

## Hydroxypropyl-Distarch Phosphate from Tapioca Starch Reduces Zinc and Iron Absorption, but not Calcium and Magnesium Absorption, in Rats

Taro Kishida, Yuka Nakai and Kiyoshi Ebihara<sup>1</sup>

Department of Biological Resources, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790, Japan

**ABSTRACT** Male rats were fed a fiber-free, purified diet containing either gelatinized tapioca starch that was not modified chemically (TS, 50 g/kg diet) or gelatinized chemically modified tapioca starch (CMS, 50 g/kg) for 21 d. TS was used as the control. The six kinds of gelatinized hydroxypropyl distarch phosphate (HDP) from tapioca with two different degrees of substitution (DS) and three different degrees of cross-linking (DC) were used as CMS sources. The wet weight and moisture of fecal output of the rats fed HDP with higher DS were 100 and 20% greater than that in the control rats, respectively. The weights of cecal wall and cecal contents were also 30 and 50% higher in the rats fed HDP with higher DS than those in the control rats. The pH of the cecal contents was more acidic in the rats fed HDP with higher DS than that in the control rats. Fecal excretion of bile acids was 40% higher in the rats fed HDP with higher DS than in the control rats. These effects of HDP were only slightly affected by the DC. The plasma cholesterol concentration was 16% lower in the rats fed HDP with higher DS and highest DC than in the control rats. The concentrations of liver lipids and plasma triglycerides and the cecal pool of organic acids were not affected by diet. The apparent absorptions of Ca and Mg were not affected by diet, but those of Zn and Fe were 75 and 70% lower in the rats fed HDP with higher DS than in the control rats. These results suggest that the physiological effects of HDP depend on the DS but not on the DC. *J. Nutr.* 131: 294–300, 2000.

**KEY WORDS:** • *tapioca starch* • *hydroxypropyl distarch* • *phosphate* • *physiological effect* • *rats*

Chemically modified starches (CMS)<sup>2</sup> are more resistant to retrogradation, have higher viscosity and are more stable in response to acid and high temperature than is native starch. Therefore, CMS are increasingly used by the food industry to improve the physical properties of various food items, including baby food. The consumption of CMS is increasing as the consumption of processed foods increases. Many investigators have observed that CMS have reduced susceptibility to enzymes *in vitro* and *in vivo* compared with the corresponding unmodified starch (1–5,51,52). Therefore, CMS would be expected to exert physiological effects similar to those of dietary fiber. The presence of CMS in the gut digesta may influence the digestion of other nutrients and absorption of other compounds. CMS may influence cholesterol and bile acid metabolism and the absorption of minerals as a result of fermentation. It has been reported that the large intestine may represent a major site of Ca and Mg absorption when acidic fermentations take place (6). Therefore, CMS would be expected to exert physiological effects similar to those of dietary fiber.

The physiological effects of dietary fibers are due to their physico-chemical properties. The enzymatic susceptibility of a CMS is affected by the type of modification, such as substitu-

tion or cross-linking. However, there are only a few reports on whether different types of CMS have different physiological effects. Hydroxypropyl distarch phosphate (HDP), a starch that can be modified by a combination of substitution and cross-linking, is one of the CMS used extensively in the food industry. Therefore, we evaluated the physiological effects of six different types of HDP from tapioca starch that have two different degrees of substitution and three different degrees of cross-linking.

### MATERIALS AND METHODS

**Types of HDP.** The following six kinds of gelatinized HDP with two degrees of substitution (LS or HS) and three degrees of cross-linking (LC, MC or HC) were used: LS-LC, LS-MC, LS-HC, HS-LC, HS-MC and HS-HC (Table 1). The hydroxypropyl substituents were introduced by causing the tapioca starch to react with propylene oxide at pH 9–10 in the presence of Na<sub>2</sub>SO<sub>4</sub>. Cross-linking of the etherified starch was performed with Na<sub>3</sub>(PO<sub>3</sub>)<sub>2</sub> (0.02, 0.12 or 0.5 g/100 g etherified starch) at pH 9–10. All derivatives, as well as the unmodified tapioca starch, were delivered to the rats in a gelatinized form. The DS was measured according to the method of Johnson (7). The DSP of each HDP was measured using a slight modification of the method described by Leach et al. (8). Briefly, 1 g of a dry HDP sample was accurately weighed and placed into a 50-mL graduated centrifuge tube. After adding 1 mL of methanol, distilled water that had been kept at 25°C was added while mixing with a glass rod until the total volume was 50 mL. The graduated centrifuge tube was occasionally shaken in a water bath at 25°C over 20 min to prevent the precipitation of HDP. After centrifugation at 1200 × g at room

<sup>1</sup> To whom correspondence should be addressed.

