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Effects of Germinated Soybean on Protein Efficiency Ratio in Rats

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Abstract: The objective of this study was to evaluate the effects of germinated soybean on protein efficiency ratio (PER) by feeding male Sprague-Dawley rats with 10% protein in 4 experimental diets containing casein (control), soy protein isolate (SPI), ungerminated soybean (UGS) and germinated soybean (GS). PER was weight gained of rats consumed experimental diet divided by protein intake. The diets were prepared from 4 formulas by AOAC, with the following composition: 10% test protein, 8% oil, 5% water, 5% AIN-mineral, 1% AIN-vitamin, 1% cellulose, 35% sucrose and 35% corn starch. The results showed that PER were not significantly different among the rats fed with UGS (2.18±0.22) and GS (2.18±0.16) diets. The findings indicated that 2 days germinated soybean produced the same effect as ungerminated soybean on PER and growth rate in rats.

Key words: Germinated soybean, ungerminated soybean, protein efficiency ratio (PER), growth rate, experimental rats

INTRODUCTION

Soybean is a very good source of plant protein (Brunsgaard *et al*., 1994). In China the word for soybean is ta-tou, which means “greater bean” (Simoons, 1991). Soybeans have played an integral part in Asian culture, both as food and as medicine, for many centuries. In the West, soybeans are best known for their protein content but increasingly, soyfoods are being recognized as having potential roles in the prevention and treatment of chronic diseases, most notably cancer and heart disease. There are also potential roles for soyfoods with respect to osteoporosis and kidney disease (Messina, 1995).

Germinated beans can be used to replace, in part, wheat flour without affecting baking properties (Hsu *et al*., 1973). Germination processes have been developed in some countries to overcome some of the disadvantages associated with ungerminated soybeans, such as undesirable flavor and odor and the presence of trypsin inhibitors (McKinney *et al*., 1958; Suberbie *et al*., 1981; Vanderstoep, 1981). Germination may also result in an increase in nutritive value relative to ungerminated seeds (Fordham *et al*., 1975). Several studies confirmed the benefits of soybean germination (Fordham *et al*., 1975; Kylen and McCready, 1975). However, limited data have been published relating to germinated soybean and their PER and growth rate in experimental rats. Therefore, this study was undertaken to determine the effects of germinated soybean on PER and growth rate compared with ungerminated soybean in rats.

MATERIALS AND METHODS

Preparation of germinated and ungerminated soybean flour: Soybean samples (2 x 5 kg) were washed and soaked in water (7.500 mL) for 10-12 h. The soybeans were then 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 soybeans 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 4 experimental diets by the AOAC method (Horwitz and Latimer, 2006) with the following composition: 10% protein, 8% soy oil, 5% water, 5% AIN-mineral, 1% AIN-vitamin, 1% cellulose, 35% sucrose and 35% corn starch (Table 1) (Mesomya *et al*., 2013).

Animals: Three-week-old weanling male Sprague-Dawley rats were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The rats with a mean initial weight of 50-60 g were used. They were randomly divided into four groups of 10 rats (one control group and three test groups). All rats were housed in individual stainless steel metabolic cages in

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an experimental controlled environment at 20-22°C, 60% relative humidity and 12 h light-dark cycle and were given free access to the diet and water for a 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.

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) (Table 2 and 3).
Casein, soy protein isolate, ungerminated soybean and germinated soybean were analyzed for proximate analysis by the AOAC method (Horwitz and Latimer, 2006) (Table 4).

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

Corrected PER of the four experimental diets containing 10% casein (control), soy protein isolate (SPI), ungerminated soybean (UGS) and germinated soybean (GS) are shown in Table 5.
Protein efficiency ratio (PER) of germinated soybean (GS) diet (2.18±0.16) was not significantly different from that of ungerminated soybean (UGS) diet (2.18±0.22) and both were significantly higher than that of soy protein isolate (SPI) diet (1.84±0.11). However, PER of GS diet, UGS diet and SPI diet were significantly lower than that of casein diet (2.50±0.16). GS, UGS and SPI diets contain protein from soybean which is plant protein but casein diet consists of animal protein from cow's milk and it was already well known that animal protein such as egg or milk are a good quality source of nitrogen (Pellet and Young, 1980). Soybean is also a very good source of plant protein (Brunsgaard *et al*., 1994) but the value of soy protein has been undervalued due to the limiting amino acid methionine (Messina, 1995). Furthermore, the high crude fiber and fat content of UGS and GS flour could impede digestion and absorption of feed intake resulting in reduced bioavailability of the diets. Protein quality is a measure of bioavailability and its evaluation is a means of determining the capacity of food protein and diets to satisfy metabolic demands for amino acids and nitrogen (Gilani, 2012).

