

Effect of germinated and heated soybean meals on plasma cholesterol and triglycerides in rats*

V Chandrasiri, HM Bau **, C Villaume, F Giannangeli, L Méjean

INSERM U 308, 38 rue Lionnois, F-54000 Nancy, France

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Summary — Soybean may be useful in diets for the prevention of cardiovascular disease and the treatment of type II hyperlipoproteinemia as it lowers blood cholesterol levels. However, unpleasant organoleptic qualities and the presence of antinutritional substances hinder its use. Some of these problems may be partially solved by germinating the seeds or heating the meals. The effects of the duration of soybean germination and of heating the meal were studied in Wistar rats. Dietary meal composition, plasma cholesterol and triglyceride levels were evaluated after feeding rats with various soybean meal or casein diets containing 10% protein for 6 weeks. Plasma cholesterol and triglyceride levels were 0.81 ± 0.11 and 0.82 ± 0.23 g/l respectively after the casein diet and 0.90 ± 0.10 and 0.51 ± 0.17 g/l after the raw soybean diet. Soybean germination had a hypercholesterolemic effect (1.05 ± 0.11 g/l after 5 d). Heating the raw meal or germinated soybean meal did not affect cholesterol levels, though it suppressed the hypotriglyceridemic effect. The triglyceride-lowering effect of soybean was probably caused by the presence of thermolabile substances or by the quantity of food ingested. The unexpected increase in blood cholesterol levels may have been due to the effect of the low dietary protein levels.

soybean / casein / cholesterol / triglycerides / germination / heating

Résumé — **Effect of farines de soja germé et chauffé sur le cholestérol et les triglycérides plasmatiques chez le rat.** *Le soja est un aliment qui peut s'avérer intéressant dans la prévention des maladies cardiovasculaires et pendant le traitement des hypertipoprotéïnémies de type II, puisqu'un effet hypocholestérolémiant lui est habituellement attribué. Cependant, son utilisation se heurte à des difficultés dues à sa saveur et à l'existence de substances antinutritionnelles. La germination des graines ou le chauffage des farines élimine partiellement ces problèmes. Les influences de la durée de germination des graines de soja et du chauffage des farines obtenues ont été étudiées sur le rat Wistar. La composition des farines, la cholestérolémie et la triglycémie ont été évaluées après 6 semaines de consommation de diverses farines de soja ou de caséine incluses dans des régimes comportant 10% de protéines. La cholestérolémie et la triglycémie après ingestion du régime à base de caséine étaient respectivement de $0,81 \pm 0,11$ et $0,82 \pm 0,23$ g/l. Après remplacement de la caséine par de la farine de soja cru, elles devenaient $0,90 \pm 0,10$ et $0,51 \pm 0,17$ g/l. La germination du soja a induit un effet hypercholestérolémiant ($1,05 \pm 0,11$ g/l après 5 jours). Le chauffage des farines de soja cru ou germé a été sans effet sur la cholestérolémie, alors que l'effet hypotriglycémiant était supprimé. Il semblerait que l'effet hypotriglycémiant obtenu avec le soja soit dû à des substances thermolabiles, ou à la quantité d'aliment ingérée. L'effet hypercholestérolémiant, plus inattendu, pourrait être en liaison avec le faible taux de protéines de l'alimentation.*

soja / caséine / cholestérol / triglycérides / germination / chauffage

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** Correspondence and reprints.

INTRODUCTION

Because of its composition, soybean may play a useful role in diets proposed for the prevention or treatment of cardiovascular diseases. Several studies have evaluated the effect of soybean protein on blood cholesterol levels in man and in animals (Verrillo *et al*, 1985; Forsythe *et al*, 1986; Mercer *et al*, 1987). The experimental conditions, however, were variable and no generally accepted explanation has yet been given for this effect. Certain authors have suggested that it was due to the lysine/arginine ratio (Kritchevsky *et al*, 1982), or the presence of other amino acids (Katan *et al*, 1982), while others have proposed that the fiber (Sharma, 1987) or saponin (Oakenfull *et al*, 1984) contents, the presence of lecithin (Jenkins *et al*, 1983), the polyunsaturated/mono-unsaturated fatty acid ratio (Dunn and Liebman, 1986), or the level of phytosterols (Pollak and Kritchevsky, 1981) or trace elements (Samman and Roberts, 1984) were involved.

