**Note**

**Effect of Dietary Protein from Proso Millet on the Plasma Cholesterol Metabolism in Rats**

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Proso millet (Panicum miliaceum) is a more minor crop than foxtail, finger and pearl millets, but it is grown for human consumption in some Asian and African countries. Recently, we have reported that the nutritional quality of proso millet protein is poor but the quality can be greatly improved by supplementing with lysine and threonine.

Many studies have shown that vegetable proteins, especially soy protein, have the ability for lowering the level of plasma cholesterol, while proteins from animal sources, usually casein, induce hypercholesterolemia in experimental animals as well as humans.

Many foods or mixed diets of proso millet with other cereal grains have been reported. In Japan, some people have eaten proso millet as a mixed diet at the rate of 10% to 20% with rice. Therefore, it is interesting to learn whether its protein has any cholesterol-lowering action in plasma or liver, because no studies about this have been reported. The present study examines the effect of proso millet protein on cholesterol metabolism in rats.

The proso millet (Panicum miliaceum) used in this study was of yellow glutinous type which was described in the previous paper. A protein concentrate of the proso millet was prepared according to the method of Murata et al. to make a diet with 20% protein, in which crystallized bacterial α-amylase (Rakuto Kasei Industry Co., Ltd., Ootu, Shiga) was used to digest the starch in proso millet. The concentrate was treated 3 times with a mixture of chloroform and methanol (2:1) and twice with methanol to extract the oil. The protein content of the concentrate was 40.3% (mean value of three preparations) as measured by the Kjeldahl method. Proso millet will subsequently be referred to as millet in this study.

Male Wistar rats of 4 weeks of age, weighing about 70 g, were purchased from Shizuoka Agricultural Cooperative for Laboratory Animals (Hamamatsu, Shizuoka) and housed individually in stainless-steel cages in a room maintained at 22°C. They were fed with a diet containing 20% casein for 3 days. After that, the rats were divided into three groups of 6 to 7 each and fed with the experimental diets for 21 days. The experimental diets contained (g/100 g): oil, 1; vitamin mixture, 1; mineral mixture, 4; choline chloride, 0.15. The diets of casein, millet protein and soy protein isolate (SPI, Fuji Pro R, Fuji Seiyu Co., Osaka) contained 27 g of casein (Oriental Yeast Co., Tokyo), 54.1 g of millet protein concentrate and 23.6 g of SPI/100 g, respectively. Corn starch was used to adjust the total amount to 100 g, their protein contents being 21.5, 21.1 and 19.9 g/100 g of diet as measured by the Kjeldahl method. The vitamin and mineral mixtures were prepared according to the National Research Council. The millet protein diet was supplemented with 1.38% L-lysine monohydrochloride and 0.46% L-threonine to simulate the amino acid composition of casein, which was done on the basis of the amino acid composition of proso millet, because rats do not grow entirely without a supplement of these amino acids. SPI was treated 4 times with methanol to extract the saponin. Each diet and water were given ad libitum. Then, the rats were starved overnight and blood was collected from the liver portal vein of the rats after anesthetizing with diethyl ether. The blood obtained was placed into tubes containing heparin and the plasma rapidly separated. And liver was perfused with saline and quickly removed. These samples were stored at −20°C until needed for analysis.

The liver and plasma lipids were extracted according to the method of Folch et al., and the extracts were used for the measurement of their total cholesterol by the method of Zlatkis et al. The high-density lipoprotein (HDL)-cholesterol in the supernatant obtained by treating with heparin-Mn2+ precipitating reagent was determined with a commercial kit (Wako Pure Chemical Ind., Ltd., Oskaka) based on an enzymatic method. The difference between the total cholesterol and HDL-cholesterol was assumed to be low-density lipoprotein (LDL)- and very low-density lipoprotein (VLDL)-cholesterol.

Table 1 shows the changes in food intake and body-weight gain, and the concentrations of cholesterol and triglyceride in the plasma and liver of rats given diets containing casein, millet protein and SPI as the protein sources. The food intake of rats receiving the diet of SPI for the experimental period of 21 days showed a higher value (332.3 g) than those of the rats given casein and millet protein. The body-weight gain of the rats in the three dietary groups was generally similar. The plasma cholesterol concentration (67.4 mg/100 ml) of the rats fed with the diet of SPI was significantly lower than that (82.9 mg/100 ml) of the rats receiving the casein diet (p < 0.05), as has been shown in many studies, while that of the rats given the diet of millet protein supplemented with lysine and threonine showed a significantly higher value than the other two (p < 0.05). Similarly, the value of plasma HDL-cholesterol in the rats given millet protein also was highest among these three dietary groups.
Table 1. Effect of Dietary Protein Sources on Food Intake and Body-weight Gain, and the Concentration of Cholesterol and Triglyceride in Plasma and Liver

<table>
<thead>
<tr>
<th>Dietary protein</th>
<th>Mill protein</th>
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<tbody>
<tr>
<td></td>
<td>Casein*</td>
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<tr>
<td>Food intake (g)***</td>
<td>284.5 ± 4.4*</td>
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<tr>
<td>Body-weight gain (g)***</td>
<td>100.4 ± 3.2</td>
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<tr>
<td>Plasma lipids (mg/100 ml)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>82.9 ± 5.6*</td>
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<tr>
<td>HDL-cholesterol</td>
<td>49.7 ± 3.0*</td>
</tr>
<tr>
<td>LDL-VLDL-cholesterol</td>
<td>33.2 ± 5.0</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>88.6 ± 10.7</td>
</tr>
<tr>
<td>Liver lipids (mg/g)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.51 ± 0.2</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>8.63 ± 1.3</td>
</tr>
</tbody>
</table>

* Mean ± SE for 6 rats.  
** Mean ± SE for 7 rats.  
*** Results for an experimental period of 21 days.  
Values not sharing common superscripts were significantly different (p<0.05).

Although both the triglyceride concentrations in the plasma and liver of the rats given the diet of millet protein was lower compared with those of the rats fed with the casein diet, the difference was not statistically significant. This trend of declining triglyceride concentration in liver and plasma is consistent with observations in rats fed with soy protein. Also, these three dietary proteins did not affect the cholesterol concentration in the liver. It has been indicated that no significant increase in serum cholesterol was observed in rats given wheat gluten diets supplemented with lysine and threonin. However, the present study with proso millet protein supplemented with these amino acids produced different results, showing a clear elevation of HDL-cholesterol. To study the changes of HDL-cholesterol in relation to dietary protein sources is important because it is thought that plasma HDL acts to transport cholesterol from peripheral tissues, and that its concentration is inversely related to the risk of coronary heart disease. Therefore, the results shown in this study may suggest that a diet of proso millet protein supplemented with lysine and threonine would favorably affect the cholesterol metabolism in rats. However, this should not be concluded from the limited experimental data presented here without more detailed studies. Further, the effect of the amount of these amino acids added to a diet on cholesterol metabolism should be also examined together with the effect of starch in this millet.

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References