Effect of Casein and Soy Protein Isolate on Vitamin B₆ Nutritional Status in Rats

**Key Words**
- Vitamin B₆ nutritional status
- Casein
- Soy protein isolate
- Xanthurenic acid
- Erythrocyte transaminase

**Abstract**
Two experiments were conducted to test the effect of casein and soy protein isolate (SPI) on the nutritional status of vitamin B₆ in rats. Adult Long-Evans rats were fed with a casein or SPI diet at a 40% protein level with (control) or without (B₆-deficient) 7 mg of pyridoxine/kg diet. Vitamin-B₆-deficient rats were depleted of B₆ with (experiment 1) or without (experiment 2) deoxypyridoxine. In experiment 1, each rat was loaded with 150 mg of DL-tryptophan after 5 weeks of pair feeding. The rats on the vitamin-B₆-deficient SPI diet (SPI-B₆) excreted twice the amount of urine xanthurenic acid in 24 h than did the rats on the vitamin-B₆-deficient casein (casein-B₆) diet (p < 0.05). In experiment 2, L-tryptophan was loaded in a 20-mg dose at the end of each week. The excretion of xanthurenic acid was higher in the SPI-B₆ group than in the casein-B₆ group over the 5-week period of the experiment (p < 0.05). Erythrocyte transaminase (EGOT and EGPT) activities were lower, while EGOT and EGPT indexes were higher in the SPI-B₆ group than in the casein-B₆ group (p < 0.05). The results suggest that the source of dietary protein significantly influenced the status of B₆ nutrition in these rats.

It is well known that the vitamin B₆ requirement is positively correlated with the quantity of protein intake [1]; thus, more vitamin B₆ is required for higher protein intake. However, little is known about whether different sources of dietary protein affect the requirement for vitamin B₆. Kazemi and Kratzer [6] observed that chicks fed with a soybean diet required more vitamin B₆ for optimum growth than did those fed with a safflower or cottonseed diet. On the other hand, Kretsch et al. [7] found that more vitamin B₆ was required to normalize xanthurenic acid excretion in vitamin-B₆-depleted young women fed animal protein than in subjects fed plant protein. Fisher et al. [4] investigated the effect of protein quality on the vitamin B₆ status in rats. They found that rats fed an amino acid mixture which simulated low-quality-protein maize had lower serum pyridoxal phosphate, total liver vitamin B₆ and urinary 4-pyridoxic acid than did rats fed an amino acid mixture which simulated high-quality protein.

While studying the effects of vitamin B₆ deficiency on serum and liver lipids in rats [8], we found that the degree of vitamin B₆ deficiency was more severe when soy protein isolate (SPI), as compared with casein, was used as the protein source. In the present study, the vitamin B₆ nutritional status of rats, judged mainly by the amount of urinary xanthurenic acid excretion after tryptophan loading, was compared between casein and soy protein test.
The Source of Dietary Protein Influencing the Status of B₆ Nutrition

diet groups. The results suggest that different kinds of dietary protein may result in differences in the vitamin B₆ nutritional status in the rat.

Materials and Methods

Animals and Diets

In experiment 1, 24 male Long-Evans rats weighing 113–134 g were divided into 4 groups containing 6 animals each and randomly assigned to one of four semisynthetic diets: the vitamin-B₆-deficient casein (casein-B₆) diet, vitamin-B₆-adequate casein (casein+B₆) diet, vitamin-B₆-deficient SPI (SPI-B₆) diet or vitamin-B₆-adequate SPI (SPI+B₆) diet. The rats were housed individually in stainless-steel cages with raised screen bottoms and supplied with tap water ad libitum. The vitamin-B₆-deficient rats were depleted of pyridoxine by feeding a pyridoxine-free diet supplemented with 0.05% deoxypyridoxine (Sigma, St. Louis, Mo., USA) for 5 weeks. In experiment 2, 24 male Long-Evans rats 91–116 g in weight were group-fed and treated as in experiment 1, but without deoxypyridoxine added to the pyridoxine-free diet.

