Pancreatic protease secretion profiles after spontaneous feeding of casein or soybean protein diet in unrestrained conscious rats

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Under unrestrained physiologic conditions, the exocrine pancreatic secretion of digestive enzymes were observed with chronic bile-pancreatic duct cannulated rats. Bile-pancreatic juice (BPJ) was intermittently collected after spontaneous feeding of an 8% casein or an 8% soybean protein isolate (SPI) diet under recirculation of BPJ to the duodenum. The operative damage of cannula implantation was recovered for 5 days on a 25% casein diet, which was examined by food consumption and the basal secretion (pancreatic secretion during fasting) of total protein and trypsin. After feeding of both the test diets, the secretions of total protein, trypsin, chymotrypsin, and carboxypeptidase A were increased rapidly and about two-fold from the basal secretion. There were peak values at 15 min in the SPI group and at 30 min in the casein group. In the casein-fed rats, the protein and protease secretions maintained high levels for the initial 150 min, but in the SPI fed rats those secretions were decreased from 15 min after feeding. The protein and protease secretions tended to be higher in the casein group than in the SPI group after 30 min, and effect of 'diet' was significant in analysis of variance. The secretion profiles of protein and three proteases were similar, and specific activities of three proteases were not increased significantly in both the groups. We conclude that these low-protein diets are potent stimulators of the exocrine pancreatic secretion after spontaneous feeding in unrestrained rats.

Keywords. exocrine pancreas; pancreatic protease; dietary protein; conscious rat

Introduction

There are many observations about the control mechanism of exocrine pancreatic secretion by autonomic nervous systems and hormones, such as cholecystokinin,[1-5] but it is not enough to use nutritional approaches to solve this mechanism. In rats, intact protein is known to be responsible for the control of pancreatic secretion,[6-8] which is exerted by a mechanism of the negative feedback inhibition by pancreatic protease activities in the small intestinal lumen.[9-11] Different chemical properties of protein sources may have distinct individuality as a stimulator of releasing pancreatic secretagogues.[12,13]

We previously observed that oligo-L-methionine (6-10 L-methionine peptide mixture), which is a slightly digestible peptide, was absorbed faster when added to a low-casein diet than when added to a low-soybean protein isolate (SPI) diet at early stages of feeding.[14,15] The finding suggests that casein stimulates the exocrine pancreatic secretion more strongly than SPI.

We developed chronic bile-pancreatic duct cannulated rats whose bile-pancreatic juice (BPJ) can be collected under an unrestrained condition after spontaneous feeding of test diets. Using the cannulated rats, we could investigate the control mechanism of the exocrine pancreatic secretion with fully composite diet in the whole animal level. The purpose of this study is to evaluate the pancreatic secretagogue-stimulating activities of the casein and soybean protein

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diet under unanesthetized and unrestrained conditions.

Materials and methods

Diets

Compositions of test diets are shown in *Table 1*. Casein and soybean protein isolate diets were made to contain 8% protein (protein content = N × 6.25). That is, the test diet contains 9.4% of casein material (ALACID; New Zealand Dairy Board, Wellington, NZ) or 9.6% soybean protein isolate (SPI; Fujipro R; Fuji Oil Co., Osaka, Japan). The SPI used in this experiment contained no or slight trypsin-inhibitor activity. Nitrogen contents of both materials were estimated by the Kjeldahl method.

Animals

Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan), were fed a 25% casein-sucrose diet (stock diet) for 5 days. After a 24-hour fast, the rats, weighing 220–250 g, were anesthetized by i.p. injection of sodium pentobarbital (40 mg/kg body weight; Abbott Co., North Chicago, IL, USA), and operated on to implant cannulas into the common bile-pancreatic duct and duodenum. A small midline incision was made, the duodenum was drawn out, and a tip of polethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume Seisakusyo, Tokyo, Japan) was inserted into the common bile-pancreatic duct. The tip of the catheter was connected to a silicone tube (I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning Co., Kanagawa, Japan) to lead subcutaneously behind the neck. Another silicone catheter (I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning) for BPJ returning to the lumen was placed through the duodenum fistula 1 cm proximal to the ampulla of Vater. The duodenum catheter was connected to the bile-pancreatic duct catheter behind the neck to maintain the flow of BPJ during a time of recovery from the operative damage.

