Food Sci. Technol. Res., **6** (4), 280–283, 2000

A Resistant Protein, Sericin Improves Atropine-Induced Constipation in Rats

Masahiro SASAKI[1], Hideyuki YAMADA[1] and Norihisa KATO[2]

[1]*Technology Department, Seiren Co., Ltd., 1-10-1, Keya, Fukui 918-8560, Japan*
[2]*Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan*

Received March 9, 2000; Accepted June 29, 2000

In this study we found that dietary supplementation of 4% of a silk protein, sericin, to a 20% casein diet significantly reduced apparent protein digestibility in rats compared to a 24% casein diet, and that the pattern of amino acid composition of feces from the rats fed the sericin-supplemented diet was similar to that of the amino acid composition of sericin; the pattern of amino acid composition of feces from rats fed the control (casein) diet was different from that of the amino acid composition of casein. *In vitro* experiments with pepsin and pancreatin also showed that the digestibility of sericin was markedly lower than that of casein. We further examined the possibility that consumption of sericin suppresses constipation because of its low digestibility along with high water-holding capacity. The results indicated that consumption of the 4% sericin supplemented diet suppressed atropine-induced constipation in rats. These results suggest that a resistant protein, sericin could represent a useful agent for the treatment of constipation.

Keywords: sericin, protease, protein digestibility, resistant protein, constipation

The protein sericin is the main constituent of silk (20–30% of the total cocoon weight), enveloping the fibroin with successive sticky layers (Fournier, 1979). When cocoon is used for silk textiles, most of the sericin is removed from the cocoon and disposed of without any use. Recently, we have found that sericin has antioxidant action and inhibitory action of tyrosinase (Kato *et al*., 1998). Sericin is known to have a skin moisturizing and antiwrinkle effect (Voegeli *et al*., 1993), apparently due to its antioxidant action and high content of serine (30–33%). These characteristic effects of sericin make this protein a valuable natural ingredient for food and cosmetic industry.

In our preliminary experiment, we found that sericin is resistant to several proteases. This was confirmed in this study by the indication of low protein digestibility in rats fed sericin-supplemented diets. It is well known that dietary fiber raises fecal weight and fecal water content associated with an improvement in constipation (Eastwood, 1992). We have found that consumption of a buckwheat protein product improves atropine-induced constipation in rats because of its low digestibility (Kayashita *et al*., 1995). This product has several physiological functions including hypocholesterolemic effect, anti-obese effect, and a preventive effect against mammary carcinogenesis (Kayashita *et al*., 1996; Kayashita *et al*., 1997; Kayashita *et al*., 1999; Kato *et al*., 2000). These functions have been postulated to be at least in part governed by a mechanism involving its lower digestibility (Kayashita *et al*., 1996; Kayashita *et al*., 1997; Kayashita *et al*., 1999; Kato *et al*., 2000). Drawing an analogy to resistant starch, we have speculated that a buckwheat protein product may be beneficial for health as a "resistant protein" (Kayashita *et al*., 1997; Kato *et al*., 2000). In this study, we postulated that sericin consumption suppresses constipation because of its low digestibility and high content of serine which may be associated with its high water-retaining capacity. Thus, our study was conducted to examine this hypothesis by determine whether consumption of sericin suppresses the atropine-induced constipation in rats.

E-mail: m.sasaki@seiren.co.jp

Material and Methods

Materials Casein was purchased from Oriental Yeast Co. (Tokyo). From cocoons of *Bombyx mori*, sericin was prepared by the method described elsewhere (Kato *et al*., 1998). The composition of amino acids of casein and sericin was determined by an amino acid analyzer (LC-10A, Amino Acid Analyzer, Shimadzu, Kyoto)

Animal experiment 1 Male Sprague-Dawley rats weighing 98–105 g (Charles River Japan Inc., Yokohama) were housed individually in metal cages in a room with controlled temperature (23–24°C) and 12-h light (08:00–20:00): dark cycle. The animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University. All rats had free access to a commercial stock diet (MF, Oriental Yeast Co., Tokyo) for 3 days and then to the experimental diets for 2 weeks (9 animals per diet group). The composition of experimental diets is described in Table 1. Feces were collected for the final 3 days of the experimental period. The fecal composites were dried to a constant weight, ground to a fine powder, and their nitrogen content was determined by an elemental analyzer (2400 CHN, Perkin Elmer, Norwalk, CT). The apparent digestibility of dietary protein was calculated as: apparent protein digestibility (%)=[(nitrogen intake−fecal nitrogen)/nitrogen intake]×100. The composition of fecal amino acids was determined by an amino acid analyzer after acid hy-drolysis for 22 h with 6 N HCl. The data were analyzed by Student's *t*-test. Results were considered significant at $p<0.05$.

