Effects of Dietary Protein and Soybean Saponins on Plasma Cholesterol Concentration in Chicks

Hiroshi UEDA

Faculty of Agriculture, Ehime University
Matsuyama-shi 790

(Received January 29, 1992)

Abstract Effects on plasma cholesterol concentration of protein source, protein content and soybean saponins in the diet were investigated in chicks. Soybean protein isolate fortified with methionine (SPI), or casein with arginine, methionine and glycine (CAA) was used as protein source. Seven-day-old Single Comb White Leghorn male chicks were freely fed the experimental diets for 10 days in all trials. Dietary protein source unaffected the plasma total cholesterol when chicks were fed a 18% protein diet without added cholesterol. Supplementation of 1% cholesterol significantly elevated the plasma total cholesterol in chicks fed the CAA diet, but the replacement of CAA with SPI suppressed that elevation. Soybean saponins at most 1% added to the CAA diet containing 18% protein unaffected the plasma total and high density lipoprotein (HDL)-cholesterol, irrespective of dietary cholesterol addition. Addition of 2% cholesterol markedly increased the plasma total cholesterol in chicks fed the SPI diet containing 10% protein with the decrease of HDL-cholesterol. A similar result was observed in chicks fed the SPI diet containing 18% protein, but the effect of added cholesterol was less significant. Likewise, chicks given the CAA diet plus 1% cholesterol showed a decrease in both total and HDL-cholesterol in plasma with the increase of dietary protein content from 10% to 42%. These results indicated that the hypocholesterolemic effect of SPI was not attributed to saponins or non-protein portions.

Anim. Sci. Technol. (Jpn.) 63 (8) : 793-799, 1992.

Key words : plasma cholesterol, soybean saponins, chick

In the course of studies to evaluate the nutritive value of leaf protein concentrate (LPC), it was observed that alfalfa LPC lowered plasma cholesterol concentration in chicks¹⁹⁻²¹⁾. This effect was partially attributed to saponins, since crude saponins extracted from alfalfa was effective in lowering the elevated plasma cholesterol level by dietary cholesterol loading¹⁸⁾, whereas alfalfa LPC after extracting saponins had little effect²¹⁾.

The hypocholesterolemic effect was observed in many plant proteins and that of soybean protein was well established in many spe-

cies^{1, 3, 5, 10, 23)}. However, mechanisms by which dietary protein may affect cholesterol metabolism have not been elucidated. Based on a difference in the amino acid content between soybean protein and casein, for instance, a high ratio of arginine to lysine or high cystine content relative to methionine in soybean protein^{3, 10)}, more attention has been paid to amino acid composition^{2, 3, 5, 6, 10)}. However, it was also known that feeding a mixture of amino acids equivalent to soybean protein was less effective in lowering plasma or serum cholesterol concentration than that of intact

protein^{2,6,7)}. Furthermore, feeding an amino acid mixture suppressed the increased fecal steroid excretion which was observed in rats by ingestion of intact soybean protein and considered as one of the possible factors causing hypocholesterolemic effect⁶⁾. These results may indicate that the cholesterol-lowering effect of soybean protein was not totally accounted for by the amino acid composition.

There were some reports emphasizing that saponins played an important role in the hypocholesterolemic effect of plant proteins^{7,8,12,13,17,18)}, but origin of saponins used in those experiments was not always soybean saponins. Thus, little information is available on the role of soybean saponins^{8,12)}.

In order to evaluate the role of soybean saponins in hypocholesterolemic effect of soybean protein, effects of dietary protein source, protein content and soybean saponins on plasma cholesterol concentration were studied with chicks.

Materials and Methods

Day-old Single Comb White Leghorm male chicks were maintained on a commercial chick starter ration for 6 days in electrically heated battery brooders. On day 7, the necessary number of chicks was selected from the stock after a 3-hour fasting. They were distributed into experimental groups of the uniform body weight and kept individually in metabolism cages. At least five replicates were allotted to each dietary treatment. They were given the experimental diet and water freely for 10 days.

As protein sources, soybean protein isolate (SPI) added with DL-methionine at the level of 1.5% crude protein and casein-amino acid mixture (CAA; 90.9% milk casein, 5.4% L-arginine HCl, 1.7% DL-methionine and 2.0% glycine) were used. Other dietary ingredients consisted of 4% corn oil, 3% cellulose powder, 20% glucose, 5.63% mineral mixture²²⁾, 0.08% vitamin mixture²²⁾, 0.2% choline chloride and cornstarch to bring up to 100%. Other sup-

plements were added at the expense of cornstarch.