The protein sources of the test diets were SPI (Ralston Purina, St. Louis, Mo., USA) and vitamin-free casein (Sigma). SPI was further extracted with ethanol using a Soxhlet apparatus to remove as much pyridoxine as possible. The compositions of the test diets are shown in table 1. The vitamin-B₆ control groups were given 7 mg of pyridoxine/kg diet. No pyridoxine was added to the vitamin-B₆-deficient diet. In experiment 1, all groups were pair fed; in experiment 2, casein-B₆ and SPI-B₆ were pair fed, and casein+B₆ and SPI+B₆ were pair fed separately. Food intake was recorded daily, and body weight was recorded twice a week.

In experiment 1, a tryptophan loading test was conducted 2 days before the end of the experiment by giving 150 mg DL-tryptophan per rat orally. In experiment 2, each rat was given 20 mg L-tryptophan (E. Merck, Darmstadt, Germany) orally at the end of each week. The rats were then placed individually in metabolic cages for 24-hour urine collection; otherwise, the feeding conditions were the same as before the transfer. Urine specimens were collected in flasks containing 1–2 ml of toluene for preservation. At the end of an experimental period, the rats were fasted overnight and sacrificed by decapitation. Blood samples were collected in tubes containing heparin and centrifuged to sediment erythrocytes.

Measurement of Erythrocyte Transaminase Activities

The 24-hour urine specimens were filtered through Whatman No. 1 filter paper, and xanthurenic acid was analyzed using the method of Wachstein and Gudaitis [15]. For erythrocyte transaminases activity assay, erythrocytes were washed twice with cold saline (0.9% NaCl) and recentrifuged at 1,000 g for 20 min at 4°C. To prepare the hemolysate, 0.1 ml of packed erythrocytes was added to a test tube containing 3.9 ml of double-deionized water and lysed by freezing and thawing for 3 times. The hemolysates were then assayed for gluconeogenesis transaminase (GOT) and glutamic pyruvate transaminase (GPT) activities in the presence or absence of additional pyridoxal phosphate. Briefly, 0.9 ml of the hemolysate was incubated with 0.1 ml of 0.8 mM pyridoxal phosphate or 0.1 ml of deionized water for 30 min at 30°C; GOT and GPT activities were measured using commercially available kit reagents (Wako, Tokyo, Japan) [5].

Table 1. Composition of the basal experimental diets (g/100 g diet)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Casein group</th>
<th>SPI group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>43</td>
<td>–</td>
</tr>
<tr>
<td>SPI</td>
<td>–</td>
<td>48</td>
</tr>
<tr>
<td>Vitamin mixture¹</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mixture²</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Lard</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cod liver oil³</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Corn starch</td>
<td>37</td>
<td>32</td>
</tr>
</tbody>
</table>

¹ One gram of the vitamin mixture supplied thiamin 0.5 mg, riboflavin 1.0 mg, niacin 4.0 mg, folic acid 0.2 mg, Ca-pantothenate 4.0 mg, biotin 0.02 mg, cyanocobalamin 0.002 mg, inositol 25 mg, menadione 0.5 mg and choline-HCl 75 mg.
² The mineral supplement contained 0.01% aluminium ammonium sulfate, 11.18% calcium biphosphate, 6.86% calcium carbonate, 30.83% calcium citrate, 0.08% cupric sulfate, 1.53% ferric ammonium citrate, 3.52% magnesium carbonate, 3.83% magnesium sulfate anhydride, 0.01% manganese sulfate, 12.47% potassium chloride, 0.04% potassium iodide, 21.87% phosphate dibasic, 7.71% sodium chloride and 0.5% sodium fluoride.
³ Vitamins A and D were supplied with cod liver oil.

Results

The body weight gain and food intake of the rats in the 2 experiments are shown in table 2. No statistically significant difference was observed between the weight gain or feed efficiency between the test groups in experiment 1. In experiment 2, the weight gain and feed efficiency of the vitamin-B₆-deficient groups were significantly lower than those of the respective control groups (p < 0.05). However, there was no significant difference between the SPI-B₆ group and casein-B₆ group.

After 5 weeks of pair feeding, the SPI-B₆ group excreted about twice the amount of urinary xanthurenic acid than did the casein-B₆ group as determined by a 150-mg DL-tryptophan loading test, while both control groups excreted much less xanthurenic acid than did the vitamin-B₆-deficient groups (table 3). The amount of urinary xanthurenic acid excretion after 20 mg L-tryptophan loading.