Increasing the dietary intake of soybean gives rise to certain problems due to its unpleasant organoleptic qualities and the presence of antinutritional substances (Bau, 1987).

Some of these problems may be solved by germination or heating processes, which improve the taste, odor and nutritional qualities of seeds (Chen *et al*, 1975; Bau and Debry, 1979; Hofsten, 1979).

We therefore studied the effects of the duration of germination and heating on soybean protein, lipid, trace element, dietary fiber and phytosterol composition, and on the plasma triglycerides and cholesterol levels of rats fed for 6 weeks with diets differing only by the nature of the protein source.

MATERIALS AND METHODS

Protocol

Twenty-one-day-old male Wistar rats weighing 50–55 g at the start of the study were placed in individual metabolism cages and fed the diets described below *ad libitum* for 42 d. The diets were identical in composition, except for the nature of the protein source (Osborne *et al*, 1919). Each diet was tested on a group of 10 rats and was composed of raw whole soybean (Kingsoy, ONIDOL, Toulouse, France) meal (WM), heated soybean meal (HWM), meal prepared with 1, 3 or 5-day germinated seeds (GM1, GM3, GM5) or heated GM5 meal (HGM5). Each diet had a total protein content of 10% as this study formed part of a larger investigation on the protein efficiency ratio. For each diet, the quantity of meal necessary to supply 10% of proteins was weighed. The lipid content of each diet was adjusted to 9% with pure commercial soybean oil (Lesieur) taking into account the percentage of lipids in the meals. Five percent cellulose, 5% salt mixture and 2% vitamins (UAR, Villemoisson sur Orge) were then added, and the diets were made up to 100% with corn starch (UAR, Villemoisson sur Orge). Body weight gain, detailed meal composition and the seed germination procedure have been described elsewhere (Chandrasiri *et al*, 1987). The effect of soybean protein was compared with that of casein.

At the end of the study, fasting rats were anesthetized with ether under a glass bell and decapitated. Blood was collected on a mixture of EDTA and INIPROL, a protease inhibitor (Choay Laboratories, Paris). It was then centrifuged at 4 °C and the plasma stored in aliquot fractions at –20 °C until assayed.

Assays

In the diets

Phytosterols were extracted from sample of the soybean meals used to prepared the test diets and assayed according to the method described by Smith *et al* (1987). Trace elements were analyzed using the method of Clegg *et al* (1981)

with slight modifications: ashing was performed with a nitric-perchloric acid (4/1) mixture instead of nitric acid alone, and an atomic absorption spectrophotometer (Perkin-Elmer, model 400) was used. The nitrogen and lipid contents were determined according to the AOAC (1980); ethanol-soluble carbohydrates were extracted with 80% alcohol and assayed using the anthrone method (Roe, 1955). Fibers were measured according to Southgate (1969).

In plasma

Plasma cholesterol and triglyceride levels were determined enzymatically using commercial kits (Boehringer Mannheim).

Data analysis

The meal composition data (tables I, II and III) were means of triplicates with coefficients of variation of less than 5%. Results were compared by a one-way ANOVA test and Fisher's test using a MacIntosh computer and the Statview program. Values of $P < 0.05$ were considered to be significant.

RESULTS

Body weight gain and the proximate compositions of the meals used to prepare the

Table I. (a) Dietary meal composition^a (in %).

Sample	Proteins	Lipids	Ethanol soluble carbohydrates	Hemicellulose	Cellulose	Lignin
WM	41.6	20	14.1	15.8	3.6	1.3
HWM	—	—	—	—	—	—
GM1	42.0	20.2	11.1	15.4	3.4	0.6
GM3	44.2	17.2	8.2	14.5	2.4	traces
GM5	46.6	16.2	2.8	13.3	0.4	traces
HGM5	—	—	—	—	—	—
Casein	83.1	—	—	—	—	—

(b) Body weight gain and protein intake (in g).