Animal experiment 2 This experiment was designed according to the protocol established by Kayashita *et al*. (1995). Male Sprague-Dawley rats weighing 90–107 g (Japan Charles River, Yokohama) were given free access to a commercial stock diet (MF, Oriental Yeast Co., Tokyo) from 10:30 to 13:30 every

Table 1. Composition of experimental diets.

Ingredient	Animal experiment 1		Animal experiment 2	
	Control (g/kg)	Sericin (g/kg)	Control (g/kg)	Sericin (g/kg)
Casein[a)]	240	200	240	200
Sericin[b)]	0	40	0	40
L-Cystine	1	1	1	1
Corn oil	100	100	100	100
Cellulose powder	50	50	0	0
Vitamin mixture (AIN-93)[c)]	10	10	10	10
Salt mixture (AIN-93G)[c)]	35	35	35	35
Sucrose	200	200	200	200
Cornstarch	364	364	414	414

[a)] Casein (N×6.25) 870 g/kg
[b)] Sericin (N×6.25) 929 g/kg
[c)] AIN-93 (Reeves *et al*., 1993)

day for 12 days. Thereafter they (22 animals per diet group) were fed an experimental diet (10:30 to 13:30) for 7 days. Composition of the experimental diets is presented in Table 1. Half of the rats were given an intraperitoneal injection of atropine (0.5 mg/kg) at 13:30 on days 7, while the remaining animals were given an injection of saline. Since atropine treatment depresses food intake, atropine was injected at the end of this feeding period (13:30). In our preliminary experiment, the depression in fecal excretion by atropine at this dose of 0.5 mg/kg was observed for 12 h after the injection. Thus, feces were collected for 12 h (13:30–19:30 and 19:30–01:30) after the injection of atropine. Collection was done frequently to prevent the feces drying out and they were immediately weighed. Samples were dried at 60°C for 24 h and again weighed. The data were analyzed by two-way ANOVA and by Duncan's multiple-range test (Duncan, 1957).

In vitro experiment *In vitro* digestion of casein and sericin with pepsin and pancreatin was performed in triplicate according to the method of Iwami *et al*., (1986). Briefly, 500 mg of net protein (casein, sericin) was suspended in 100 ml of distilled water, adjusted to pH 2.0 with dilute HCl, and incubated at 37°C with 5 mg porcine pepsin (Nacalai Tesque, Kyoto). After 24 h of incubation, the peptic digest was adjusted to pH 8.5 with $NaHCO_3$, to which porcine pancreatin (Sigma, St. Louis, MO) was added. The reaction mixture containing 10 mg of peptic digest and 0.2 mg of pancreatin in 9 ml of 0.12 M $NaHCO_3$ was incubated at 37°C for 24 h. At appropriate intervals, samples were taken during digestion with pepsin and with pancreatin and were deproteinized by trichloroacetic acid (TCA). After centrifugation, this acid-soluble fraction was adjusted to pH 8.5 with $NaHCO_3$ and then allowed to react with 2,4,6-trinitrobenzenesulfonic acid at 37°C for 2 h. The process of protein digestion (free amino group) was evaluated by measuring the increase in absorbance at 420 nm.

Results

Animal experiment 1 Final body weight and food intake were unaffected by dietary manipulation. Sericin supplementation caused a slight elevation in fecal dry weight and marked elevation in fecal nitrogen, resulting in lower apparent protein digestibility (Table 2). The data in Fig. 1 shows the similarity of amino acid pattern between sericin and the feces from the sericin-fed animals. However, the amino acid pattern of casein was quite different from that of the feces from the casein-fed animals which had not received sericin (Fig. 1). Actually, the composition of fecal amino acids from the sericin-fed rats was well correlated with that of the amino acids of sericin ingested (r=0.928, p<0.01), while the composition of fecal amino acids from the rats fed control diets was not correlated with that of the amino acids of casein ingested (r=0.393, p>0.05).

