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Effects of Raw Potato Starch with High Resistant Starch Levels on Cecal Fermentation Properties in Rats

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Summary The effects of potato starch, isolated from Snowden (SD) and Kitahime (KH) varieties, on cecal fermatation properties in rats were evaluated. In high-amylose cornstarch (HAS), SD and KH groups, cecal acetate and total short-chain fatty acid concentrations were increased and cecal pH was lowered compared to control (CON) group. Further, cecal immunoglobulin A levels were increased and cecal ammonia-nitrogen, *p*-cresol, skatole and indole concentrations were lowered in HAS, SD and KH groups compared to the CON group. Therefore, potato starch might possess beneficial intestinal fermentation properties.
Key Words immunoglobulin A, toxic metabolite, short-chain fatty acid, pH

Some starches resist small intestinal digestion and they are categorized as resistant starches (RS) (*1*). Similar to dietary fiber, RS also enter the large bowel, where it is fermented by microbiota. This leads to the formation of short-cain fatty acids (SCFA), lowering of colonic pH and stimulation of the beneficial bacteria proliferation (*1*). The fermentation process, resultant metabolites and modification of colonic environment, has been found to suppress the development of toxic metabolites (*2*) and to increase the secretion of immunoglobulin A (IgA) from lymphocytes (*3*, *4*). The toxic metabolites such as ammonia and *p*-cresol which are produced by microbial protein degradation in the colon are found to influence the incidence of colorectal cancers (*5*–*7*). IgA is known to improve the intestinal immune function through blocking luminal bacterial attachment to epithelial cells (*8*). Therefore, the proper regulation of the intestinal microbial composition and the fermentation is important for the host health.

RS are classified into 4 types based on its physical and chemical properties, where high-amylose cornstarch (HAS) is considered to be a RS type 2 (*9*). The consumption of HAS is widely reported to promote SCFA production and lower colonic pH (*10*, *11*). In addition, rats fed HAS diet showed lower concentration of colonic toxic metabolites (*12*, *13*) and higher luminal IgA expression (*3*, *11*). Hense, HAS is one of the major RS sources with well characterized prebiotic properties.

Potato, a major dietary source of starch is widely consumed as a staple food and its starch is being used as a raw material or additive in the processing industry. Raw potato starch is considerd to be a source of RS type 2 (*9*) and it is well known that its consumption promotes SCFA production and lowers pH in the colon (*14*, *15*). However, effect of potato starch consumption on stimulation of IgA secretion and toxic metabolites production is less well appreciated. Therefore, the effects of raw potato starch on intestinal fermentation properties including toxic metabolites production and IgA secretion in rats were evaluated in the present study.

Materials and Methods

Materials. Starch isolated from two varieties of potato, Snowden (SD) and Kitahime (KH) were used in this study along with HAS (HS-7, J-Oil Mills, Tokyo, Japan) as the positive control. Potato starch was isolated as previously described (*16*).

RS and phosphorus content. RS content in HAS and isolated potato starches was determined by AOAC 2002. 02. Phosphorus contents in the starch isolated from SD and KH were reported to be 695 ppm and 852 ppm, respectively (*16*).

Animals and diets. The experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine (approval no. 28–87). Twenty male Fischer 344 rats (7-wk-old) were purchased from Charles River Laboratories (Yokohama, Japan). The rats were treated and maintained according to the "Guide for the Care and Use of Laboratory Animals". After a 7 d acclimation period, the rats were randomly assigned to four groups and fed one of the experimental diets (Oriental Yeast Co., Ltd., Tokyo, Japan) formulated based on the AIN-93G diet (Table 1) for 4 wk. Care and dissection of the rats were conducted as previously described (*17*).

Cecal SCFA analysis. The cecal SCFA concentrations in the rat cecal content were determined using high-performance liquid chromatography (HPLC; LC-10AD, Shimadzu, Kyoto, Japan). Samples for HPLC

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Table 1. Composition of experimental diets.