In Experiment 1, the effect of dietary protein source (CAA vs. SPI) and cholesterol addition (0 vs. 1%) on chick performance and plasma total cholesterol concentration was studied using the diets containing 18% crude protein. In Experiment 2, the effect of dietary soybean saponins on performance and plasma total and high density lipoprotein (HDL)-cholesterol levels in chicks fed the CAA diet containing 18% crude protein with or without 1% cholesterol was determined. Experiments 3 and 4 were conducted to examine the effect of protein content on chick performance and plasma total and HDL-cholesterol levels. The 10% and 18% protein diets containing SPI with or without 2% cholesterol were used in Experiment 3. In Experiment 4, the CAA diets containing 10% to 42% protein with 8% increment were used and all diets were supplemented with 1% cholesterol.

After weighing body weight at the end of the experiment, blood was taken by heart puncture from 3-hour fasted chicks using heparinized syringes in all trials. Blood plasma was obtained by centrifugation at 3,000 rpm for 15 minutes. Chemical and statistical analyses were described elsewhere¹⁸⁻²¹⁾.

Results

The result of Experiment 1 is given in Table 1. The dietary protein source and cholesterol addition unaffected chick performance. The cholesterol added in the diet significantly increased plasma total cholesterol in chicks fed the CAA diet but not in chicks fed the SPI diet.

The result of Experiment 2 is shown in Table 2. The dietary addition of cholesterol and soybean saponins did not cause an appreciable effect on chick performance. The 1% cholesterol addition significantly increased total cholesterol level but soybean saponins had no effect. Both variables affected plasma HDL-cholesterol level but consistent tendency

Protein, Saponin and Plasma Cholesterol

Table 1. Performance and plasma total cholesterol levels in chicks fed casein-amino acid mixture (CAA) or soybean protein isolate (SPI) with or without 1% cholesterol¹⁾

Treatment	Body weight gain (g/chick)	Feed intake (g/chick)	Gain/feed (g/100 g)	Plasma total cholesterol (mg/100 ml)
CAA ²⁾	82.4	165.2	49.9	187 ^a
SPI ³⁾	83.7	165.4	50.6	186 ^a
CAA+1% cholesterol	83.8	165.4	50.6	347 ^b
SPI+1% cholesterol	78.7	170.4	46.2	197 ^a
Pooled SE	3.0	4.2	1.3	6

¹⁾ Values are means for 5 chicks each. Means with different superscript letters are significantly different at 5% level.

²⁾ Containing 90.9% milk casein, 5.4% L-arginine HCl, 1.7% DL-methionine and 2.0% glycine.

³⁾ Added with DL-methionine at the level of 1.5% of crude protein.

Table 2. Performance and plasma total and high density lipoprotein(HDL)-cholesterol levels in chicks fed casein-amino acid mixture supplemented with soybean saponins together with or without 1% cholesterol¹⁾

Treatment	Body weight gain (g/chick)	Feed intake (g/chick)	Gain/feed (g/100 g)	Plasma total cholesterol (mg/100 ml)	Plasma HDL-cholesterol (mg/100 ml)
1) None	93.7 ^{ab}	181.2	51.8	209 ^a	145 ^{bc}
2) As 1+0.5% saponins	87.0 ^a	173.7	50.0	201 ^a	154 ^c
3) As 1+1.0% saponins	90.2 ^{ab}	187.0	48.2	185 ^a	134 ^{ab}
4) As 1+1.0% cholesterol	97.5 ^{ab}	179.8	54.2	358 ^b	121 ^a
5) As 4+0.25% saponins	100.2 ^{ab}	188.2	53.6	382 ^b	133 ^{ab}
6) As 4+0.5% saponins	91.3 ^{ab}	173.0	52.8	379 ^b	142 ^{bc}
7) As 4+1.0% saponins	103.8 ^b	185.7	55.8	398 ^b	143 ^{bc}
Pooled SE	3.3	5.5	2.0	21	4

¹⁾ Values are means for 6 chicks each. Means with different superscript letters are significantly different at 5% level.

Table 3. Performance and plasma total and high density lipoprotein(HDL)-cholesterol levels in chicks fed diet soybean protein isolate, providing 10% and 18% protein, supplemented with or without 2% cholesterol (Chol)¹⁾

Protein (%)	Chol (%)	Body weight gain (g/chick)	Feed intake (g/chick)	Gain/feed (g/100 g)	Plasma total cholesterol (mg/100 ml)	Plasma HDL-cholesterol (mg/100 ml)
10	—	56.8 ^a	167.8	33.9 ^a	242 ^a	224 ^c
10	2.0	62.4 ^a	170.2	36.7 ^a	652 ^b	151 ^a
18	—	88.4 ^b	186.4	47.4 ^b	216 ^a	184 ^b
18	2.0	85.6 ^b	171.6	49.9 ^b	294 ^a	139 ^a
Pooled SE		2.5	5.6	0.9	24	7

¹⁾ Values are means for 5 chicks each. Means with different superscript letters are significantly different at 5% level.