Sample	Daily body weight gain	Daily protein intake
WM	1.4 ± 0.3	1.1 ± 0.1
HWM	3.7 ± 0.3	1.6 ± 0.1
GM1	2.1 ± 0.4	1.3 ± 0.2
GM3	1.3 ± 0.4	1.0 ± 0.1
GM5	1.1 ± 0.4	0.9 ± 0.1
HGM5	2.7 ± 0.3	1.3 ± 0.1
Casein	4.1 ± 0.4	1.5 ± 0.1

^a Dry weight basis; WM: raw whole soybean meal; HWM: heated soybean meal; GM1, GM3, GM5: 1, 3 or 5-day germinated soybean meal; HGM5: heated GM5.

Table II. Trace element contents^a.

Sample	Zinc	Copper	Zn/Cu
WM	47.7	13.66	3.49
GM1	54.45	11.74	4.64
GM3	56.7	12.82	4.42
GM5	54.9	12.58	4.36

^a Dry weight basis ($\mu\text{g/g}$); WM: raw whole soybean meal; GM1, GM3, GM5: 1, 3 or 5-day germinated soybean meal.

diets are given in table I. Increasing the duration of germination led to an increase in the protein content, a decrease in the lipid, hemicellulose, cellulose and lignin contents and a large fall in the ethanol-soluble carbohydrate content of the meal.

The trace element content of the meals is shown in table II. There was an irregular change in Cu and Zn with the increasing duration of germination. The Zn/Cu ratio increased with germination time.

Phytosterol contents are shown in table III. The phytosterol contents of sunflower seeds and rapeseed oils are also given for comparison. Germination of soybean seeds led to an increase in almost all phytosterols, and especially in β -sitosterol.

The effects of the different diets on blood cholesterol and triglyceride levels as a function of germination time and heat treatment are shown in table IV. The statistical significance of differences between pairs of diets is also given.

Variance analysis showed that the diet had a global effect on cholesterol ($F_{6,63} = 12.5$) and triglyceride levels ($F_{6,63} = 4.37$).

Analysis of variance attributable to germination for diets WM, GM1, GM3 and GM5 showed that germination time affected both cholesterol ($F_{3,36} = 9.83$) and triglyceride levels ($F_{3,36} = 3.47$). Compared with the casein diet, the crude soybean meal diet led to a non-significant increase in cholesterol and a significant decrease in triglycerides. The heated meal produced a very large increase in triglycerides, while cholesterol was unchanged. Similarly, the

Table III. Phytosterol content^a of soybean oils prepared after different germination durations.

Phytosterol	WM	GM1	GM2	GM3	GM5	Sunflower oil	Rapeseed oil
γ -tocopherol	9.3	12.4	12.3	10.5	12.3		
α -tocopherol	10.1	6.9	7.2	6.3	16.1		
Δ -7avenasterol	14.1	9.9	17.1	11.2	18.5		
Campesterol	82.8	71.8	67.0	84.7	94.9	11.2	40.0
β -sitosterol	147.1	174.8	150.3	180.5	222.7	69.5	56.1
Δ -7 stigmasterol	14.9	15.2	16.6	17.3	18.6	10.3	1.6
Δ -5 avenasterol	15.1	12.4	14.8	16.4	21.6	4.6	4.1
Stigmasterol	81.4	67.8	99.6	100.5	99.9	8.2	0.6
Brassicasterol							10.8

^a mg/100 g (dry weight basis); WM: raw whole soybean meal; GM1, GM2, GM3, GM5: 1, 2, 3, 5-day germinated soybean meals.

Table IV. Effect of diet on plasma cholesterol and triglyceride levels (mean \pm SD).

Diet	Cholesterol (g/l)	Triglycerides (g/l)
WM	0.90 \pm 0.10 ^{a,e}	0.51 \pm 0.17 ^a
HWM	0.83 \pm 0.14 ^a	0.85 \pm 0.26 ^{b,e}
GM1	0.98 \pm 0.19 ^{b,e}	0.83 \pm 0.40 ^{b,f}
GM3	1.26 \pm 0.19 ^c	0.64 \pm 0.41 ^{a,b,c}
GM5	1.05 \pm 0.11 ^{b,d}	0.42 \pm 0.07 ^{a,c}
HGM5	1.14 \pm 0.17 ^{c,d}	0.96 \pm 0.39 ^{d,e,f}
Casein	0.81 \pm 0.11 ^a	0.82 \pm 0.23 ^{b,d}

Means not sharing the same superscript letter in the same column are significantly different at $P < 0.05$. WM: raw whole soybean meal; HWM: heated soybean meal; GM1, GM3, GM5: 1, 3 or 5-day germinated soybean meals; HGM5: heated GM5.

heated germinated seed meal (HGM5) induced a large increase in triglyceride levels, whereas it did not affect cholesterol.