After a 24-hr fast, operated rats were divided into two groups and were given 2 g of the powdered test diets within the individual cages for 30 min after twice sampling BPJ in the fasting state. A polyethylene tube (SP 28; Natsume Seisakusyo) was connected to the pancreatic duct catheter, and BPJ was diverted through the polyethylene tube under a head of 5 cm from the cage bottom. Bile-pancreatic juice was collected for 3 min at 30 and 60 min before feeding and 15, 30, 60, 90, 120, 150, and 180 min after feeding of 2 g test diets. Bile-pancreatic juice was recirculated into the duodenum through the catheter continuously except each 3 min for sampling. The effects of BPJ diversion for 3 min may be negligible. The rats could freely move in the individual cages throughout the experimental period. In order to confirm the recovery of the exocrine pancreatic function from the operative damage, the BPJ was collected at 17:00 every day after the operation (under feeding of the stock diet from 20:00 to 8:00) in a separate experiment. The experiments were performed in the room controlled at 23° ± 2° C and 12-hr light: dark cycle (8:00–20:00, light period).

Table 1 Composition of diets

	Stock diet	8% Casein diet	8% SPI diet
	%	%	%
Casein[a]	25.0	9.4	—
SPI[a]	—	—	9.6
Sucrose	63.1	78.5	78.3
Corn oil[b]	5.0	5.0	5.0
Mineral mixture[c]	4.0	4.0	4.0
Vitamin mixture[d]	1.0	1.0	1.0
Granulated vitamin E[e]	0.1	0.1	0.1
Choline chloride	2.0	2.0	2.0

[a] Casein and SPI contained 13.7% and 13.4% nitrogen, respectively.
[b] Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to the corn oil.
[c] The mineral mixture is identical to the mineral mixture 2 (MM2) described by Ebihara, Imamura, and Kiriyama.[16] It provided (mg/kg diet): Ca, 4491; P, 2997; K, 3746; Mg, 375; Fe, 38.0; I, 0.31; Mn, 81.1; Zn, 25.9; Cu, 15.3; Na, 4342; Cl, 6678; Se, 0.27; Mo, 1.12; Cr, 0.49; B, 0.35; V, 0.22; Sn, 1.05; As, 1.20; Si, 15.7; Ni, 3.00; F, 2.71; Co, 0.20.
[d] The vitamin mixture was prepared in accordance with the AIN-76 mixture[17] except that menadione and L-ascorbic acid were added to make 5.81 μmol/kg[18] and 284 μmol/kg[19] diet, respectively.
[e] Vitamin E granule (Yuvela, Eisai Co., Tokyo) supplied 423 μmol all-rac-α-tocopheryl acetate per kg diet.

Analyses

The BPJ was adequately diluted with 0.1% Triton X-100 solution and activities of trypsin, chymotrypsin, and carboxypeptidase A (CPA) were measured. Zymogens of trypsin and chymotrypsin were activated by purified enterokinase (Sigma Chemical Co., St. Louis, MO, USA) and CPA was activated by purified trypsin (Sigma Chemical Co.). Trypsin,[20] chymotrypsin[21] and CPA[22] activities were estimated photometrically using synthetic substrates, Nα-p-toluenesulfonyl-L-arginine methyl ester (TAME), N-benzoyl-L-tyrosine ethyl ester (BTEE) and carbobenzoxy-glycyl-L-phenylalanine (ZGP), respectively. Protein content was quantified by modified Lowry's method.[23,24] Values in *Figures 2–6* are the amount of protein or the enzyme unit released from the pancreas for 3 min. Values of basal secretion are average of two collections before feeding of a test diet.

Statistical analyses

The statistical analyses were performed by one-way and two-way analysis of variance (ANOVA) (time and diet). The significant differences among means were determined by Duncan's multiple range test ($P < 0.05$). Values given are mean ± SEM.