Table 4. Performance and plasma total and high density lipoprotein(HDL)-cholesterol levels in chicks fed diet containing graded levels of casein-amino acid mixture supplemented with 1% cholesterol¹⁾

Protein (%)	Body weight gain (g/chick)	Feed intake (g/chick)	Gain/feed (g/100 g)	Plasma total cholesterol (mg/100 ml)	Plasma HDL-cholesterol (mg/100 ml)
10	75.2 ^a	176.2 ^{bc}	42.7 ^a	564 ^d	166 ^d
18	99.7 ^b	180.6 ^c	55.2 ^b	368 ^c	121 ^c
26	104.5 ^b	155.3 ^{abc}	67.2 ^c	267 ^b	99 ^b
34	112.0 ^b	151.3 ^{ab}	74.0 ^d	216 ^{ab}	88 ^{ab}
42	93.7 ^b	136.3 ^a	71.4 ^{cd}	196 ^a	80 ^a
Pooled SE	4.0	6.1	1.4	14	3

¹⁾ Values are means for 6 chicks each. Means with different superscript letters are significantly different at 5% level.

was not obtained.

The result of Experiment 3 is presented in Table 3. Body weight gain and gain/feed ratio were only affected by SPI content. When 2% cholesterol was added to the 10% protein diet, plasma total cholesterol significantly increased. It was also increased in chicks fed the 18% protein diet, but the elevation was slight as compared with the low protein diet and the difference was not significant. HDL-cholesterol level tended to decrease both by increasing protein content and by adding cholesterol, but their interaction was significant as shown in Table 3.

The result of Experiment 4 is given in Table 4. Body weight gain and gain/feed ratio were the lowest in chicks fed the 10% protein diet. Increasing the CAA content increased both parameters and the maximum was obtained by feeding 34% crude protein diet. While, feed intake showed the contrary tendency. Plasma total and HDL-cholesterol levels decreased as protein content increased. This effect was most obvious between chicks fed the 10% to 26% crude protein diet.

Discussion

Neither protein source (Table 1) nor protein content (Table 3) in the diet affected the plasma total cholesterol in chicks fed the cholesterol-free diets. While, the SPI diet

acted as a hypocholesterolemic as compared with the CAA diet when 1% cholesterol was added to the diet (Table 1). Similar results were reported in chick^{4,5)} and rat³⁾ trials, whereas in rabbits a hypocholesterolemic effect of SPI can be easily induced without adding cholesterol to the diet^{2,14)}.

It was reported that 1% soybean saponins prevented the hypercholesterolemia in rats caused by 1% cholesterol addition although it unaffected the plasma cholesterol when they were given a cholesterol-free diet⁸⁾. A similar result was obtained with rats given 1.3% soybean saponins¹²⁾. These results disagreed with the present result as shown in Table 2 where soybean saponins had no effect on plasma cholesterol level even when cholesterol was added in the diet. The saponin content of SPI itself was 0.44%¹²⁾ and that of the SPI diet formulated to provide 18% protein was reported to be 0.08%¹³⁾. Thus, the levels of soybean saponins added to CAA diet, 1% at most, would be enough to evaluate the saponin effect.

The hypocholesterolemic effect of soybean protein was accompanied by the increased fecal steroid excretion^{1,6,10,11)}, but the mode of the increased excretion was reported to be somewhat different between soybean protein and soybean saponins. Soybean saponins increased the fecal excretion of bile acids but did not affect the

fecal excretion of neutral steroids in rats fed the cholesterol-free diet⁸⁾. Similarly, it was reported that the difference was in only fecal bile acids excretion when rats were given cholesterol-enriched soy flour diets differing in saponin content¹⁷⁾. While, the experiments with rats fed the cholesterol-free diet showed that feeding SPI increased the fecal excretion of neutral steroids alone¹¹⁾ or of bile acids (or acidic steroids) as well as neutral steroids^{1,6,10,11)}. These results indicated that soybean saponins did not account entirely for the hypocholesterolemic effect of SPI, although it has been uncertain whether data obtained in rats are case with chicks. There might be species difference as reported by RESHEF *et al.*⁹⁾ who showed that the ingestion of alfalfa saponins increased fecal excretion of cholesterol in mice but not in Japanese quail.