Seed germination and duration of germination induced complex variations in triglyceride and cholesterol levels as compared to raw soybean meal or casein.

Germinated soybean seeds induced higher cholesterol levels than casein or raw meal. One-day germinated seeds induced a significant increase in triglycerides. When the germination time was extended, the triglyceride levels were significantly lower than those observed with the casein diet.

DISCUSSION

The hypocholesterolemic effect of dietary soybean protein in comparison with casein has been attributed to phytosterols, lecithin, the Lys/Arg ratio, dietary fibers, the poly/mono unsaturated fatty acid ratio or

saponins (Pollak and Kritchevsky, 1981; Katan *et al*, 1982; Kritchevsky *et al*, 1982; Jenkins *et al*, 1983; Oakenfull *et al*, 1984; Samman and Roberts, 1984; Dunn and Liebman, 1986; Sharma, 1987).

Our results showed that dietary intake of germinated soybean protein led to a significant increase in plasma cholesterol, whereas no decrease was observed with raw soybean meal.

In rats, the hypocholesterolemic effect generally described with soybean diets was usually obtained with diets containing 20% protein. These results cannot therefore be compared to our results obtained with 10% protein diets as variations in the dietary protein content may modify the nature of the dietary effect on plasma cholesterol. Leszczynski *et al* (1987) has shown that plasma cholesterol and phospholipids fell in female chicks fed a 24.8% protein diet in comparison with a 12.8% protein diet. Sugiyama *et al* (1986) also reported that the effect on plasma cholesterol of the same methionine supplement was reversed when the dietary protein content was increased from 10 to 25%.

Modifications in the micronutrient composition of the soybean meals tested may explain the metabolic effect observed after feeding with meals obtained from germinated soybean seeds.

During germination, there was an increase in the zinc levels with little change in copper so that the Zn/Cu ratio in the diets increased with germination. As dietary mineral levels are good indicators of plasma mineral levels which influence plasma cholesterol levels (Katya-Katya *et al*, 1984; Klevay, 1975), such an increase in the Zn/Cu ratio may have been responsible for the concomitant increase in plasma cholesterol.

Phytosterols have a hypocholesterolemic effect through their action on intestinal cholesterol absorption (Pollak and Kritch-

evsky, 1981) and/or synthesis. Soybean oil is naturally rich in campesterol, β -sitosterol and stigmasterol compared to rapeseed and sunflower seed oils. Prolonged germination leads to an increase in phytosterols, and especially β -sitosterol. This increase may promote a decrease in plasma cholesterol.

Compared with a casein diet, a soybean diet may be hypocholesterolemic due to its dietary fiber content (Sharma, 1987) so that the decrease in fiber content during germination has a hypercholesterolemic effect.

In brief, prolonged germination modifies the levels of some of the parameters affecting plasma cholesterol levels. There was an increase in the Zn/Cu ratio (hypercholesterolemic effect), and phytosterol content (hypocholesterolemic effect) and a decrease in the fiber content (hypercholesterolemic effect).

However, as the plasma cholesterol level increased in our study using 10% protein diets, the influence of the Zn/Cu ratio and the decreased dietary fiber content were not overcome by the increased phytosterol content. This is not really unexpected since phytosterols have no hypocholesterolemic effect in rats on a cholesterol-free diet (Sugano *et al*, 1977).

Our results demonstrate that dietary soybean meals have a global effect on plasma triglyceride levels. Dietary intake of a raw soybean meal led to a significant decrease in the triglyceride levels in comparison with the casein diet. Similar results have been reported (Jenkins *et al*, 1983) with a soybean protein concentrate in 10% protein diets in rats, and with a soybean protein isolate in 20% protein diets in rats (Sugano *et al*, 1982). Tsai *et al* (1987) also obtained such an effect with soybean fiber. Our results using germinated soybean meal cannot be satisfactorily explained by the fiber content, as the hypotriglyceridemic

effect was suppressed after one day of germination when there was no change in fiber content. When germination was prolonged, the hypotriglyceridemic effect occurred despite the decrease in fiber content.