Results

Changes in the body weight, food intake, and the secretions of total protein and trypsin during postoperative days on a stock diet are shown in *Figures 1 and 2*. The food intake was increased successively and the body weight gain was similar to that during preoperative days. Pancreatic secretions of total protein and trypsin were increased gradually after the operation and both the secretions reached plateau levels on the fifth day *(Figs. 2A and 2B)*.

Changes in total protein, trypsin, chymotrypsin, and CPA secretions after feeding of an 8% casein or an 8% SPI diet were shown in *Figures 3–6*. The secretions of total protein and three proteases reached peak values 15 min after feeding of the SPI diet and 30 min after feeding of the casein diet. The increments of protein and these protease secretions from 30 to 180 min of the casein-fed rats tended to be greater than those of the SPI-fed rats. The protein and enzyme secretions in the casein group maintained high levels for the initial 150 min after feeding, but the secretions in the

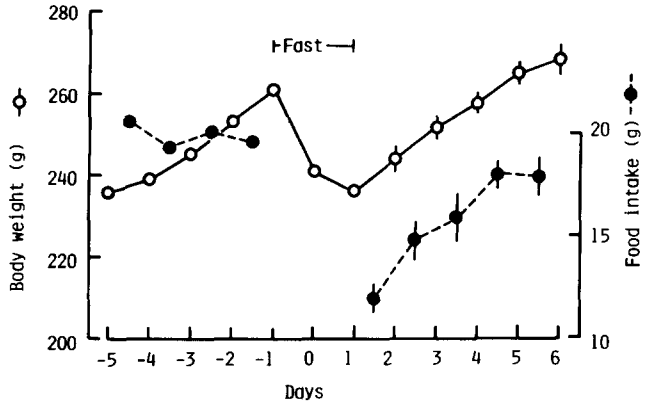

Figure 1 Changes in the body weight and the intake of a 25% casein diet after the operation for implantation of cannula. On day 0, the operation was performed on the rats. The values represent mean ± SEM (n = 14).

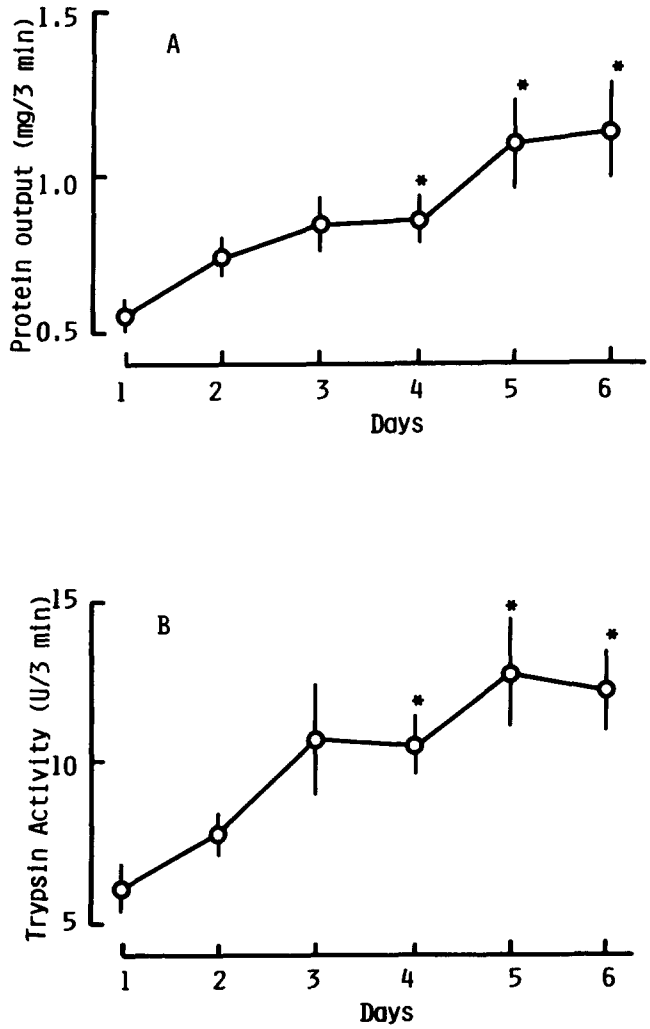

Figure 2 Changes in total protein secretion (A) and trypsin secretion (B) after the operation for implantation of cannula. The values represent mean ± SEM of the amount of secretion for 3 min (n = 14). The bile-pancreatic juice was collected every day at 17:00 (fed the stock diet from 20:00 to 8:00). Asterisks represent the significant differences from the values of the first day after the operation (*$P < 0.05$).