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

	Casein diet (g)	SPI diet (g)	UGS diet (g)	GS diet (g)
Soy flour	-	1236.25	2853.88	2716.65
Casein	1184.27	-	-	-
Soy oil	798.52	796.44	734.53	732.25
Water	488.93	487.46	482.84	484.35
AIN-min	496.03	495.10	485.13	486.96
AIN-vit	100.00	100.00	100.00	100.00
Cellulose	99.30	100.00	83.62	82.67
Sugar	3416.48	3392.38	2630.00	2698.56
Corn starch	3416.47	3392.37	2630.00	2698.56

min : Mineral mixture
vit : Vitamin mixture
SPI : Soy protein isolate
UGS : Ungerminated soybean
GS : 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)	148.60
Day 1-germinated	183.72
Day 2-germinated	209.84
Day 3-germinated	195.87
Day 4-germinated	178.77
Day 5-germinated	131.30

Table 3: Content of GABA in the four experimental diets

Experimental diet	GABA (mg/100 g)
Casein	<0.25
Soy protein isolate	<0.25
Ungerminated soybean	27.27
Germinated soybean	25.07

Table 1-3, From Mesomya *et al*. (2013)

Table 4: Proximate analysis of casein and soy

	Protein	Moisture	Fat	Ash	Crude fiber
Sample	g/100 g wet weight				
Casein	84.44	9.35	1.25	3.35	0.59
SPI	80.89	10.14	2.88	3.96	0.00
UGS flour	35.04	6.01	22.94	5.21	5.74
GS flour	36.81	5.76	24.94	4.80	6.38

SPI : Soy protein isolate
UGS : Ungerminated soybean
GS : Germinated soybean

Table 5: Means±standard deviation (SD) of corrected PER of casein diets and three soybean experimental diets

Experimental diets	Corrected PER
Casein	2.50±0.16[a]
SPI	1.84±0.11[c]
UGS	2.18±0.22[b]
GS	2.18±0.16[b]

Values are means±SD, N = 10. Values in a column with different superscripts are significantly different at $p<0.05$.
SPI : Soy protein isolate
UGS : Ungerminated soybean
GS : Germinated soybean

Both germination time and temperature had influences on the composition and concentration of bioactive compounds in germinated soybean flour (Paucar-Menacho *et al*., 2010). However, PER of soybean seeds with 0, 1 and 3 germination days presented no significant differences among them (Jimenez *et al*.,

Table 6: Means±standard deviation (SD) of body weight, protein intake and PER in rats

Experimental diets	Initial body weight (g)	Final body weight (g)	Protein intake (g)	PER
Casein	64.33±2.79[a]	194.67±17.40[a]	37.16±2.88[a]	3.50±0.22[a]
SPI	64.60±3.73[a]	147.15±9.31[c]	32.02±1.65[c]	2.57±0.16[c]
UGS	64.35±3.27[a]	168.09±21.63[b]	33.58±3.71[b]	3.06±0.31[b]
GS	64.28±3.41[a]	170.85±21.80[b]	34.74±4.27[b]	3.05±0.23[b]

Values are means±SD, N = 10. Values in a column with different superscript are significantly different at $p<0.05$.
SPI: Soy protein isolate UGS: Ungerminated soybean GS: Germinated soybean

1985). PER of UGS and GS diets were significantly higher than that of SPI diet because UGS and GS were both soybean meal but SPI was the processed product of soybean. Reviews by Rakosky (1970) and Bressani (1975) suggested that the nutritional quality of soy protein may decrease during processing to soy protein isolate. Supplementation with methionine leads to an improve nutritional quality of soy products for both humans and rats (Rakosky, 1970; Bressani, 1975; Torun *et al*., 1981) but it is possible that processing of soy protein results in a loss of sulfur amino acids and threonine, thus causing a more severe deficiency of sulfur amino acids and threonine in the processed products relative to soybean meal. PER of GS diet was not significantly different from that of UGS diet indicating that germination of soybean did not produce significant effects on PER and growth rate in rats in this case.

Weight gain, protein intake and PER were significantly higher in rats fed casein diet than in those fed the other soy proteins (Table 6). Protein quality of GS diet was similar to that of UGS diet, but was significantly higher than that of SPI diet. Final weight and PER in rats fed GS diet was not significantly different from that fed UGS diet indicating that GS and UGS did not produce different effects on PER and growth rate in rats. Although variations in GABA content of soybean were seen among ungerminated and at different germination times (Table 2), the content of GABA in both UGS and GS diets were similar (25.07, 27.27 mg/100 g) and were much higher than those in the SPI and the control casein diets (<0.25 mg/100 g) (Table 3). Therefore it could not be concluded whether GABA in GS and UGS diets produced any different effect on PER and growth rate in rats.

Conclusion: This research showed that neither 2-days germinated soybean nor ungerminated soybean versus casein affected the PER and growth rate in rats. However PER of GS, UGS and SPI diet were significantly lower than that of casein diet.

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