E-mail: ebihara@agr.ehime-u.ac.jp

<sup>2</sup> Abbreviations: CMS, chemically modified starch; DC, degree of cross-linking; DS, degree of substitute; DSP, degree of swelling power; HDP, hydroxypropyl distarch phosphate; SCFA, short-chain fatty acid.

TABLE 1

Properties of tapioca starch (TS) and hydroxypropyl distarch phosphate (HDP)<sup>1</sup>

Starch	Moisture	Degree of substitution (DS) <sup>2</sup>	Amylase-resistant starch <sup>3</sup>	Degree of swelling power (DSP) <sup>4</sup>
	g/100 g		g/100 g	
TS	2.2	—	0.9	—
LS-LC	3.5	0.05	20.9	19.9
LS-MC	3.8	0.05	24.5	12.3
LS-HC	4.8	0.05	28.0	7.6
HS-LC	2.8	0.23	55.7	11.5
HS-MC	2.0	0.23	56.9	9.4
HS-HC	7.0	0.23	59.5	7.6

<sup>1</sup> TS, gelatinized tapioca starch not chemically modified; HDP, gelatinized hydroxypropyl distarch phosphate from TS. LS-LC, LS-MC, LS-HC, HS-LC, HS-MC and HS-HC are HDP with two degrees of substitution (DS; LS or HS) and three degrees of cross-linking (DC; LC, MC or HC).

<sup>2</sup> DS, moles of hydroxypropyl groups introduced per mole of anhydroglucose.

<sup>3</sup> Amylase-resistant starch is starch that is not hydrolyzed to glucose by amylolytic enzymes (termamyl and amyloglucosidase).

<sup>4</sup> DSP is the ability of HDP to swell in water. The DSP of each HDP was calculated as follows: Solubility (%) =  $A \cdot 10^{-1}$ , DSP =  $B/(1000 - 10 \cdot S)$ , where A is total sugar content in the supernatant (mg), B is precipitate (mg) and S is solubility (%).

temperature for 30 min, the volume of the aqueous supernatant was measured. Then, the total sugar content in the aqueous supernatant was analyzed with phenol/H<sub>2</sub>SO<sub>4</sub> reagent (9). The weight of the precipitate of swollen granules was measured. The DSP of each HDP was calculated as follows:

$$\text{Solubility (\%)} = A \cdot 10^{-1}$$

$$\text{DSP} = B/(1000 - 10 \cdot S)$$

where A is total sugar content in the supernatant (mg), B is precipitate (mg) and S is solubility (%).

The degree of swelling power (DSP) decreased with increasing DC. Kainuma et al. (10) showed that there is an inverse relationship between the DC and the DSP of starch granules. Therefore, the DC of the three HDPs increased in the following order: LC < MC < HC.

**Measurement of the level of amylase-resistant starch.** Amylase-resistant starch is starch that cannot be hydrolyzed to glucose by amylolytic enzymes. To determine the level of amylase-resistant starch in each HDP, 1-g triplicate samples were incubated with heat-stable  $\alpha$ -amylase (Termamyl 120L; Novo Nordisk, Copenhagen, Denmark) at pH 6.0 at 100°C for 30 min and then allowed to cool. After cooling, the pH was adjusted to 4.5 by HCl, and the samples were incubated with amyloglucosidase (Sigma A-9913; Sigma Chemical, St. Louis, MO) at 60°C for 30 min. After the incubation, the amount of glucose in the hydrolysate was enzymatically determined using pyranoseoxidase (Determiner GL-E; Kyowa Medix, Tokyo, Japan). The concentration of amylase-resistant starch was calculated as follows:

Amylase-resistant starch (g/100 g)

$$= (1 - G \cdot 0.9 \cdot \text{wt. sample}^1) \cdot 100$$

where wt. sample is the initial weight (g), and G is the weight of the hydrolysate (g).

**Animals and diets.** This study was approved by the Laboratory Animal Care Committee of Ehime University, and the rats were maintained in accordance with the "Guidelines for the Care and Use of Laboratory Animals" of Ehime University.

Male Wistar rats (Japan SLC, Hamamatsu, Japan) with an initial

weight of ~80 g were used in these experiments. The rats were housed individually in cages with screen bottoms of stainless steel in a room maintained at  $23 \pm 1^\circ\text{C}$  with a 12-h light/dark cycle (light, 0700–1900 h). The rats were acclimated by feeding a commercial solid diet (MF; Oriental Yeast, Osaka, Japan) for 7 d. After acclimation, the rats were divided into seven groups of six rats each (each group had a similar mean body weight). The composition of diets used in the experiment is shown in Table 2. The rats were given free access to the experimental diet and water for 21 d. The body weight and food intake were recorded daily for each rat in the morning before the diet was replaced. Then, the condition of the feces of each rat was observed.