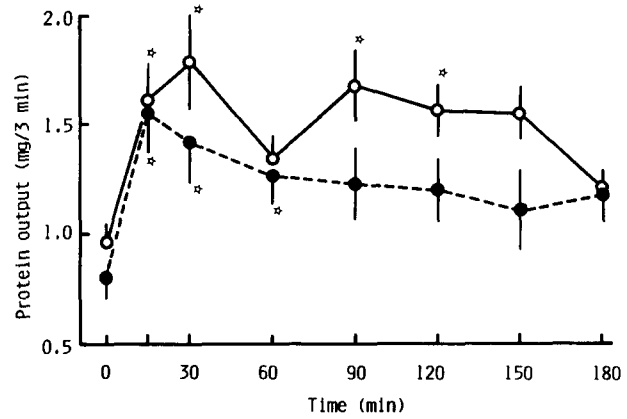

Figure 3 Changes in total protein secretion after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM of the amount of secretion for 3 min (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effects of time and diet were significant ($P < 0.01$). Asterisks represent the significant differences from basal secretion (Time 0; average value of the secretion 30 and 60 min before feeding) in each group (*$P < 0.05$).

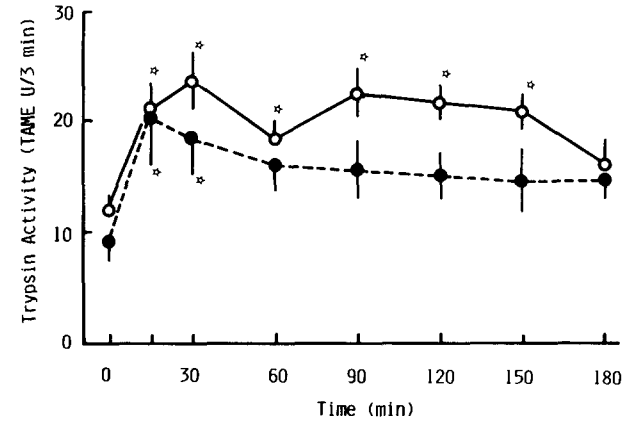

Figure 4 Changes in trypsin secretion after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM of the amount of secretion for 3 min (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effects of time and diet were significant ($P < 0.01$). Asterisks represent the significant differences from basal secretion in each group (*$P < 0.05$).

SPI group gradually fell from 15 min after feeding. Two-way ANOVA shows that diet influenced significantly in total protein, trypsin, and chymotrypsin secretions, and not in CPA secretion. The secretion profiles of trypsin, chymotrypsin, and CPA were similar to the profile of protein.

The changes in specific activities of trypsin, chymotrypsin, and CPA in both the diet groups (*Figures 7–9*) were not significant.

The final body weights of rats used in this experiment were 233 ± 2 g and 234 ± 2 g and the intakes of test diets were 1.78 ± 0.07 g and 1.81 ± 0.05 g for 30 min in the casein and the SPI groups, respectively.

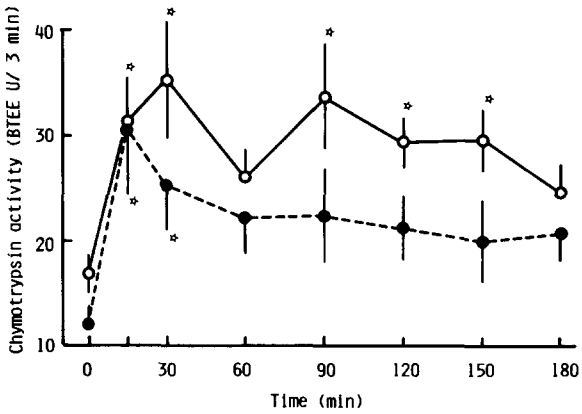

Figure 5 Changes in chymotrypsin secretion after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM of the amount of secretion for 3 min (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effects of time and diet were significant ($P < 0.01$). Asterisks represent the significant differences from basal secretion in each group ($^*P < 0.05$).

