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Note

Buckwheat Protein Extract Suppression of the Growth Depression in Rats Induced by Feeding Amaranth (Food Red No. 2)

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Dietary fiber has an ameliorative effect on the toxicity of amaranth (Food Red No. 2). To test the possibility that a buckwheat protein extract (BWPE) has dietary fiber-like activity by virtue of its low digestibility, we examined the influence of BWPE on amaranth toxicity in rats. The results show that BWPE-containing diet suppressed the growth depression induced by the dietary addition of 5% amaranth.

Key words: buckwheat protein; amaranth; growth; rat

The quality of buckwheat protein is higher than that of any other plant proteins examined.[1] Although little is known about other nutritional aspects of this protein, we have recently found that feeding a buckwheat protein extract (BWPE) markedly reduced serum cholesterol in rats fed on a cholesterol-enriched diet.[2] This effect is ascribed to enhanced excretion of fecal neutral sterols.*[1] During these studies, we observed elevated fecal weight and elevated fecal nitrogen. In addition, BWPE had an ameliorative effect on atropine-induced constipation in rats.[3] These effects appear to be similar to those of dietary fiber.[4] BWPE may have dietary fiber-like activities by virtue of its lower digestibility. Dietary fiber is well known to suppress the toxicity of some chemicals, including that of amaranth (Food Red No. 2).[5] To test the possibility that BWPE has dietary fiber-like activities, we examined in this study if BWPE intake could suppress the growth depression in rats induced by a dietary addition of amaranth.

Male Sprague-Dawley rats (Hiroshima Laboratory Animal Center, Hiroshima, Japan) weighing approximately 90 g were used. The room temperature was kept at 24°C on a 12 h light–dark cycle (lights on 8:00 a.m.–8:00 p.m.). All the rats had free access to deionized water and the experimental diets. The rats were fed, in a two-way plan, a diet containing casein or BWPE (20% as the protein source) with or without the addition of amaranth (Hosogaya Chemical Co., Ltd., Tokyo, Japan). Amaranth was added to the diets at the level of 5% according to the previous study,[5] the composition of the experimental diets being shown in Table I. BWPE was prepared according to the method described elsewhere.[6] Food consumption and body weight were recorded everyday. After 3 wk of consuming the diets (5 animals per diet group), the animals were lightly anesthetized with diethylether and killed by decapitation between 1:00 p.m. and 3:00 p.m. The tissues were immediately excised and weighed. All results were tested for statistical significance by two-way ANOVA and Duncan's multiple-range test.[7]

With the rats not receiving amaranth, there was no difference in the growth between the casein group and BWPE group (Table II). The addition of amaranth to the casein diet caused a significant reduction in growth compared to that from the casein diet without amaranth. This significant effect on growth was observed after feeding for 7 d. In contrast, the addition of amaranth to the BWPE diet caused no growth retardation when compared to the effect from the BWPE diet without amaranth. Food intake was not significantly affected by dietary manipulation, but amaranth addition to the casein diet resulted in lower nutritional efficiency. However, amaranth addition to the BWPE diet had no influence on nutritional efficiency. Amaranth intake resulted in 121% and

Table I. Composition of the Experimental Diets[1]

Ingredient	Casein diet (%)	BWPE diet (%)
BWPE[2]	0	38.1
Casein[3]	23.0	0
Sucrose	20.0	20.0
α-Corn starch	37.0	26.1
Corn oil	10.0	5.8
Cellulose powder	5.0	5.0
Salt mixture[4]	3.5	3.5
Vitamin mixture[4]	1.0	1.0
Choline bitartrate	0.2	0.2
DL-Methionine	0.3	0.3

[1] Amaranth was added to these diets at the level of 5%.
[2] BWPE ($N \times 6.25$) 52.5%.
[3] Casein ($N \times 6.25$) 87.0%.
[4] AIN 76.[7]

Table II. Effect of Amaranth (Am) on the Growth, Food Intake, and Nutritional Efficiency in Rats Fed on the Casein Diet and BWPE Diet