**Sampling and analytical procedures.** Before the rats were killed, feces were collected on the final 3 d of the experimental period from each rat. The feces were freeze-dried, weighed and milled. The level of nitrogen (N) in the diet and feces of each rat was analyzed in duplicate for each collection according to the Kjeldahl method (11). The apparent digestibility of protein ( $N \times 6.25$ ) was calculated by measuring the N content in the food and feces. To determine the levels of Ca, Zn, Fe and Mg, the powdered feces (~70 mg) and diet (~500 mg) were wet-ashed in HNO<sub>3</sub>/HClO<sub>4</sub> (3:1). The concentrations of Zn, Fe and Mg in the ashed solutions were measured by atomic absorption spectrophotometry (AA-6400F; Shimadzu, Kyoto, Japan) after dilution with deionized water. The calcium concentration in the ashed solutions was measured by atomic absorption spectrophotometry after dilution with 10 mol lanthanum chloride/L. The apparent absorption of Zn, Fe, Mg or Ca was calculated as the difference between the dietary intake and fecal excretion of Zn, Fe, Mg or Ca, respectively. Fecal steroids were extracted with a mixture of chloroform/methanol (1:1, v/v) at 70°C for 60 h (12). Total fecal bile acids were determined enzymatically by the 3 $\alpha$ -hydroxysteroid dehydrogenase assay method of Sheltaway and Losowsky (13) using taurocholic acid as the standard.

Blood was collected from the abdominal aorta of rats under sodium pentobarbital (50 mg/kg body mass, Nembutal; Abbot Laboratories, North Chicago, IL) anesthesia in a blood collection tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) that contained heparin as an anticoagulant. The plasma was separated by centrifugation at  $1400 \times g$  at 4°C for 15 min and was stored at  $-50^\circ\text{C}$  until analysis. The liver was removed, weighed and stored at  $-50^\circ\text{C}$  for further analysis.

After blood collection, the cecum was removed and weighed. The contents were transferred to a cooled 50-mL vial and homogenized under CO<sub>2</sub> gas. The water content of the cecal content was determined as the difference between the wet mass and the dry mass of the cecal content after freeze-drying. The cecal pH was measured immediately after removal with a compact pH meter using a sampling sheet (model C-1; Horiba, Tokyo, Japan; calibrated at 20°C). The cecal wall was flushed with ice-cold saline (9 g NaCl/L, 4°C), blotted onto filter paper and weighed. The level of cecal ammonia was

TABLE 2

Compositions of control and test diets

Ingredient	Control diet	Test diet
	g/kg	
Casein	200	200
Corn oil	50	50
Mineral mixture <sup>1</sup>	40	40
Vitamin mixture <sup>2</sup>	10	10
Sucrose	650	650
Gelatinized tapioca starch	50	—
Gelatinized HDP <sup>3</sup>	—	50

<sup>1</sup> Based on AIN-76 (50).

<sup>2</sup> The AIN-76 vitamin mixture used in this study contained 20 g of choline bitartrate/100 g.

<sup>3</sup> Hydroxypropyl distarch phosphate (HDP) prepared from tapioca starch.

determined spectrophotometrically in the deproteinized [4 mL of 0.25 mol sulfuric acid and 50 g sodium tungsten dihydrate per L, 1:1 (v/v), for ~50 mg contents] supernatant (1500 × g, 10 min) of the cecal content (14).