Table 2. Effect of dietary sericin on fecal nitrogen and apparent protein digestibility in rats (animal experiment 1).

	Control	Sericin
Final body wt (g)	221±2	223±3
Food intake (g/3days)	61.7±1.1	64.2±1.5
Nitrogen intake (g/3days)	2.07±0.04	2.16±0.05
Fecal dry wt (g/3days)	3.83±0.10	4.33±0.18*
Fecal nitrogen (g/3days)	0.068±0.004	0.200±0.009**
Apparent protein digestibility	96.7±0.2	90.7±0.4**

Mean±SE (n=9). Significantly different from the control group by Student's t-test (*p<0.05, **p<0.01).

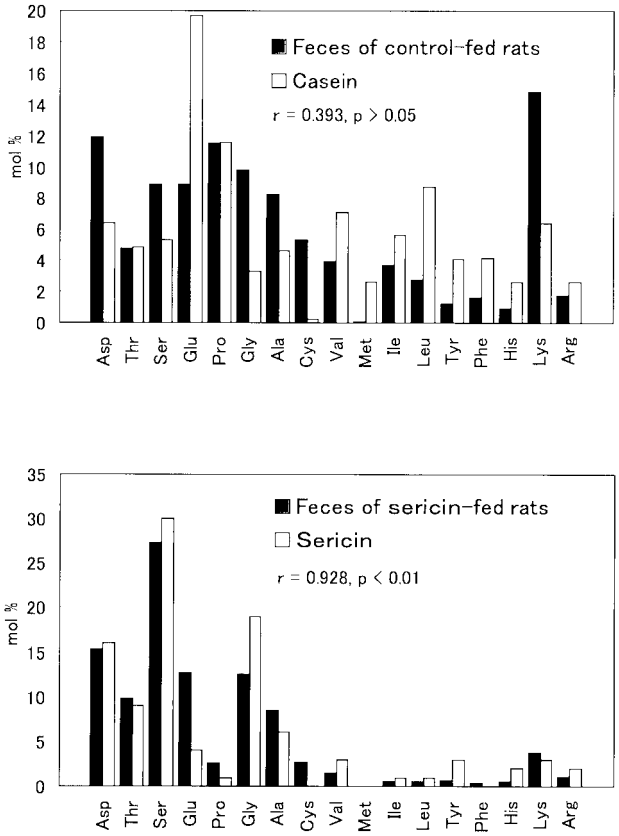

Fig. 1. The amino acid composition of the feces (control group and sericin group) and of ingested proteins (casein and sericin) (animal experiment 1). Individual values represent means of 9 animals. Error bars of the fecal data were too small to show.

Animal experiment 2 There was no difference in the gains in body weight or food intake for 7 days between the control group and the sericin group (data not shown, p>0.05). In the rats receiving saline, fecal wet weight and dry weight was unaffected by dietary treatment (Table 3); in those which had not received sericin, atropine injection caused a marked depression in the fecal wet and dry weights for 6 h and in the dry weight for 12 h (p<0.05). Atropine injection had no influence on fecal wet or dry weight in sericin fed rats (p>0.05). The fecal water content was

Table 3. Effect of atropine on the fecal weight in rats fed experimental diets (animal experiment 2).

	Saline		Atropine		Two-way ANOVA		
	Control	Sericin	Control	Sericin	Protein	Atropine	Interaction
Fecal wet wt (g)							
0–6 h	0.25 ± 0.05^{a}	0.25 ± 0.03^{a}	0.12 ± 0.03^{b}	0.27 ± 0.03^{a}	$p<0.05$	n.s.	$p<0.05$
0–12 h	0.42 ± 0.05^{ab}	0.46 ± 0.05^{a}	0.28 ± 0.04^{b}	0.48 ± 0.05^{a}	$p<0.05$	n.s.	n.s.
Fecal dry wt (g)							
0–6 h	0.17 ± 0.03^{a}	0.14 ± 0.01^{a}	0.07 ± 0.02^{b}	0.17 ± 0.02^{a}	n.s.	n.s.	$p<0.01$
0–12 h	0.27 ± 0.03^{a}	0.26 ± 0.02^{a}	0.18 ± 0.03^{b}	0.29 ± 0.03^{a}	n.s.	n.s.	$p<0.05$
Fecal water content (%)							
0–6 h	31.7 ± 3.0^{b}	38.9 ± 3.4^{ab}	32.9 ± 1.6^{ab}	39.6 ± 1.5^{a}	$p<0.05$	n.s.	n.s.
0–12 h	33.5 ± 2.2^{b}	40.0 ± 1.5^{a}	35.4 ± 1.5^{ab}	40.4 ± 1.5^{a}	$p<0.05$	n.s.	n.s.