Ingredients (g/kg diet)	CON	HAS	SD	KH
Casein	200	200	200	200
L-Cystine	3	3	3	3
Sucrose	100	100	100	100
Soybean oil	70	70	70	70
t-Butylhydroquinone	0.014	0.014	0.014	0.014
Cellulose	50	50	50	50
Mineral mixture[1]	35	35	35	35
Vitamin mixture[1]	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
Cornstarch	300	—	—	—
HAS	—	300	—	—
SD	—	—	300	—
KH	—	—	—	300
α-Cornstarch	229.486	229.486	229.486	229.486

[1] According to AIN-93G.
CON, control; HAS, high-amylose cornstarch; SD, Snowden; KH, Kitahime.

Table 2. Feed intake, body weight and cecal parameters in the rats fed specific diets for 4 wk.

	CON	HAS	SD	KH
Feed intake (g/4 wk)	392±7[a]	338±8[b]	389±6[a]	401±14[a]
Final body weight (g)	238±2[a]	218±7[b]	229±4[ab]	226±4[ab]
Cecal content				
Weight (g)	2.37±0.10[b]	10.6±1.8[a]	13.3±1.6[a]	13.8±1.2[a]
pH	7.67±0.06[a]	5.81±0.10[b]	5.61±0.10[b]	5.86±0.11[b]
SCFA (μmol/g content)				
Acetate	92.9±9.2[c]	157±19[b]	216±14[a]	163±9[ab]
Propionate	16.9±1.8	9.32±3.27	7.87±2.63	11.4±2.7
n-Butyrate	2.83±0.31[b]	21.4±7.4[a]	6.47±1.89[ab]	9.82±2.13[ab]
Total	113±10[b]	188±24[a]	230±15[a]	184±9[a]
IgA (mg/g content)	0.14±0.05[b]	0.39±0.02[a]	0.26±0.01[ab]	0.31±0.04[a]
Toxic metabolite (μg/g content)				
Ammonia-nitrogen	669±57[a]	447±68[b]	331±32[b]	425±42[b]
p-Cresol	317±40[a]	120±21[b]	82.7±13.4[b]	81.7±5.1[b]
Skatole	321±23[a]	79.3±17.9[b]	52.3±7.6[b]	45.6±4.5[b]
Indole	163±22[a]	75.0±18.0[b]	42.1±3.5[b]	64.9±13.0[b]

Data are expressed as mean±SE, $n=5$. [a-c] Mean values within a row with unlike superscript letters are significantly different ($p<0.05$), as determined by ANOVA paired with Tukey's test. CON, control; HAS, high-amylose cornstarch; SD, Snowden; KH, Kitahime.

were prepared as previously described (*18*). The analytical conditions were as follows: column, RSpak KC-811 (8.0 mm×300 mm, Shodex, Tokyo, Japan); eluent and flow rate, 2 mM perchloric acid at 1.0 mL/min; column temperature, 47°C; reaction reagent and flow rate, ST3-R (10×diluted, Shodex) at 0.5 mL/min; UV detector wavelength, 430 nm.

Cecal IgA analysis. The IgA levels in the rat cecal content suspensions were determined using a rat IgA ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's instructions.

Cecal ammonia-nitrogen analysis. The ammonia-nitrogen concentration in the rat cecal content suspensions was determined using a commercially available kit (Wako Pure Chemical Industry, Ltd., Osaka, Japan) according to the manufacturer's instructions.

Cecal p-cresol, skatole and indole analysis. The cecal *p*-cresol, skatole and indole concentrations in the cecal content were determined using HPLC (LC-10AD, Shimadzu) and samples for HPLC were prepared as previously described (*19*).

Statistical analysis. Significant differences amongst the 4 groups were determined by analysis of variance (ANOVA) paired with Tukey's test (SPSS version 17, IBM Corporation, Armonk, NY, USA). Correlations between the parameters were assessed using Pearson's correlation analysis. A p value less than 0.05 was considered as statistically significant.

Results

RS content

RS contents in HAS, SD and KH starches were 35.2%, 54.3% and 61.8% respectively.

Feed intake and body weight

Feed intake was significantly lower ($p<0.05$) in the rats fed HAS diet than the rats fed CON, SD and KH diets, while final body weight was lower ($p<0.05$) in the rats fed HAS diet than the rats fed CON diet (Table 2).

