Note

Hypocholesterolemic Activity of Bagasse Alkaline Extract in Rats Fed a High Cholesterol Diet

Nobuhiro FUKUDA,¹⁾ Maki MACHIDA,
Hirosuke OKU and Isao CHINEN

Laboratory of Biochemistry and Nutrition,
Department of Agricultural Chemistry, Faculty of
Agriculture, University of the Ryukyus,
Okinawa 903-01, Japan

¹⁾Laboratory of Food Science and Nutrition,
Department of Biological Resources, Faculty of
Agriculture, Miyazaki University,
Miyazaki 889-21, Japan

Received June 19, 1989

Of the various non-nutritive dietary fibers (DF), water soluble DF (SDF) is considered to be effective in lowering serum cholesterol levels in man and in experimental animals.^{1~6)}

In recent communications, we have reported that addition of 0.5% to 3% bagasse alkaline extract fraction B following a high cholesterol diet for 2 weeks, significantly reduced serum cholesterol from 17.4 to 33.3% without influencing food intake or growth.^{7,8)} However, in these experiments the effects of fraction B on fecal excretion of neutral and acidic steroids could not be ascertained. This study deals with the effect of longer term (8 weeks) feeding of fraction B on the concentrations of serum and liver lipids and also with more detailed estimates on fecal steroids excretion, since the hypocholesterolemic activity of SDF is mostly associated with the increased fecal excretion of either neutral or acidic steroid.^{1~3)}

Male Wistar rats were obtained from Ryukyu Biotec (Okinawa). The composition of the basal diet was (in %): vitamin-free casein, 20; Fats, 10 (lard, 9 and corn oil, 1); mineral mixture, 4-vitamin mixture, 1; choline chloride, 0.15; cellulose powder, 4; cholesterol, 0.5; sodium cholate, 0.15; and sucrose to 100. Vitamin and mineral mixtures were those of Harper. Fraction B at the 1% level was added at the expense of sucrose. Bagasse alkaline extract fraction B was prepared as described previously.^{7,8)} Rats were given free access to the diets and water for 8 weeks, with body weight and food consumption being recorded every other day. Feces were collected for 2 days before blood was withdrawn from the tail vein at 2, 4, 6, and 8 weeks, and lyophilized. The animals were killed by decapitation and serum and liver lipids were extracted as described by Folch *et al.*⁹⁾ Cholesterol, triglyceride, and

phospholipid were measured by methods described elsewhere.^{7,8)} Fecal neutral and acidic steroids were analyzed by gas-liquid chromatography and enzymatically, respectively, as described previously.^{7,8)} The data were analyzed for statistical significance using Student's *t*-test.¹⁰⁾

Table I summarizes the effects of fraction B on the serum and liver lipids. There was no difference in food intake and weight gain during 8-week feeding in the two groups. Although the weights of major viscera such as heart, lung, kidney, spleen, adrenals, and testes were similar, liver weight was lowered significantly in rats fed fraction B, indicating that the fraction B prevented liver enlargement due to dietary cholesterol. The fraction B feeding caused a significant reduction in the concentrations of serum total and free cholesterol compared to those of rats fed control diet, while the ester ratio remained unchanged. Serum were analyzed every 2 weeks for total cholesterol with the cholesterol B-test Wako (Wako Chemicals) during the study period. The concentrations of serum total cholesterol in rats fed with fraction B were consistently lower than those observed in control rats at all times and the concentrations at 2, 4, and 8 weeks were significantly different (Table II). There was a difference in serum total cholesterol levels at 8 weeks as

Table I. EFFECTS OF DIETARY BAGASSE ALKALINE EXTRACT FRACTION B ON SERUM AND LIVER LIPIDS

	Control (8)	Fraction B (8)
Food intake (g/day)	14.3 ± 0.4	14.2 ± 0.2
Initial	76 ± 2	76 ± 1
Body weight (g)		
Final	338 ± 8	329 ± 5
Liver weight (g/100 g body wt)	5.7 ± 0.1	5.3 ± 0.1*
Serum lipids (mg/dl)		
Total	324.0 ± 7.9	290.4 ± 10.4*
Cholesterol		
Free	46.2 ± 1.2	41.1 ± 1.7*
E (%)	85.7 ± 0.4	85.8 ± 0.2
Triglyceride	235.3 ± 18.9	208.3 ± 23.2
Phospholipid	213.4 ± 6.7	194.9 ± 6.7
Liver lipids (mg/g)		
Total	63.8 ± 1.5	60.3 ± 2.2
Cholesterol		
Free	3.0 ± 0.2	3.0 ± 0.2
E (%)	95.3 ± 0.2	94.9 ± 0.3
Triglyceride	45.7 ± 3.5	44.6 ± 3.0
Phospholipid	26.3 ± 0.4	27.4 ± 0.8

Numbers in parentheses represent the number of rats and results are expressed as the mean ± SE.

* Significantly different from the control group at *p* < 0.05.