Mean±SE ($n=11$). n.s.: not significant. Within a row, values followed by different letters are significantly different by Duncan's multiple-range test ($p<0.05$).

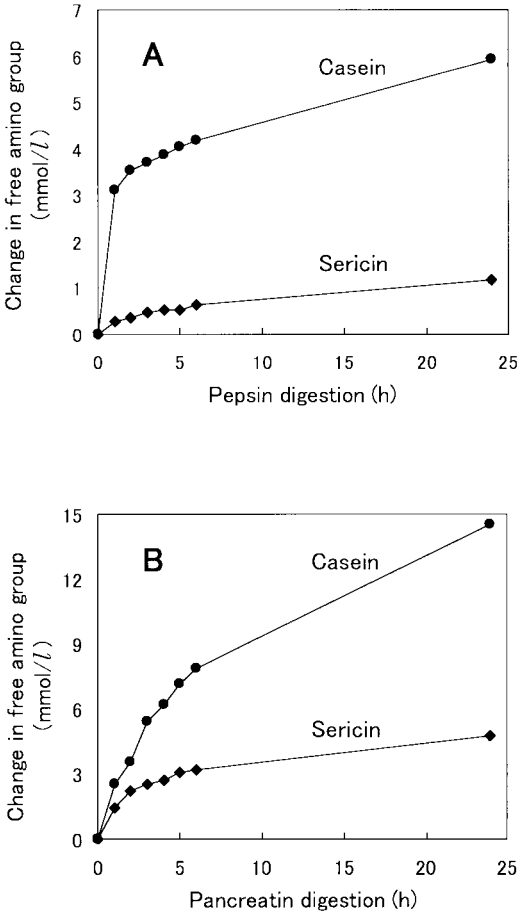

Fig. 2. Courses of pepsin digestion (A) and pancreatin digestion (B) of casein and sericin (*in vitro* experiment). Individual values represent means of assays performed in triplicate. Error bars of the data were too small to show. Analysis by *t*-test revealed significant difference at all time points between casein and sericin ($p<0.01$).

higher in the sericin group than in the control group regardless of atropine injection (ANOVA analysis, $p<0.05$).

In vitro experiment The *in vitro* digestibility of casein and sericin with pepsin and pancreatin was assessed by the change in free amino group in the incubation mixture. The production of the free amino group from sericin was lower than that of casein (Fig. 2), indicating a lower digestibility of sericin understood. Addition of 0.05% sericin to the incubation mixture containing casein caused no suppression in the concentration of the free amino group (data not shown), suggesting that sericin does not inhibit the activity of the proteases at the concentration of 0.05%.

Discussion

This study demonstrated that sericin was resistant to pepsin and pancreatin, and the apparent protein digestion in rats fed a sericin-supplemented diet was also lower than that in rats fed a casein diet without supplementation of sericin. Since we have failed to find that sericin inhibits the activity of the proteases, its low digestibility appears to be due to its protease resistant property, and not to its protease inhibitory effect. This possibility was also supported by the evidence that the composition of fecal amino acids in the sericin-fed rats was very similar to that of sericin ingested. This finding on fecal amino acids further implies that sericin is not easily fermented in the large bowel. In addition, we have observed that sericin is resistant to the four proteases, protease type XXVII (Sigma), papain, trypsin and thermolysin (Sasaki *et al.*, unpublished data); the reason for this resistance is unknown at present. It is necessary to examine whether the high content of serine in sericin relates to this property. It seems possible that the property might be involved in a defense mechanism of the cocoon against the invasion of several organisms.