The levels of cecal organic acids (acetic, propionic, *n*-butyric, succinic and lactic acids) were measured using HPLC (LC-6A; Shimadzu, Kyoto, Japan) by the internal standard method. Approximately 300 mg of the cecal content was homogenized by ultrasonication (USC-6; Iwaki Glass Ltd., Chiba, Japan) in 2 mL of 10 mmol sodium hydroxide/L aqueous solution, containing 0.5 g crotonic acid/L (Nakarai, Kyoto, Japan) as an internal standard, in an ice-water bath, and then centrifuged at 10,000 × g for 15 min. The fat-soluble substances in the supernatant were removed by extraction with chloroform. Neutralization of the cecal content with sodium hydroxide prevented the extraction of the individual target organic acid or crotonic acid by chloroform. The aqueous phase was filtered through a membrane filter (cellulose acetate, pore size 0.45 μm; DISMIC-13cp; Toyo Roshi, Ltd., Tokyo, Japan). These samples were subject to HPLC for analysis of the organic acids. The organic acids were separated with an ion exclusion column and detected according to the postcolumn pH-buffered electroconductivity detection method (16) using an H-type cation exchanger column (shim-pack SCR-102H, 8 mm i.d. × 30 cm long; Shimadzu, Kyoto, Japan), column temperature 45°C, with a mobile phase of 2 mmol perchloric acid/L (flow rate: 1 mL/min, 45°C). They were then reacted with a commercial detection reagent (ST3-R; 15 mmol disodium hydrogen phosphate/L), 5 mmol *p*-toluene sulfonic acid/L aqueous solution (flow rate, 0.8 mL/min, 45°C), an electroconductivity detector of positive polarity at 45°C (CDD-6A; Shimadzu) and a detection reagent of 20 mmol bis-Tris/L aqueous solution containing 5 mmol *p*-toluene sulfonic acid/L and 100 μmol EDTA/L (flow rate 0.8 mL/min, 45°C).

The levels of triglyceride and total cholesterol in the plasma were enzymatically determined with commercial kits (Triglyceride E-Test Wako and Cholesterol E Test Wako; Wako Pure Chemical Industries, Osaka, Japan). The level of liver total lipids was determined gravimetrically after extraction according to the method of Folch et al. (15). The levels of liver triglyceride and total cholesterol were also measured. Lipids were extracted from 500 mg liver with chloroform/methanol (2:1, v/v) according to the method of Folch et al. (15). After extraction, the volume of the lipid solution was adjusted to 20 mL with the same solution of chloroform/methanol (2:1, v/v). One mL of this extract was dried under a nitrogen stream, and the obtained residue was mixed with 100 μL isopropyl alcohol containing 100 g Triton X-100/L (Wako Pure Chemical Industries). Thirty μL of this mixture was mixed with 3 mL of aqueous enzyme solution according to the standard procedure of the assay kit (Triglyceride E-Test Wako and Cholesterol E-Test Wako; Wako Pure Chemical Industries), and the triglyceride and cholesterol concentrations were determined colorimetrically. In a preliminary study, 30 μL of isopropyl alcohol containing 100 g Triton X-100/L did not affect the enzymatic reactions (data not shown).

**Statistical analysis.** Data are expressed as means ± SEM. The statistical significance of a difference between the control group and each HDP group was evaluated by one-way analysis of variance followed by Student-Newman-Keuls test using the Super ANOVA statistical software package (Abacus Concepts, Berkeley, CA).

The effects of the DS and DC were analyzed by two-way ANOVA, using a computer software package (StatView Version 4.5; Abacus Concepts), and the difference among groups was examined using Duncan's new multiple range test when the *F*-value was significant. Differences were considered to be significant at *P* < 0.05. Correlation coefficients were determined by linear regression (17).

## RESULTS

The content of amylase-resistant starch in the LS and HS diets was markedly greater than that in the TS diet, which was used as the control (Table 1). The level of amylase-resistant starch in the HS diets was more than twice as much as that in the respective LS diet with the same DC. The level of amylase-resistant starch slightly increased with increasing DC.

Loose stools or diarrhea was observed in the rats fed the LS

and HS diets, but intestinal adaptation took place during the 1st wk of feeding. The severity of diarrhea was not affected by the DS of the HDP, but increased with increasing DC. Body mass gain, food intake and feed efficiency were not affected by the type of diet (Table 3). The weight of the cecal contents and cecal wall of the rats fed the HS diets was significantly higher than the respective weight in the control rats and was also significantly higher than the respective weight in the rats fed the LS diets. The moisture in the cecal contents of rats fed the LS-MC, LS-HC or HS diets was significantly lower than that in the control rats, and the moisture in the cecal contents of rats fed the HS diets was significantly lower than that in the rats fed the LS diets; however, the level of moisture in the cecal content was not related to the DC. The wet weight of the fecal output of the rats fed the LS-HC diet or the HS diets was significantly higher than that of the control rats. The wet weight of the fecal output of the rats fed the HS diets was significantly greater than that of the rats fed the LS diets, and it increased with increasing DC. The moisture of the fecal output was significantly higher in the rats fed the LS-HC diet or the HS diets than in the control rats. The moisture absorbed in the colon was significantly lower in the rats fed the LS-HC or HS diets than in the control rats, and the moisture absorbed in the colon of the rats fed the LS and HS diets gradually decreased with increasing DC.