Cecal parameters

The cecal contet weight was significantly higher ($p<0.05$) in the rats fed HAS, SD and KH diets than the rats fed CON diet (Table 2). The cecal pH was significantly lower ($p<0.05$) in the rats fed HAS, SD and KH diets than the rats fed CON diet (Table 2).

The cecal acetate and total-SCFA concentrations were significantly higher ($p<0.05$) in the rats fed HAS, SD and KH diets than the rats fed CON diet (Table 2), and the cecal acetate concentration in the SD group was significantly higher compared to the HAS group. The cecal propionate concentration amongst the groups was not significantly different ($p>0.05$), and the cecal *n*-butyrate concentration was significantly higher ($p<0.05$) in the rats fed HAS than the rats fed CON diet (Table 2).

The cecal IgA levels were significantly higher ($p<0.05$) in the rats fed HAS and KH diets than the rats fed CON diet (Table 2). Its levels in the rats fed SD diet tended to be higher ($p=0.096$) compared to the CON group but not significant.

The cecal ammonia-nitrogen, *p*-cresol, skatole and indole concentrations were significantly lower ($p<0.05$) in rats fed HAS, SD and KH diets than the rats fed CON diet (Table 2).

Discussion

In the current study, the effect of raw potato stach on the intestinal fermentation properties in rats were examined. HAS and potato starch isolated from SD and KH contained high RS levels, and the cecal content weights in these groups were higher similar to previous studies (*14*, *15*). Further, it has been reported that starch with lower digestibility increases the amount of digesta in the distal gut, and a large volume of digesta is known to provide a favourable environment for microbial growth in the distal gut by providing nutrients to gut bacteria (*3*, *20*). Therefore, a large amount of non-digestible materials in SD and KH diets might have increased the cecal digesta volume.

Consumption of HAS and potato starch diets increased acetate and total-SCFA productions and lowered pH in rat cecum similar to previous studies (*14*, *15*). However, cecal propionate concentration was not different among the groups in the current study. The exact reason is unclear, but fermentable carbohydrates are known to alter the intestinal microbial composition and subsequently impact the amount of intestinal fermentation metabolites (*3*, *21*). Further, the microbial propionate production pathway is different from acetate and butyrate pathways (*22*). Therefore, cecal microbial composition in all groups should be determined in order to understand the underlying mechanisms.

The cecal IgA levels were increased in HAS, SD and KH groups, further, Pearson's correlation analysis also showed positive corralations with the cecal acetate ($r=0.435$; $p<0.10$), *n*-butyrate ($r=0.554$; $p<0.05$) and total-SCFA ($r=0.478$; $p<0.05$) concentrations and a negative correlation with the cecal pH ($r=-0.677$; $p<0.01$) for the cecal IgA levels. It was previously reported that the IgA secretion in cecum was increased in rats fed HAS diet accompanied with the increased cecal SCFA production (*3*). Moreover, cecal IgA levels were increased in rats with lower cecal pH (*4*). Therefore, in the current study, secretion of cecal IgA in potato starch groups might have been stimulated by the increased cecal SCFA and lowered cecal pH.

Cecal toxic metabolite concentrations were lowered in HAS, SD and KH groups. Ammonia, *p*-cresol, skatole and indole in colon, are formed following the deamination and α or β elimination reactions by bacterial activities (*5*, *23*). Deamination occurs mainly at neutral or even alkaline pH conditions (*24*). Several previous in vitro studies reported that ammonia-nitrogen concentration was reduced in HAS group with increased SCFA production and lowering of pH (*25*), further, *p*-cresol and indole concentrations were also found to be reduced at lower pH (*26*). Moreover, similar observations were reported in rats fed HAS diet, in agreement with the increased SCFA content and lower pH (*12*, *13*). Therefore, the cecal toxic metabolite productions in potato starch groups might have been suppressed by the lower cecal pH caused by higher SCFA production in the current study.

In conclusion, our observations suggested that the consumption of potato starch could stimulate IgA secretion and suppress toxic metabolite productions, as a result of increased cecal SCFA production and lowering of cecal pH similar to HAS. Therefore, potato starch might have possessed favorable physicochemical properties, which might have influenced intestinal fermentation and the subsequent improvement of the intestinal environment.

Disclosure of State of COI

No conflicts of interest to be declared.

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