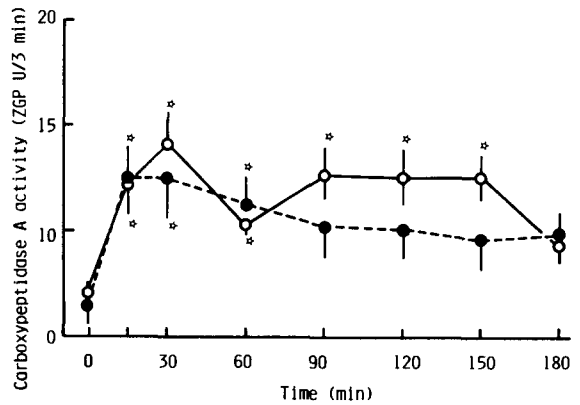

Figure 6 Changes in carboxypeptidase A secretion after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM of the amount of secretion for 3 min (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effect of time was significant ($P < 0.01$) and that of diet was not significant. Asterisks represent the significant differences from basal secretion in each group ($^*P < 0.05$).

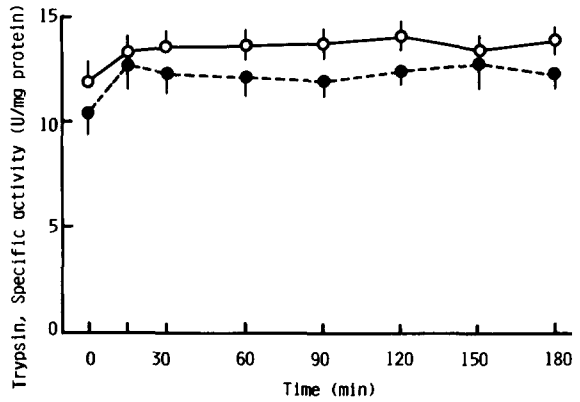

Figure 7 Changes in trypsin specific activities after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effect of diet was significant ($P < 0.01$) and that of time was not significant.

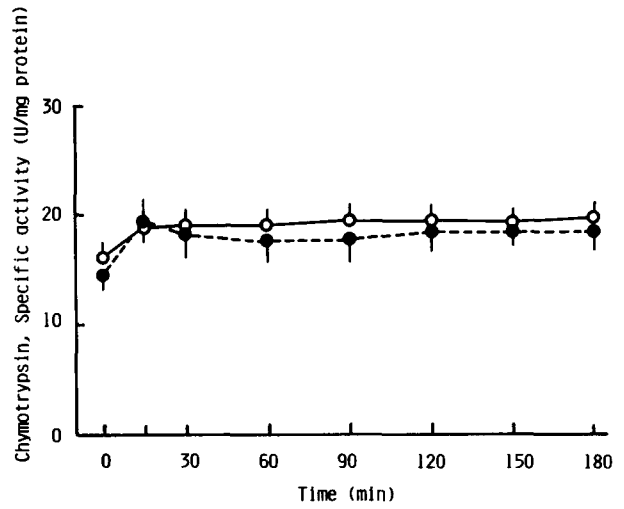

Figure 8 Changes in chymotrypsin specific activities after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effects of diet and time were not significant.

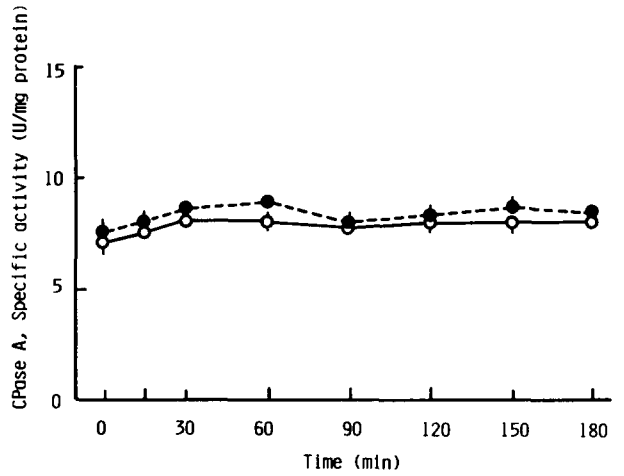

Figure 9 Changes in the carboxypeptidase A (CPase A) specific activities after feeding of an 8% casein diet (open circle) and an 8% SPI diet (closed circle). The values represent mean ± SEM (n = 12 in casein group, n = 11 in SPI group). The results of two-way ANOVA show the effect of time was significant ($P < 0.05$) and that of diet was not significant.