Different authors have demonstrated the presence of lipase inhibitors in various germs and seeds (Satouchi and Matsushita, 1976; Gargouri *et al*, 1984; Wang and Huang, 1984; Lairon *et al*, 1985; Glenn and Chapman, 1987). These authors suggested that certain proteins reacting with lipids may affect intestinal lipolysis and thus influence lipid absorption and metabolism. Such a mechanism may partially explain the results obtained here for the difference between casein and raw soybean meal as well as the effect of germination (increase in lipase inhibitor with germ development).

Heat treatment of both raw soybean meal (WM) and germinated soybean meal (GM5) led to a decrease in the hypotriglyceridemic effect with no effect on cholesterolemia. These results suggest that the factor involved in the hypotriglyceridemic effect is thermolabile.

On the other hand, hepatic secretion of triglycerides incorporated in hepatic lipoproteins reflects the dietary triglyceride intake, and rats fed with raw or germinated soybean had a lower daily food intake than rats fed with heated soybean or casein (table I). Studies with pair-fed rats will be performed to estimate the effect of food intake on blood lipids.

CONCLUSION

Lower triglyceride levels were obtained in rats fed 10% soybean protein diets than in those fed casein. Prolonging seed germination produced variations in the main factors considered to be responsible for the

hypocholesterolemic effect such as the Zn/Cu ratio and fiber content. Heated flours still retained their hypercholesterolemic effect, whereas the hypotriglyceridemic effect was suppressed suggesting that the hypotriglyceridemic effect obtained with soybean was due to thermolabile factors. However, it may also be related to the food intake. The hypercholesterolemic effect was apparently produced by mechanisms associated with the dietary protein content, experimental conditions (species and age of animals, duration of experiments), and composition of the meals.

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REFERENCES

- AOAC (1980) *Official Methods of Analysis*. Assoc Official Analytical Chemists, Washington, DC, 13th edn
- Bau HM, Debry G (1979) Germinated soybean protein products chemical and nutritional evaluation. *J Am Oil Chem Soc* 56, 160-162
- Bau HM (1987) Les constituants biochimiques de quelques graines d'oléagineux et de légumineuses. Thèse de Docteur ès Sciences, Université de Nancy I, 169 p
- Chandrasiri V, Bau HM, Villaume C, Giannangeli F, Lorient D, Mejean L (1987) Effet de la germination de la graine de soja sur la composition et la valeur nutritionnelle de sa farine. *Sci Alim* 7 (VIII), 139-150
- Chen LH, Hells CE, Fordham JR (1975) Germinated seeds for human consumption. *J Food Sci* 40, 1290-1294
- Clegg MS, Keen CL, Lonnerdal B, Hurley LS (1981) Influence of ashing techniques of the analysis of trace elements in animal tissue. I. Wet ashing. *Biol Trace Elem* 3, 107-115
- Dunn C, Liebman M (1986) Plasma lipid alterations in vegetarian males resulting from the substitution of tofu for cheese. *Nutr Res* 6, 1343-1352
- Forsythe WA, Green MS, Anderson JJB (1986) Dietary protein effects on cholesterol and lipoprotein concentrations: a review. *J Am Coll Nutr* 5, 533-549
- Gargouri Y, Julien R, Pieron G, Verger R, Sarda L (1984) Studies on the inhibition of pancreatic and microbial lipases by soybean proteins. *J Lipid Res* 25, 1214-1221
- Glenn W, Chapman JR (1987) A proteinaceous competitive inhibitor of lipase isolated from *Helianthus annuus* seeds. *Phytochemis. y* 26, 3127-3131
- Hofsten BV (1979) Legume sprouts as a source of protein and other nutrients. *J Am Oil Chem Soc* 56, 382-388
- Jenkins MY, Mitchell GV, Vanderveen JE, Adkins JS, Edwards CH (1983) Effects of dietary protein and lecithin on plasma and liver lipids and plasma lipoprotein in rats. *Nutr Rep Int* 28, 621-634
- Katan MB, Vrooman L, Hermus RJ (1982) Reduction of casein induced hypercholesterolemia and atherosclerosis in rabbits and rats by dietary glycine, arginine and alanine. *Atherosclerosis* 43, 381-391
- Katya-Katya M, Ensminger A, Méjean L, Debry G (1984) Effect of zinc supplementation on plasma cholesterol levels. *Nutr Res* 4, 633-638
- Klevay LM (1975) Coronary heart disease: the zinc/copper hypothesis. *Am J Clin Nutr* 28, 764-774
- Kritchevsky D, Tepper SA, Czarnecki SK, Klurfeld DM (1982) Atherogenicity of animal and vegetable protein. Influence of the lysine to arginine ratio. *Atherosclerosis* 41, 429-431
- Lairon D, Borel P, Termine E, Grataroli R, Chabert C, Hauton JC (1985) Evidence for a proteic inhibitor of pancreatic lipase in cereals, wheat bran and wheat gum. *Nutr Rep Int* 32, 1107-1113
- Leszczynski DE, Mason WR, Kummerow FA (1987) Dietary fatty acid and protein effects on plasma and liver lipids. *Nutr Res* 7, 413-420