As shown in Table 3, when 2% cholesterol was added to the SPI diet, total plasma cholesterol levels were significantly lower in chicks fed the 18% protein diet than those fed the 10% protein diet. This difference may result from saponins or non-protein portions retained in SPI. However, the decrease of total plasma cholesterol due to high protein feeding was also observed in chicks fed the CAA diet containing no saponins (Table 4). It was reported that the hypocholesterolemic effect of soybean flour containing 50% crude protein was similar to that of SPI containing 77.5% crude protein, irrespective of much difference in non-protein portion²³⁾. These results emphasized again that the hypercholesterolemic effect of soybean protein was due not so much to saponins or other non-protein fractions as to the structure of the protein or other factors, and if any, the effect of saponins was not primary but secondary as indicated previously¹²⁾.

As shown in Tables 2 and 3, the addition of cholesterol increased plasma total cholesterol, but HDL-cholesterol was rather decreased. While, increasing the dietary protein content lowered plasma total cholesterol in cholesterol-

fed chicks that was accompanied by the concomitant decrease of HDL-cholesterol levels. However, the change of HDL-cholesterol was small as compared with that of other lipoprotein fractions expressed as the difference between total and HDL-cholesterol levels. Therefore, low density or very low density lipoprotein cholesterol is probably involved in the change of plasma total cholesterol in chicks as reported with rats^{15,16)} and rabbits¹⁴⁾. It should be noted, however, that results of chicks did not necessarily agree with those of trials with rats^{15,16)} or rabbits^{2,14)} where serum cholesterol levels increased instead of decrease with the increase of dietary casein content.

References

- 1) DE SCHRIJVER, R., Cholesterol metabolism in mature and immature rats fed animal and plant protein. *J. Nutr.*, **120**: 1624-1632. 1990.
- 2) HUFF, M.W., R.M.G. HAMILTON and K.K. CARROLL, Plasma cholesterol levels in rabbits fed low fat, cholesterol-free, semi-purified diets: Effect of dietary proteins, protein hydrolysates and amino acid mixtures. *Atherosclerosis*, **28**: 187-195. 1977.
- 3) JACQUES, H., Y. DESHAIES and L. SAVOIE, Relationship between dietary proteins, their in vitro digestion product, and serum cholesterol in rats. *Atherosclerosis*, **61**: 89-98. 1986.
- 4) JOHNSON, D.J., G.A. LEVEILLE and H. FISHER, Influence of amino acid deficiencies and protein level on the plasma cholesterol of the chicks. *J. Nutr.*, **66**: 367-376. 1958.
- 5) KRITCHEVSKY, D., Vegetable protein and atherosclerosis. *J. Am. Oil Chem. Soc.*, **56**: 135-140. 1979.
- 6) NAGATA, Y., K. TANAKA and M. SUGANO, Further studies on the hypocholesterolaemic effect of soya-bean protein in rats. *Br. J. Nutr.*, **45**: 233-241. 1981.
- 7) NAGATA, Y., K. TANAKA and M. SUGANO, Serum and liver cholesterol levels of rats and mice fed soy-bean protein or casein. *J. Nutr. Sci. Vitaminol.*, **27**: 583-593. 1981.
- 8) OAKENFULL, D.G., D.L. TOPPING, R.J. ILLMAN and D.E. FENWICK, Prevention of dietary hypercholesterolaemia in the rat