Discussion

The exocrine pancreatic secretion was enhanced about two-fold after spontaneous feeding of low-protein diets in conscious rats, and the secretions of total protein, trypsin, and chymotrypsin in the casein group were higher than those in the SPI group from the results of ANOVA. These findings show that the exocrine pancreatic secretion responds to the dietary proteins in the low-protein diets under the physiological conditions.

Green and Nasset[25] reported that 24% soy protein diet enhances the pancreatic trypsin secretion more strongly than 24% casein diet after intragastric infusion of the diets under restrained conditions. Berger

and Schneeman[26] also demonstrate that the secretions of two carboxypeptidases after duodenum infusion of SPI are higher than those after infusion of casein. These results are in disagreement with our observations, which may be due to the difference of the feeding condition and the feeding materials. The pancreatic secretion is affected by many factors other than dietary protein; for example, gastric emptying of dietary components, gastric acid secretion, and dietary fat. The amount of protease inhibitors in SPI material also affects the pancreatic secretions.

We previously observed that the gastric emptying rate of casein and SPI is almost identical under the same conditions as the present study. That is, after a 24-hr fast, rats were given 2 g of an 8% casein or SPI diet, and after 60 min, casein and SPI remaining in the stomach were both about 50%, with the same time course of emptying.[27] The pH of gastric content after feeding of an 8% casein diet and an 8% SPI diet are 5.04 ± 0.07 and 5.64 ± 0.11, respectively ($n = 6$, $P < 0.001$). The content of protease inhibitors in SPI may be various. Richter and Schneeman reported that trypsin inhibitor activity of SPI used in their study was about four and one-half–fold higher than that of casein.[28] The SPI used in our present experiments inhibits the protease activity approximately 25% more strongly than casein.[29] This result reveals that the content of protease inhibitors in SPI used in our experiments is very low. Therefore, the discrepancy between the results of previous investigators and ours is possibly due to the content of protease inhibitors in SPI. Dietary fat, especially fatty acid, is known to enhance the pancreatic secretion in rats.[30-32] The contribution of dietary fat to increasing the pancreatic secretion in our study is unknown, but the effect of the fat on the pancreatic secretion in 8% casein- and 8% SPI-fed group may be the same.

Several authors have revealed the non-parallel secretion of pancreatic enzymes.[33-35] The specific activities of three proteases tended to be increased after feeding, but the change is not significant. Therefore, the exocrine pancreatic secretion in the protease activities is nearly parallel under the spontaneous feeding, which is advantageous to digest dietary proteins efficiently.

Volumes of bile-pancreatic juice during fasting were 103 ± 12 and 102 ± 15 μL/3 min in the rats fed the casein and SPI diets (body weight about 230 g), respectively. Petersen and Grossman[36] mentioned that the bile-pancreatic flow is disturbed under anesthetic condition and state that the bile pancreatic flow volume in the fasting state is about 2300 μL/kg for 30 min (pancreatic: 800 μL, bile: 1500 μL) in conscious rats under recirculation of BPJ. The flow volume of rats used in our study is larger than 2,300 μL/kg for 30 min, which reveals that the bile-pancreatic flow is not disturbed and the physiologic functions are maintained in rats used in this study.

We repeated similar experiments and obtained the results to show that the amount of the test diet fed during initial 30 min (feeding speed) and the final body weight (food consumption and body weight gain during the recovery period) are also factors influencing the secretion. The difference of the pancreatic secretion between the casein and SPI groups was variable, but the results did not reverse. In this series of the experiments we confirmed that the protease secretion rates were increased two-fold after feeding of the low protein diets.

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