The finding of sericin's protease resistant property together with its high water-retaining capacity prompted us to investigate whether consumption of the protein suppresses constipation in rats which have received atropine. Constipation by atropine is caused by reduced release of acetylcholine from the parasympathetic nervous system (Borody *et al.*, 1985). One reason for the constipation in human has been thought due to a similar mechanism (Borody *et al.*, 1985). This study demonstrated an improvement in atropine-induced constipation in rats by feeding sericin. Although this experiment was conducted with a cellulose-free diet believed to easily cause constipation, essentially the same results were obtained with the diets containing 5% cellulose as in animal experiment 1 (Table 2); this suggested that the anti-constipation effect of sericin holds regardless of cellulose intake. Dietary fiber raises fecal weight associated with improvement in constipation (Eastwood, 1992). This effect is believed ascribable to the water-retaining capacity of unfermented fiber (Eastwood, 1992). Our study showed higher water content in the feces of rats fed sericin, and such a rise in content might relate to the improvement in constipation. We earlier found that consumption of a buckwheat protein product markedly improved atropine-induced constipation in rats (Kayashita *et al.*, 1995). This effect was also thought to be due to its low digestibility (Kayashita *et al.*, 1995).

In general, the protease resistant property of dietary proteins might be favorable to prevent of constipation as true of dietary fibers.

In conclusion, this study demonstrated the protease-resistant property of sericin, and led to our finding that this protein suppressed atropine-induced constipation in rats, thus suggesting that sericin could be a useful agent treatment of constipation as a resistant protein. Recently, we recognized the preventive effect of dietary sericin against colon carcinogenesis in mice which is similar to the effect of dietary fiber (Sasaki *et al*., 2000). Taken together, sericin may be beneficial for health as a component of functional food.

References

Borody, T.J., Quigley, E.M., Phillips, S.F., Wienbeck, M., Tucker, R.L., Haddad, A. and Zinsmeister, A.R. (1985). Effects of morphine and atropine on motility and transit in the human ileum. *Gastroenterology*, **89**, 562–570.

Duncan, D.B. (1957). Multiple range test for correlated and heteroscedatic means. *Biometrics*, **13**, 164–176.

Eastwood, M.A. (1992). The physiological effect of dietary fiber. *Ann. Rev. Nutr.*, **12**, 19–35.

Fournier, A. (1979). Quantitative data on the *Bombyx mori* L. silkworm. *Biochimie*, **61**, 283–320.

Kato, N., Kayashita, J. and Sasaki, M. (2000). Physiological functions of buckwheat protein and sericin as resistant proteins. *Nippon Eiyo Shokuryo Gakkaishi*, **53**, 71–75 (in Japanese).

Kato, N., Sato, S., Yamanaka, J., Yamada, H., Fuwa, N. and Nomura, M. (1998). Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. *Biosci. Biotechnol. Biochem.*, **62**, 145–147.

Kayashita, J., Shimaoka, I., Nakajoh, M. and Kato, N. (1996). Feeding of buckwheat protein extract reduces hepatic triglyceride concentration, adipose tissue weight and hepatic lipogenesis in rats. *J. Nutr. Biochem.*, **7**, 555–559.

Kayashita, J., Shimaoka, I., Nakajoh, M., Kishida, N. and Kato, N. (1999). Consumption of a buckwheat protein extract retards 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in rats. *Biosci. Biotechnol. Biochem.*, **63**, 1837–1939.

Kayashita, J., Shimaoka, I., Yamazaki, M. and Kato, N. (1995). Buckwheat protein extract ameliorates atropine-induced constipation in rats. *Curr. Adv. Buckwheat Res.*, **2**, 941–946.

Kayashita, J., Shimaoka, I., Yamazaki, M. and Kato, N. (1997). Consumption of buckwheat protein lowers plasma cholesterol and raises fecal neutral sterols in cholesterol-fed rats because of its low digestibility. *J. Nutr.*, **127**, 1395–1400.

Iwami, K., Sakakibara, K. and Ibuki, F. (1986). Involvement of post-digestion 'hydrophobic' peptides in plasma cholesterol-lowering effect of dietary plant proteins. *Agric. Biol. Chem.*, **50**, 1217–1222.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C. (1993). Purified diets for laboratory rodents: Final report of the AIN ad hoc writing committee on the reformulation of the AIN76 rodent diet. *J. Nutr.*, **123**, 1939–1951.

Sasaki, M., Kato, N., Watanabe, H. and Yamada, H. (2000). Silk protein, sericin suppresses colon carcinogenesis induced by 1,2-dimethylhydrazine in mice. *Oncol. Rep.*, **7**, 1049–1052.

Voegeli, R., Meier, J. and Blust, R. (1993). Sericin silk protein: Unique structure and properties. *Cosmet. & Toiletries*, **108**, 101–108.