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Hypolipidaemic effect of maize starch with different amylose content in ovariectomized rats depends on intake amount of resistant starch

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The effect of amylose content on digestibility of starch in the small intestine and on the concentration of plasma lipid were studied in ileorectostomized rats and in ovariectomized rats, respectively. Seven kinds of starch with different amylose content (0, 27, 54, 62, 76, 79, 86 %) were used as test starch, which contained 0·4, 5·6, 37·1, 40·2, 45·6, 36·9 and 36·1 % resistant starch (RS), respectively. Rats were fed one of test diets containing 30 % test starch with different amylose content for 14 d in ileorectostomized and for 21 d in ovariectomized rats. Food intake was not significantly different among the groups. In ileorectostomized rats, the small intestinal starch digestibility decreased with increasing intakes of amylose and RS. In ovariectomized rats, body weight gain was lower on the higher amylose maize starch diets. The concentrations of plasma TAG and cholesterol decreased with increasing intake of RS. The concentrations of liver total lipids and TAG decreased with increasing intake of RS, but that of liver cholesterol did not. There was significant positive correlation between the level of sterol regulatory element-binding protein-1c mRNA and concentration of liver TAG. Total SCFA amount in the caecum increased logarithmically with increasing dry weight of caecal contents. The amount of bile acids in the small intestinal content and the excretions of bile acids and neutral steroids in faeces increased with increasing RS intake. These results show that starch rich in RS is more effective in preventing ovarian hormone deficiency-induced hyperlipidaemia.

Amylose content: Resistant starch: Ovariectomized rats: Plasma lipids: Bile acids

It is well known that lipid metabolism is influenced by sex hormones in animals and man[1,2]. Sex hormones such as oestrogen have a major impact on atherosclerotic processes. Oestrogen deficiency is associated with changes in cholesterol levels. Studies in animal models have shown that oestrogen inhibits the development of atherosclerotic lesions[3,4].

Starch consists of two types of molecule, amylose and amylopectin. The amylose content is an important factor that determines the digestibility of starch. Starches with higher amylose content are found to be more resistant to digestion. The amylose content of starches is thus the major cause of resistant starch (RS) formation.

High amylose maize starch (HAMS) has been reported to reduce plasma cholesterol concentrations in rats[5–8]. The physico-chemical properties of HAMS are affected by RS content, which are expected to influence its physiological effects. HAMS is genetic varieties of starch containing over 40 % amylose. However, it is not known if differences in the RS content in HAMS affects plasma cholesterol concentration in oestrogen-deficient rats. In addition, there have been few systematic studies of one kind of starch with different amylose/amylopectin contents.

Therefore, in the present study, we compared the influence of maize starch with different amylose content on the change in lipid metabolism associated with oestrogen deficiency in ovariectomized rats.

Materials and methods

Test starches

Waxy maize starch (AL-0), normal maize starch (AL-27) and five kinds of HAMS (AL-54, AL-62, AL-76, AL-79, AL-86) were donated from the National Starch and Chemical Co. (Chicago, IL, USA). Amylose content was measured using commercial kits (Amylose/Amylopectin Assay Kit; Biocon Japan Ltd, Nagoya, Japan). RS content was measured using commercial kits (Resistant Starch Assay Kit; Biocon Japan Ltd). Amylose content of AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 and AL-86 was 0, 26·8, 53·8, 61·6, 75·7, 78·9 and 85·8 g/100 g, respectively. The RS content of AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 and AL-86 was 0·4, 5·6, 37·1, 40·2, 45·6, 36·9 and 36·1 g/100 g, respectively.

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, cholesterol 12α-hydroxylase; HAMS, high amylose maize starch; RS, resistant starch; SREBP, sterol regulatory element-binding protein; total-C, total cholesterol.

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Animals and diets

The present 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.

Wistar rats (Japan SLC, Hamamatsu, Japan) were housed individually in screen-bottomed, stainless steel cages in a room maintained at 23 ± 1°C with a 12 h light–dark cycle (light 07.00–19.00 hours). In the experiment, rats were allowed free access to one of the following seven diets: AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 or AL-86 (Table 1). Body weight and food intake were recorded daily in the morning before replacing the food.

Experiment 1. Starch and RS digestibilities were examined in ileorectostomized rats. After acclimation to the AIN93G-based diet without cellulose for 7 d, Wistar female rats weighing about 200–230 g were subjected to ileorectostomy in which the distal ileum is anastomosed to the rectum, described previously[9]. Rats subjected to the operation were not allowed food and water for the first 24 h after operation, and were intramuscularly injected with 0·01 ml Mycillin Sol (containing procaine penicillin G (200 g/l) and dihydrostreptomucin sulphate (250 g/l); Toyo Jozo, Shizuoka, Japan) for the first 3 d after surgery. Then, rats were freely fed the AIN93G-based diet without cellulose for 10 d. Constant growth rates (5–7 g body weight gain/d) was achieved with this diet after 5 d. After postoperative recovery, rats (250–300 g) were divided into six groups (n 6) on the basis of body weight. Rats were allowed free access to one of the following six diets for 14 d: AL-27, AL-54, AL-62, AL-76, AL-79 or AL-86 (Table 1). Ileorectostomy effluents were collected for the last 4 d of the experimental period, freeze-dried and stored at −50°C until analysis. Starch and RS digestibility was calculated as follows:

$$\text{Starch digestibility (\%)} = \frac{\text{starch intake} - \text{starch in ileorectostomy effluents}}{\text{starch intake}} \times 100.$$

$$\text{RS digestibility (\%)} = \frac{\text{RS intake} - \text{RS in ileorectostomy effluents}}{\text{RS intake}} \times 100.$$

Starch and RS intakes were calculated from the food intake for the last 4 d of the experimental period, and from starch and RS content in each diet. The starch and RS contents in the diets and ileorectostomy effluents were determined using the commercial kits (Total Starch Assay Kit and Resistant Starch Assay Kit).