- Mercer NJH, Carroll KK, Giovannetti PM, Steinke FH, Wolfe BM (1987) Effects on human plasma lipids of substituting soybean protein isolate for milk protein in the diet. *Nutr Rep Int* 35, 279-287
- Oakenfull DG, Topping DL, Illman RJ, Fenwick DE (1984) Prevention of dietary hypercholesterolemia in the rat by soya bean and quillaja saponins. *Nutr Rep Int* 29, 1039-1046
- Osborne TB, Mendel LB, Ferry EL (1919) A method of expressing numerically the growth promoting value of protein. *J Biol Chem* 37, 223-229
- Pollak OJ, Kritschewsky D (1981) *Sitosterol*. S Karger, Basel, pp 220
- Roe JH (1955) The determination of sugar in blood and spinal fluid with anthrone reagent. *J Biol Chem* 212, 335-343
- Samman S, Roberts DCK (1984) The phosphorylation state of casein and its hypercholesterolaemic effect: role of divalent cations. *Atherosclerosis* 52, 347-348
- Satouchi K, Matsushita S (1976) Purification and properties of a lipase inhibiting protein from soybean cotyledons. *Agric Biol Chem* 40, 889-897
- Sharma RD (1987) An evaluation of hypercholesterolemic activity of some uncommon legumes. *Nutr Res* 7, 351-363
- Smith RL, Sullivan DM, Richter EF (1987) Determination of phytosterols in butter samples by using capillary column. *J Assoc Off Anal Chem* 70, 912-915
- Southgate DAT (1969) Determination of carbohydrates in food. II. Unavailable carbohydrates. *J Sci Food Agric* 20, 331-335
- Sugano M, Morioka H, Ikeda I (1977) A comparison of hypercholesterolemic activity of β -sitosterol and β -sitostanol in rats. *J Nutr* 107, 2011-2019
- Sugano MS, Ishiwaki N, Nagata Y, Imaizumi K (1982) Effects of arginine and lysine addition to casein and soya bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. *Br J Nutr* 48, 211-221
- Sugiyama K, Akai H, Muramatsu K (1986) Effects of methionine and related compounds on plasma cholesterol level in rats fed a high cholesterol diet. *J Nutr Sci Vitaminol* 32, 537-549
- Tsai AC, Vinik AI, Lasichak A, Lo GS (1987) Effects of soy polysaccharide on postprandial plasma glucose, insulin, glucagon, pancreatic polypeptide, somatostatin, and triglyceride in obese diabetic patients. *Am J Clin Nutr* 45, 596-601
- Verrillo A, De Teresa A, Giarrusso PC, La Rocca S (1985) Soybean protein diets in the management of type II hyperlipoproteinaemia. *Atherosclerosis* 54, 321-331
- Wang S, Huang ACH (1984) Inhibitors lipase activities in soybean and other oil seeds. *Plant Physiol* 76, 929-934