Table II. EFFECTS OF DIETARY BAGASSE ALKALINE EXTRACT ON THE COURSE OF SERUM CHOLESTEROL AND FECAL EXCRETIONS OF NEUTRAL AND ACIDIC STEROIDS

Diets	Week	Serum cholesterol (mg/dl)	Feces (Dry wt, g)	Neutral			Acidic (mg/day)
				Cholesterol	Coprostanol (mg/day)	Total	
Control (8)	2	368.7 ± 20.9	0.8 ± 0.1	15.1 ± 1.3	4.3 ± 0.7	19.4 ± 1.5	27.8 ± 2.9
	4	331.3 ± 13.8	1.1 ± 0.1	19.0 ± 1.4	10.7 ± 0.9	29.7 ± 1.6	24.2 ± 2.1
	6	301.0 ± 12.6	1.0 ± 0.1	13.8 ± 2.0	10.5 ± 0.9	24.3 ± 2.1	33.6 ± 2.9
	8	332.9 ± 17.6	1.0 ± 0.1	13.6 ± 1.3	10.0 ± 1.2	23.6 ± 2.1	39.9 ± 3.6
Fraction B (8)	2	307.2 ± 12.8*	0.9 ± 0.1	18.5 ± 0.9*	2.9 ± 0.8	21.3 ± 0.9	28.0 ± 2.1
	4	287.0 ± 11.1*	1.2 ± 0.1	20.7 ± 1.9	11.4 ± 2.1	32.0 ± 2.2	29.8 ± 0.7*
	6	280.5 ± 8.4	1.2 ± 0.1	13.4 ± 1.0	12.4 ± 1.1	25.8 ± 1.1	41.8 ± 1.5*
	8	269.6 ± 7.7**	1.2 ± 0.1	14.5 ± 0.8	13.3 ± 2.3	27.8 ± 2.6	50.2 ± 3.2

Numbers in parentheses represent the number of rats and results are expressed as the mean ± SE.

*, ** Significantly different from the corresponding control group at $p < 0.05$ and $p < 0.01$, respectively.

shown in Tables I and II. The discrepancy is ascribed to the different methods used to measure cholesterol. However, there were no effects on the other lipid classes in the serum compartment. These results agree well with those reported previously.^{7,8)} The effectiveness of fraction B in the reduction of serum cholesterol levels is comparable with that observed in rats fed SDF prepared from rice, corn, and wheat brans,⁴⁻⁶⁾ although fraction B appeared to have no effects on the concentration of liver lipids.

Table II summarizes the effects of fraction B on the fecal excretion of neutral and acidic steroids. The mechanisms responsible for the hypocholesterolemic effect of SDF, though not completely clear, are mostly considered to be exerted through the interruption of absorption of either neutral or acidic steroid.¹⁻³⁾ Feeding fraction B did not caused any apparent abnormality in fecal excretion such as constipation or diarrhea. The rate of microbial transformation of cholesterol to coprostanol was markedly depressed by feeding cholesterol after 2 weeks in both groups. However, after 4 weeks and thereafter over a third of neutral steroids were excreted as coprostanol. Fraction B appeared to have no effect on the fecal excretion of neutral steroids. On the other hand, although acidic steroid excretions into feces at 2 weeks were not different between the groups, which agrees with results reported previously,^{7,8)} the diet containing fraction B stimulated the excretion of acidic steroids compared to the control group at 4 weeks and thereafter and the excretions at 4 and 6 weeks were significantly different. It seems therefore that the cholesterol-lowering effect of fraction B is partly mediated by the increased fecal excretion of acidic steroids which may be attributable to mechanisms other than sequestering bile acids in the intestinal tract, since fraction B does not bind bile acids *in vitro*.⁷⁾ It is not clear whether the increase in the fecal excretion of acidic steroids in rats fed fraction B is due to disruption of micelle formation or due to changes in the

microbial flora in the intestine. Thus, clear information in this respect awaits future studies.

The results of this study, together with those of the preceding experiments, indicated possible usefulness of fraction B prepared from bagasse as DF for treatment of hypercholesterolemia.

References

- 1) J. W. Anderson and W. J. L. Chen, *Am. J. Clin. Nutr.*, **32**, 346 (1979).
- 2) W. J. L. Chen and J. W. Anderson, in "Dietary Fiber," ed. by G. V. Vahouny and D. Kritchevsky, Plenum Press, New York and London, 1986, pp. 275 ~ 286.
- 3) K. Tsuji, in "Dietary Fiber," ed. by S. Innami and S. Kiriyaama, Dai-ichi Shuppan, Tokyo, 1985, pp. 131 ~ 159.
- 4) Y. Ayano, F. Ohta, Y. Watanabe, K. Mita and T. Nakamura, in "Dietary Fiber," ed. by S. Innami, G. Inoue, Y. Gotoh, A. Yoshida and S. Kiriyaama, Shinohara Shuppan, Tokyo, 1983, pp. 201 ~ 215.
- 5) Y. Ayano, F. Ohta, Y. Watanabe and K. Mita, *J. Jpn. Soc. Nutr. Food Sci.*, **33**, 283 (1980).
- 6) Y. Ayano, F. Ohta, Y. Watanabe, T. Nakamura and M. Takizawa, *J. Jpn. Nutr. Food Sci.*, **35**, 431 (1982).
- 7) H. Oku, N. Fukuda, M. Mori, M. Kinjyo, H. Motonon and I. Chinen, *Nutr. Rep. Int.*, **38**, 843 (1988).
- 8) N. Fukuda, H. Oku, I. Yoshihara, M. Machida and I. Chinen, *Nippon Nōgeikagaku Kaishi*, **63**, 1599 (1989).
- 9) J. Folch, M. Lees and G. H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
- 10) R. A. Fisher, "Statistical Methods for Research Workers," 14th Ed., Oliver & Boyd, Edinburgh, Scotland, 1970, pp. 140 ~ 142.