The pH of the cecal contents in rats fed each HS diet was significantly lower than that in the control rats; however, the pH of the cecal contents in rats fed the LS diets was not (Table 4). The concentration of ammonia in the cecal contents was not affected by the diet. The concentrations of total organic acids and acetic, propionic and lactic acids in the cecal contents were not affected by the diet. However, the concentration of *n*-butyric acid in the cecal contents was significantly lower in the rats fed the LS or HS diets than in the control rats. The concentration of succinic acid in the cecal contents of rats fed the HS-HC diet was significantly lower than that in the control rats.

The apparent protein digestibility in the rats fed the HS-MC or HS-HC diet was lower than that in the control rats (Table 5). The plasma cholesterol concentration in the rats fed the HS-HC diet was significantly lower than that in the control rats and was significantly lower than that in the rats fed the LS diets. The plasma triglyceride concentration, the liver weight and concentrations of liver total lipids, cholesterol and triglyceride were not affected by the diet (data not shown). The levels of bile acids in the cecal contents and fecal excretion of bile acids were higher in the rats fed the HS diets than in the control rats and in the rats fed the LS diets.

The apparent absorptions of Ca and Mg were not affected by diet (Table 6). However, the apparent absorption of Zn in the rats fed the HS-MC or HS-HC diet was significantly lower than that in the control rats and in the rats fed the LS diets. The apparent absorption of Fe in the rats fed the LS or HS diets was significantly lower than that in the control rats. The apparent absorption of Fe slowly decreased with increasing DC. The apparent absorption of Fe in the rats fed the HS diets was significantly lower than that in the rats fed the LS-LC or LS-MC diet.

## DISCUSSION

The *in vitro* digestibility of gelatinized hydroxypropyl starches decreases with increasing substitution of hydroxypropyl groups (18, 19). On the other hand, it has been reported that cross-linking with phosphate has no effect on the *in vivo* digestibility of HDP from tapioca starch (20) or potato starch



*Effect of the degree of substitution and cross-linking in hydroxypropyl distarch phosphate (HDP) on body mass gain, food intake, wet mass of cecal contents, cecal wall weight, fecal output and apparent moisture absorption in the colon of rats<sup>1,2</sup>*

<sup>1</sup> Rats were fed a diet that each contained a test starch (5 g/100 g diet) for 21 d.  
<sup>2</sup> Each value represents the means  $\pm$  SEM,  $n = 6$ . In each column, means with different superscript letters are significantly different between groups fed HDP ( $P < 0.05$ ). \* Means are significantly different ( $P < 0.05$ ) than means in the control group.  
<sup>3</sup> Feces were collected on the last 3 d of the experimental period.  
<sup>4</sup> Moisture absorbed in the colon (M) was calculated with the following equation:  $M = (C - F) \times 100/C$ , where C is the moisture in the cecal content and F is the moisture in the feces.  
<sup>5</sup> Control group was fed a diet containing TS as a test starch.  
<sup>6</sup> LS-LC, LS-MC or LS-HC group was fed a diet containing HDP with DS of 0.05 and lowest DC, HDP with DS of 0.05 and middle DC or HDP with DS of 0.05 and highest DC as a test starch.  
<sup>7</sup> HS-LC, HS-MC or HS-HC group was fed a diet containing HDP with DS of 0.23 and lowest DC, HDP with DS of 0.23 and middle DC or HDP with DS of 0.23 and highest DC as a test starch.  
<sup>8</sup> Two-way ANOVA: DS, significant influence of the degree of substitution ( $P < 0.05$ ); DC, significant influence of the degree of cross-linking ( $P < 0.05$ ). NS, not significant ( $P > 0.05$ ).

increasing DS and with increasing DC in rats fed hydroxypropylated potato starch or HDP (DS = 0.01) from potato starch at 100 g/kg (19). In the present study, diarrhea was observed in the rats fed all LS and HS diets, but the degree of diarrhea was not affected by the DS. Cecal enlargement was observed in the rats fed the HS diets but not in the rats fed the LS diets. However, among the rats fed the HS diets, the degree of cecal enlargement was not affected by the DC. It has been demonstrated that enlargement of the cecum is due to an increased load of osmotically active substances in the caudal part of the

*Effect of the degree of substitution and cross-linking of hydroxypropyl distarch phosphate on the pH of the cecal contents and cecal pool of principal organic acids in rats<sup>1,2</sup>*

<sup>1</sup> Rats were fed a diet that each contained a test starch (5 g/100 g diet) for 21 d.  
<sup>2</sup> Each value represents the means  $\pm$  SEM,  $n = 6$ . In each column, means with different superscript letters are significantly different between groups fed HDP ( $P < 0.05$ ). \* Means are significantly different ( $P < 0.05$ ) than means in the control group. NS,  $P > 0.05$ .  
<sup>3</sup> For definitions, see Table 3.  
<sup>4</sup> Total = acetic + propionic + *n*-butyric + succinic + lactic acids.