- by soya bean and Quillaja saponins. *Nutr. Rep. Int.*, **29**: 1039-1046. 1984.
- 9) RESHEF, G., B. GESTETNER, Y. BIRK and A. BONDI, Effect of alfalfa saponins on the growth and some aspects of lipid metabolism of mice and quails. *J. Sci. Food Agric.*, **27**: 63-72. 1976.
- 10) SAUTIER, C., K. DIENG, C. FLAMENT and C. DOUCET, Effects of whey protein, casein, soya-bean and sunflower proteins on the serum, tissue and faecal steroids in rats. *Br. J. Nutr.*, **49**: 313-319. 1983.
- 11) SAUTIER, C., C. DOUCET, C. FLAMENT and D. LEMONNIER, Effects of soy protein and saponins on serum, tissue and feces steroids in rats. *Atherosclerosis*, **34**: 233-241. 1979.
- 12) SUGANO, M., S. GOTO, Y. YAMADA, Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. *J. Nutr.*, **120**: 977-985. 1990.
- 13) TERAFUNTUWAT, S. and I. TASAKI, Effect of dietary saponin on the performance and plasma cholesterol level of chicks and the alleviation of saponin toxicity by cholesterol supplementation. *Jpn. J. Zootech. Sci.*, **57**: 524-533. 1986.
- 14) TERPSTRA, A.H.M., L. HARKES and F.H. VAN DER VEEN, The effect of different proportions of casein in semipurified diets on the concentration of serum cholesterol and the lipoprotein composition in rabbits. *Lipid*, **16**: 114-119. 1981.
- 15) TERPSTRA, A.H.M., G. VAN TINTELEN and C.E. WEST, The effect of semipurified diets containing different proportions of either casein or soybean protein on the concentration of cholesterol in whole serum, serum lipoproteins and liver in male and female rats. *Atherosclerosis*, **42**: 85-95. 1982.
- 16) TERPSTRA, A.H.M., G. VAN TINTELEN and C.E. WEST, The hypocholesterolemic effect of dietary soy protein in rats. *J. Nutr.*, **112**: 810-817. 1982.
- 17) TOPPING, D.L., R.P. TRIMBLE, R.J. ILLMAN, J.D. POTTER and D.G. OAKENFULL, Prevention of dietary hypercholesterolemia in the rat by soy flour high and low in saponins. *Nutr. Rep. Int.*, **22**: 513-519. 1980.
- 18) UEDA, H. and M. OHSHIMA, Effects of alfalfa saponin on chick performance and plasma cholesterol level. *Jpn. J. Zootech. Sci.*, **58**: 583-590. 1987.
- 19) UEDA, H. and M. OHSHIMA, Nutritive value of alfalfa leaf protein concentrate prepared from low saponin variety in chicks and pigs. *Jpn. J. Zootech. Sci.*, **60**: 561-566. 1989.
- 20) UEDA, H., M. OHSHIMA and I. AKITOMO, Nutritive value and hypocholesterolemic effect of alfalfa leaf protein concentrates prepared from two different varieties in chicks. *Jpn. J. Zootech. Sci.*, **58**: 347-355. 1987.
- 21) UEDA, H., M. OHSHIMA and M. KAMADA, The effects of processing methods and cholesterol addition on the nutritive value of leaf protein concentrates in chicks. *Japan. Poult. Sci.*, **23**: 195-202. 1986.
- 22) UEDA, H., H. YOKOTA and I. TASAKI, Alleviatory effect of glycine on the methionine toxicity in chicks. *Japan. Poult. Sci.*, **16**: 113-120. 1979.
- 23) YADAV, N.R. and I.E. LIENER, Reduction of serum cholesterol in rats fed vegetable protein or an equivalent amino acid mixture. *Nutr. Rep. Int.*, **16**: 385-389. 1977.

ヒナの血漿コレステロール濃度に及ぼす飼料の タンパク質とダイズサポニンの影響

上 田 博 史

愛媛大学農学部, 松山市 790

ダイズタンパク質の血漿コレステロール降下作用の機構を知るために、飼料のタンパク質源、ダイズサポニンおよびタンパク質含量がニワトリヒナの血漿コレステロール濃度に及ぼす影響を検討した。7日齢の単冠白色レグホン雄ヒナを用い、10日間の飼育試験を4回行った。タンパク質源として、粗タンパク質の1.5%のDL-メチオニンを補足した分離ダイズタンパク質とカゼイン-アミノ酸混合物(90.9%カゼイン, 5.4%L-アルギニン塩酸塩, 1.7%DL-メチオニンおよび2.0%グリシン)を用いた。試験1では両タンパク質源から18%タンパク質飼料を調製し、タンパク質源と1%コレステロール添加の影響を検討した。その結果、ヒナの血漿総コレステロールはカゼイン-アミノ酸混合物にコレステロールを添加した場合にのみ有意な増加を示した。この血漿コレステロールの増加は最大1%のダイズサポニンを添加しても抑制されなかった(試験2)。ダイズタンパク質飼料に2%のコレステロールを添加すると、10%タンパク質飼料では18%タンパク質飼料と異なり血漿コレステロールの上昇が認められた。コレステロール無添加飼料ではタンパク質含量の影響はみられなかった(試験3)。タンパク質含量の影響はカゼイン-アミノ酸混合物に1%のコレステロールを添加しても認められ、とくにタンパク質含量を10%から26%に増加させると、血漿中の総コレステロールおよび高密度リポタンパク質コレステロールは1/2に減少した(試験4)。以上の結果はダイズタンパク質給与でみられた血漿コレステロール降下作用にダイズサポニンあるいはタンパク質中に残存する非タンパク態部分が関与していないことを示した。

日畜会報, 63 (8) : 793-799, 1992