtained in day 2-germinated soybean which was then chosen for preparation of experimental diets in this research.

Guo *et al*. (2011) found that soaking at 30°C for 4 h was the most effective way to obtain soybean seeds with sufficient moisture, satisfactory germination and GABA accumulation. The suitable stress opportunity was dark culture for 2 days with distilled water and then hypoxia stress in aerated culture medium for 2 days in a dark incubator at 30°C. Under these conditions, the maximum GABA content (224 mg per 100 g dry weight) was 3.5 times higher than that in the initial sample with aeration treatment (0 h) and 12.5 times higher than that in the raw material (Guo *et al*.,2011).

It is well established that in different experimental species, soybean protein isolate (SPI) versus casein in the diet decreases serum cholesterol concentration (Carroll, 1982). Table 4 shows that the serum cholesterol level of rats fed with the experimental diet made from SPI (73.10±13.32 mg/dL) was significantly lower ($P < 0.05$) than of those fed with diet made from casein (89.40±10.47 mg/dL). In the rat, the hypocholesterolemic effect of soybean protein has been observed mainly in high-density lipoprotein cholesterol (HDL-c) (Park *et al*., 1987) . The serum HDL-c level of rats fed with the experimental diets made from 10% SPI, 10% UGS, 10% GS, 20% GS and 28% GS were significantly lower ($P < 0.05$) than those from rats fed with the 10% casein control diet (Table 4).

The serum cholesterol , triglyceride, HDL-c and LDL-c levels of rats fed with the experimental diets made from 10% UGS and 10% GS were not significantly different (Table 4). Cholesterol level in rats fed either with 10% UGS or 10% GS (84.20±5.73 and 81.90±8.06 mg/dL,

Table 1: Composition of six experimental diets (10 kg)

	10% Case in diet (g)	10% SPI diet (g)	10% UGS diet (g)	10% GS diet (g)	20% GS diet (g)	28% GS diet (g)
Soy Flour	-	1236.25	2853.88	2716.65	4889.88	6791.5
Casein	1184.27	-	-	-	-	-
Soy oil	798.52	796.44	734.53	732.25	678.05	630.62
Water	488.93	487.46	482.84	484.35	471.83	460.88
Min.	496.03	495.1	485.13	486.96	476.53	467.4
Vit.	100	100	100	100	100	100
Cellulose	99.3	100	83.62	82.67	68.8	56.67
Sugar	3416.48	3392.38	2630	2698.56	1657.46	746.47
Corn starch	3416.47	3392.37	2630	2698.56	1657.45	746.46

Min. = Mineral mixture Vit. = Vitamin mixture
10% Casein = 10% Protein - casein
10% SPI = 10% Protein - soy protein isolate
10% UGS = 10% Protein - ungerminated soybean
10% GS = 10% Protein - germinated soybean
20% GS = 20% Protein - germinated soybean
28% GS = 28% Protein - germinated soybean

Table 2: Content of GABA in raw soybean, ungerminated soybean and 1-5 days germinated soybean

Soybean	GABA (mg 100 g)
Raw	27.79
Ungerminated (soaked)	148.6
Day 1-germinated	183.72
Day 2-germinated	209.84
Day 3-germinated	195.87
Day 4-germinated	178.77
Day 5-germinated	131.3

Table 3: Content of GABA in the six experimental diets

Experimental diet	GABA (mg 100 g)
10% Protein-casein	<0.25
10% Protein-soy protein isolate	<0.25
10% Protein-ungerminated soybean	27.27
10% Protein-germinated soybean	25.07
20% Protein-germinated soybean	56.4
28% Protein-germinated soybean	76.99

Table 4: Means±standard deviation of serum cholesterol, triglyceride, HDL-c and LDL-c concentration of experimental rats fed with the six experimental diets

Experimental diet	Cholesterol	Triglyceride (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
10%Casein	89.40 ± 10.47^{a}	110.40 ± 29.45^{a}	50.70 ± 7.58^{a}	16.60 ± 4.94^{b}
10%SPI	73.10 ± 13.32^{b}	58.20 ± 13.13^{bc}	40.30 ± 5.10^{b}	23.40 ± 5.01^{a}
10% UGS	84.20 ± 5.73^{a}	73.20 ± 13.15^{b}	41.90 ± 4.09^{b}	27.50 ± 4.69^{a}
10% GS	81.90 ± 8.06^{a}	63.20 ± 8.09^{bc}	42.10 ± 7.01^{b}	27.50 ± 5.42^{a}
20% GS	67.50 ± 6.05^{b}	54.60 ± 16.07^{c}	40.60 ± 3.80^{b}	16.00 ± 2.35^{b}
28% GS	57.40 ± 6.25^{c}	30.30 ± 5.25^{d}	39.50 ± 4.83^{b}	11.80 ± 2.69^{c}

Values are Means±SD, N = 10, Values in a column with different superscript are significantly different at p<0.05
10%SPI = 10% Protein-soy protein isolate
10%UGS = 10% Protein-ungerminated soybean
10% GS = 10% Protein-germinated soybean
20% GS = 20% Protein-germinated soybean
28% GS = 28% Protein-germinated soybean

respectively) did not differ from that in rats fed with 10% casein (89.40±10.47 mg/dL) whereas triglyceride (73.20±13.15 and 63.20±8.09 mg/dL, respectively) and HDL-c (41.90±4.09 and 42.10±7.01 mg/dL, respectively) levels were significantly lower than those in rats fed with the control diet (110.40±29.45 and 50.70±7.58mg/dL, respectively). Increasing concentration of germinated soybean protein in the diet to 28% GS, however, resulted in significant decrease of serum cholesterol (57.40±6.25 mg/dL), triglyceride (30.30±5.25 mg/dL) and LDL-c (11.80±2.69 mg/dL) to the levels lower than those in experimental rats fed with 10% casein, 10% SPI, 10% UGS, 10% GS and 20% GS diets.

Conclusions: Soybean protein is one of the vegetable proteins examined most extensively for the hypocholesterolemic effects, and it exerts a cholesterol - lowering effect both in humans and in experimental animals (Carroll and Kurowska, 1995; Huang *et al*., 1993). However the present research showed that at 10% protein, neither ungerminated soybean nor germinated soybean versus casein affected the serum cholesterol level in rats. At 28% protein-germinated soybean, the serum cholesterol, triglyceride and LDL-c levels of rats were significantly lower ($P < 0.05$) than those of rats fed with the diets made from 10% casein, 10%SPI, 10%UGS, 10% GS and 20% GS. The data indicated that the experimental diet with 28% protein germinated soybean could reduce serum cholesterol, triglyceride and LDL-c in the experimental rats.

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