TABLE 5

*Effect of the degree of substitution and cross-linking in hydroxypropyl distarch phosphate on plasma cholesterol, cecal bile acids and fecal excretion of bile acids in rats<sup>1</sup>*

Group <sup>2</sup>	Apparent digestibility of dietary protein <sup>3</sup>	Plasma cholesterol	Cecal bile acids	Fecal excretion of bile acids
	%	μmol/L	μmol/cecum	μmol/3 d
Control	96.2 ± 0.2	2.92 ± 0.11	40.9 ± 4.3	66.7 ± 4.3
LS-LC	95.7 ± 0.5 <sup>b</sup>	2.98 ± 0.15 <sup>b</sup>	33.4 ± 4.8 <sup>a*</sup>	75.4 ± 3.0 <sup>a</sup>
LS-MC	95.5 ± 0.6 <sup>b</sup>	2.89 ± 0.09 <sup>b</sup>	33.0 ± 3.9 <sup>a*</sup>	75.5 ± 3.6 <sup>a</sup>
LS-HC	95.3 ± 0.9 <sup>b</sup>	2.88 ± 0.09 <sup>b</sup>	33.8 ± 3.5 <sup>a*</sup>	72.3 ± 3.1 <sup>a</sup>
HS-LC	92.4 ± 0.9 <sup>a*</sup>	2.80 ± 0.12 <sup>ab</sup>	42.4 ± 2.2 <sup>b</sup>	92.6 ± 3.4 <sup>b*</sup>
HS-MC	92.1 ± 0.7 <sup>a*</sup>	2.74 ± 0.12 <sup>ab</sup>	41.1 ± 3.9 <sup>b</sup>	92.3 ± 3.8 <sup>b*</sup>
HS-HC	91.8 ± 0.8 <sup>a*</sup>	2.44 ± 0.20 <sup>a*</sup>	45.3 ± 2.4 <sup>b</sup>	94.4 ± 3.0 <sup>b*</sup>
ANOVA	DS	NS	DS	DS

<sup>1</sup> Each value represents the means ± SEM,  $n = 6$ . In each column, means with different superscript letters are significantly different between groups fed HDP ( $P < 0.05$ ). \* Means are significantly different ( $P < 0.05$ ) than means in the control group. NS,  $P > 0.05$ .

<sup>2</sup> For definitions, see Table 3.

<sup>3</sup> Apparent digestibility of dietary protein (D) was calculated with the following equation:  $D = (I - F) \times 100/I$ , where  $I$  is N intake and  $F$  is the fecal N excretion. The amounts of N ingested and excreted in feces were analyzed by the Kjeldahl method (see text).

intestinal tract (22, 23). In the case of carbohydrates, it would primarily be associated with short-chain fatty acids (SCFA) produced by the gut microflora from the undigested part of the carbohydrate. However, the SCFA concentration in the cecum of the rats fed the LS and HS diets did not significantly differ from that in the control rats. On the other hand, Oku et al. (24) speculated that cecal enlargement depends on the amount of maldigested materials that reach the cecum. The level of amylase-resistant starch in the HS diet was greater than that in the LS diet. The wet weight of the cecal content of the rats fed the HS diet was significantly greater than that of the control rats; however, the wet weight of the cecal content of the rats fed the LS diet was not. Therefore, the cecal enlargement caused by the HS diet may be due to increased influx of the unabsorbed starch fraction into the cecum. The cecal tissue weight of the rats fed the HS diet was heavier than that in the control rats. Til et al. (25) showed that feeding HDP (DS = 0.075) from potato starch led to an increase in the tissue weight of the cecum. Feeding nonfermentable bulk to rats predominantly increased the thickness of the muscularis externa (26). There was a positive correlation between the cecal content and cecal tissue weight among rats fed diets containing resistant starch (27). Thus, the increase in cecal content may have contributed to the heavier cecal tissue weight in the rats fed the HS diet. Til et al. (25) showed that feeding HDP (DS = 0.075) from potato starch leads to an increase in the weight of the colon. However, in the present study, the LS and HS diets did not lead to an increase in the colon tissue weight. Enlargement of the large intestine is known to readily occur in rats fed CMS (28).