Group	Gain in body wt.[1] (g/3 wk)	Nutrient intake (g/3 wk)	Nutritional efficiency
Casein	171 ± 4^{a}[2]	399 ± 11	0.43 ± 0.01^{a}
BWPE	174 ± 9^{a}	366 ± 12	0.48 ± 0.01^{a}
Casein + Am	115 ± 9^{b}	364 ± 35	0.33 ± 0.04^{b}
BWPE + Am	153 ± 3^{a}	379 ± 16	0.41 ± 0.02^{a}
Two-way ANOVA			
Protein	$p < 0.01$	NS[3]	$p < 0.05$
Am	$p < 0.01$	NS	$p < 0.01$
Protein × Am	$p < 0.05$	NS	NS

[1] Initial body wt., 90 ± 1 g (mean ± SE, $n = 20$).
[2] Values are means ± SE for 5 rats per group. Within a column, values followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple-range test.
[3] NS = not significant ($p > 0.05$).

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*[1] H. Tomotake, I. Shimaoka, J. Kayashita, F. Yokoyama, and N. Nakajoh, Abstracts of Papers, Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, Sapporo, August 1995, p. 142.

85% increases in the weight (g/100 g of body weight) of the cecum of those rats fed on the casein diet and BWPE diet, respectively (effect of amaranth, $p<0.01$ by ANOVA). There was no difference in the cecum weight between the casein and BWPE groups ($p>0.05$ by ANOVA). The higher cecum weight with amaranth is consistent with the results of the previous report.[5]

The present study demonstrates that BWPE intake suppressed the toxicity of amaranth in rats, as evidenced by the data of growth and nutritional efficiency. These results appear to be similar to the effects of dietary fiber,[5] and support our hypothesis that dietary BWPE has dietary fiber-like activity. Although dietary fiber is known to elevate the cecum weight,[9] BWPE had no significant influence on cecum weight. The weight of the cecum together with its contents was also unaffected by BWPE intake (data not shown). It has been reported that dietary fiber causes an acidic pH of the cecum contents.[10] Our study indicates that BWPE had no influence on the pH value (unpublished data). These facts imply that not all responses to dietary fiber can be observed with dietary BWPE.

BWPE contains a small amount of crude fiber (0.2% of BWPE).[2] Our previous study has shown that the dietary fiber fraction extracted from BWPE by the method of Prosky *et al.*[11] had no influence on the serum cholesterol level and fecal weight (to be published). Therefore, the hypocholesterolemic activity of BWPE is not ascribable to the dietary fiber in BWPE. Dietary fiber is known to elevate cecum weight,[9] but the present study showed no influence of BWPE on cecum weight. Thus, it seems unlikely that the counteracting effect of BWPE on amaranth toxicity is ascribable to the dietary fiber in BWPE.

We have examined whether the lower digestibility of BWPE protein is related to the hypocholesterolemic activity.*[2] Partial digestion of BWPE by trypsin caused a significant loss of this activity. In these experiments, there was good correlation between fecal neutral sterols and fecal nitrogen.*[2] In view of these facts, we speculate that the hypocholesterolemic activity of BWPE is at least in part due to the lower digestibility of BWPE protein. The ameliorative effect of BWPE on amaranth toxicity may also be related to this lower digestibility which would increase the mass of the intestinal contents and reduce the concentration of amaranth in the chyme.

There is evidence that amaranth toxicity develops mainly in the upper gastrointestinal tract as a result of the decreased availability of nutrients that is caused by the rapid transit and inhibitory effect of amaranth on the digestion-absorption process.[12] The beneficial effect of dietary fiber appears to be in normalizing the rapid transit through the upper gastrointestinal tract of chyme containing amaranth.[12] A further study is necessary to examine if the effect of BWPE is also mediated by a similar mechanism.

Our recent study has demonstrated that when growing rats were fed on 10% protein diets, the quality of BWPE protein was significantly higher than that of casein, as evidenced by the data on growth, protein efficiency ratio, nitrogen balance, and amino acid score (Wijesinghe *et al.* to be published). The digestibility of BWPE protein was lower than that of casein. However, the high quality of BWPE protein seems to have made up for the deficit. These facts raise the possibility that the beneficial effect of BWPE is in part mediated by the high quality of BWPE protein. A further study will be conducted to test this possibility.

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