The apparent protein digestibility in the rats fed the HS-MC or HS-HC diet was lower than that in the control rats. A reduction in feed efficiency is generally caused by reduced digestibility of proteins and/or energy sources and by reduced retention of nutrients resulting from various metabolic disturbances. However, the feed efficiency and body mass gain in the

rats fed the HS-MC or HS-HC diet did not differ from those in the control rats. Thus, it does not appear that HS-MC and HS-HC interfere with the digestion and absorption of protein and energy sources. On the other hand, the fecal N excretion (data not shown) and fecal output were greater in the rats fed the HS-MC or HS-HC diet than in the control rats. The greater fecal N excretion is caused by reduced protein digestibility and by increased production of endogenous protein (e.g., digestive enzymes and sloughed cells) and/or by greater fecal biomass. The bacteria in the large bowel play a major role in fecal bulking and constitute a large proportion of fecal N (29). However, the amylase-resistant starch in HDP is not a good substrate for bacteria in the large bowel (1, 30). Thus, the greater fecal N excretion and the lower apparent protein digestibility in the rats fed the HS-MC or HS-HC diet may be due to increased fecal output as a result of a shorter transit time in the large bowel.

The fecal wet weights and fecal moistures in the rats fed the LS-HC and HS diets were significantly greater than those of the control rats, but those in the rats fed the LS-LC or LS-MC diet were not. If the magnitude of the laxative effect is calculated as the increase in fecal wet weight per gram of amylase-resistant starch consumed, the effect was as follows (g/g): 0.028 for the LS-LC diet, 0.049 for the LS-MC diet, 0.289 for the LS-HC diet, 0.835 for the HS-LC diet, 1.267 for the HS-MC diet and 1.310 for the HS-HC diet. The laxative effect of the LS and HS diets increased as the level of amylase-resistant starch increased ( $r = 0.969$ ,  $P = 0.0014$ ). Therefore, the increase in fecal output in the rats fed LS-HC and HS diets is due to the increases in fecal moisture and level of amylase-resistant starch. The amount of absorbed moisture in the colon of rats fed the LS-HC and LS diets was significantly lower than that in the control rats. The contents in the colon of the rats fed the LS-HC and HS diets felt stickier and more viscous to the touch compared with those of the rats fed the TS diet. Therefore, the reduced moisture absorption in the colon of rats fed the LS-HC and HS diets would be due to inhibition of diffusion.

The plasma cholesterol concentration in the rats fed the HS-HC diet was significantly lower than that in the control

TABLE 6

*Effect of the degree of substitution and cross-linking in hydroxypropyl distarch phosphate on the apparent absorption of calcium, magnesium, zinc and iron<sup>1,2</sup>*

Group <sup>3</sup>	Calcium	Magnesium	Zinc	Iron
	%			
Control	50.6 ± 1.9	62.1 ± 1.9	23.8 ± 2.5	27.3 ± 2.4
LS-LC	47.7 ± 3.0	61.5 ± 2.3	13.4 ± 3.7 <sup>b</sup>	16.3 ± 3.9 <sup>b*</sup>
LS-MC	51.6 ± 1.6	58.6 ± 1.1	19.0 ± 3.8 <sup>b</sup>	13.8 ± 2.5 <sup>b*</sup>
LS-HC	52.3 ± 1.7	62.9 ± 1.7	17.5 ± 2.3 <sup>b</sup>	10.5 ± 3.3 <sup>ab*</sup>
HS-LC	48.1 ± 1.4	67.1 ± 2.6	14.2 ± 2.3 <sup>b</sup>	7.4 ± 2.2 <sup>a*</sup>
HS-MC	53.4 ± 2.5	67.6 ± 1.7	5.4 ± 2.4 <sup>a*</sup>	7.9 ± 1.6 <sup>a*</sup>
HS-HC	50.0 ± 1.5	69.6 ± 1.1	5.2 ± 2.4 <sup>a*</sup>	6.9 ± 2.9 <sup>a*</sup>
ANOVA	NS	NS	DS	DS

<sup>1</sup> Each value represents the means ± SEM,  $n = 6$ . In each column, means with different superscript letters are significantly different between groups fed HDP ( $P < 0.05$ ). \* Means are significantly different ( $P < 0.05$ ) than means in the control group. NS,  $P > 0.05$ .

<sup>2</sup> The apparent absorption of calcium, magnesium, zinc and iron were calculated with the following formula: Apparent absorption (%) = (intake - fecal excretion) × 100/intake.

<sup>3</sup> For definitions, see Table 3.

rats, and that in the rats fed the HS-LC or HS-MC diet was slightly ( $P = 0.097$ ) but not significantly lower than that in the control rats. Because the experimental diets were not supplemented with cholesterol, the hypocholesterolemic effect of the HS diet must involve changes in endogenous sterol metabolism. One possibility is increased fecal excretion of cholesterol and bile acids. Bile acids may bind to HS in the small intestine, because starch has been shown to bind bile acids *in vitro* (31). The cecal contents and fecal excretion of bile acids in the rats fed the HS diet were significantly larger than the respective values in the rats fed the LS diet. By inhibiting the reabsorption of bile acids and enhancing fecal bile acid excretion, the HS may cause increased hepatic synthesis of bile acids, thereby reducing the serum cholesterol concentration. However, it is still unclear whether each type of HS can effectively bind bile acids. Another possible explanation concerns propionic acid production from fermentation of HS in the large bowel. When propionic acid was fed to rats, it significantly reduced the serum cholesterol concentration (32). Propionic acid infused into the cecum prevented the increase in plasma cholesterol concentration in rats fed a cholesterol-free casein diet (33). Furthermore, *in vitro* studies using rat liver cells have shown that propionic acid may attenuate hepatic cholesterol synthesis (34). On the other hand, other studies have shown that propionic acid has no hypocholesterolemic effects (35, 36). However, in the present study, the concentration of propionic acid in the cecal content was not affected by the diet. Further studies are needed to elucidate the mechanism of the hypocholesterolemic effect of HS.

A strong negative correlation has been found between the cecal pH and the cecal pool of SCFA (37). However, despite a similar cecal pool size of organic acids, the cecal pH in the rats fed the HS diet was lower than that in the control rats. Lactic acid is poorly and slowly absorbed (38, 39). Hoshi et al. (40) suggested that the higher concentration of cecal succinic acid predominantly appears to contribute to the lower cecal pH. However, in the present study, the concentration of lactic acid was not affected by the diet. Moreover, the concentration of succinic acid in the rats fed HS-HC diet was lower than that in the control rats.

A recent *in vitro* study examined carbohydrate fermentation in human fecal slurries and found that starch fermentation increased *n*-butyric acid production (41, 42). In the present study, the LS and HS diets did not increase the concentration of *n*-butyric acid compared with that in the control rats. Also, the LS and HS diets did not increase the concentration of propionic acid in comparison with that in the control rats. These are in agreement with the result of our previous study in rats fed HDP (DS = 0.01, 100 g/kg diet) from potato starch (19). The lower concentration of *n*-butyric acid can be explained by the cecal enlargement, because butyric acid is readily oxidized by cecal and colonic epithelial cells (43). Also, this lower concentration of *n*-butyric acid can be explained by the lower concentration of butyric acid-producing bacteria. The amylase-resistant starch in LS and HS may not be a good substrate for butyric acid-producing bacteria. The concentration of acetic, lactic or succinic acid in the rats fed the LS or HS diet was not higher than that in the control rats. However, in a previous study, we found that rats that were fed HDP (100 g/kg diet) with a molar substitution of 0.01 from potato starch had higher concentrations of acetic, lactic and succinic acids than the control rats fed potato starch (19). On the other hand, it has been reported that the pancreatin-indigested fractions in HDP are not good substrates for lactic acid-producing bacteria in *in vitro* bacterial fermentation

(30). The apparent digestibility of CMS and the structure of the maldigested fraction in feces after CMS administration depend on the type of modification (1). Therefore, the difference in the results between the present study and our previous study is due to the differences in the quality and quantity of HDP.

The apparent absorptions of Ca and Mg were not affected by the diet. Mineral absorption generally occurs in the upper part of the intestine. However, in rats, the large intestine has a high capacity for Mg and Ca absorption (44). Moreover, it has been reported that SCFA also contribute to enhance the absorption of Ca and Mg from the cecum (45–47). On the other hand, the apparent absorption of Zn in the rats fed the HS diet was significantly lower than that in the control rats, but the apparent absorption of Zn in the rats fed the LS diet was not. The apparent absorption of Fe in the rats fed the LS or HS diet was significantly lower than that in the control rats. Bruns and Hood (48) reported that HDP bound iron *in vitro*. Hood et al. (49) reported that feeding HDP resulted in marked depression in iron retention in iron-deficient rats.

These results show that the characteristics of amylase-resistant starch in HDP are similar to dietary fiber, with respect to increases fecal bulking and fecal excretion of bile acids, and change in pH of cecal content, decreases in absorption of Fe and Zn and an decrease in apparent protein digestibility. The dietary fiber-like effects of CMS would be affected by the type of modification. In fact, dietary fiber-like effects of HDP depend on the DS but not the DC.

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