Experiment 2. Six-month-old female Wistar rats were acclimated by feeding a commercial solid diet (Roden Lab Diet EQ; PMI, USA) for 7 d. After acclimation, rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg body; Nembutal, Abbott Laboratories, Chicago, IL, USA), and bilaterally ovariectomized, after which they were randomly divided into seven groups (n 6), and were allowed free access to one of the following diets for 21 d: AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 or AL-86 (Table 1). Before the animals were killed, faeces were collected from each rat on the final 4 d of the experimental period. The faeces were freeze-dried, weighed and milled.

On the last day of the experiment, a blood sample was collected from the neck of each rat at night into a blood collection tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA) that contained heparin as an anticoagulant. The plasma was separated by centrifugation at 1400 ***g*** at 4°C for 15 min, and was stored at −50°C until analysis. After blood collection, the liver was immediately perfused with cold saline (9 g NaCl/l), removed, washed with cold saline, blotted dry on filter paper, weighed and stored at −50°C until analysis. After the liver was removed, the small intestine and caecum were removed. The contents of the small intestine were transferred into a pre-weighed tube, freeze-dried and weighed. The caecum was weighed, then 0·4 g of the caecal contents were transferred into a tube and 2 ml 10 mmol sodium hydroxide/l was immediately added and the mixture

Table 1. Composition of diets (g/kg)

	Diet						
	AL-0	AL-27	AL-54	AL-62	AL-76	AL-79	AL-86
Casein	200	200	200	200	200	200	200
Maize oil	70	70	70	70	70	70	70
Mineral mixture*	35	35	35	35	35	35	35
Vitamin mixture*†	10	10	10	10	10	10	10
Cellulose‡	50	50	50	50	50	50	50
L-Lysine	3	3	3	3	3	3	3
Maize starch							
AL-0	632	332	332	332	332	332	332
AL-27		300					
AL-54			300				
AL-62				300			
AL-76					300		
AL-79						300	
AL-86							300
Amylose content§	0	80·4	161·4	184·8	227·1	236·7	257·4
Resistant starch content‖	2·5	18·1	112·6	121·9	138·1	112·0	109·6

* Based on AIN93G[63].
† The vitamin mixture contained 20 g choline bitartrate/100 g.
‡ Cellulose powder, PC200 (Danisco Japan Ltd, Tokyo, Japan).
§ Content of AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 and AL-86 was 0, 26·8, 53·8, 61·6, 75·7, 78·9 and 85·8 g/100 g, respectively.
‖ Content of AL-0, AL-27, AL-54, AL-62, AL-76, AL-79 and AL-86 was 0·4, 5·6, 37·1, 40·2, 45·6, 36·9 and 36·1 g/100 g, respectively.

was used for SCFA analysis; an aqueous solution containing 0·5 g crotonic acid/l was used as an internal standard. The moisture level of the caecal contents was determined as the difference between the wet mass and the dry mass of the caecal contents after freeze-drying. The caecal wall was flushed with ice-cold saline (9 g NaCl/l, 4°C), blotted on to filter paper and weighed.

Estimation of energy intake

Energy from available starch, casein and maize oil was estimated to be 16·7 kJ/g (4 kcal/g), 16·7 kJ/g (4 kcal/g) and 37·6 kJ/g (9 kcal/g), respectively. Part of the ingested maize starch that travels to the large intestine without being digested is utilized as a fermentation substrate by microflora, and is converted into various organic acids such as SCFA. The energy produced by fermentation of unavailable maize starch is estimated to be 7·1 kJ/g (1·7 kcal/g)[10]. However, the digestibility of AL-0 in the small intestine and in the large intestine was assumed to be 100 and 0 %, respectively. Energy intake in the experiment period was estimated as follows:

$$\text{Energy intake (kJ/3 weeks)} = \text{P} + \text{F} + \text{DS} + \text{UDS},$$

where P is the energy from casein = casein intake (g/3 weeks) × 16·7 kJ; F is the energy from maize oil = Maize oil intake (g/3 weeks) × 37·6 kJ; DS is the energy from starch digested in the small intestine = Starch intake (g/3 weeks) × (Starch digestibility in Expt 1)/100 × 16·7 kJ; UDS is the energy from starch digested in the large intestine = Starch intake (g)/3 weeks × (Starch digestibility in Expt 2 − Starch digestibility in Expt 1)/100 × 7·1 kJ.

Biochemical analysis

The concentrations of total-cholesterol (total-C), HDL-cholesterol and TAG in the plasma were determined enzymatically using commercial diagnostic kits (Cholesterol E-Test Wako, HDL Cholesterol Test Wako and Triglyceride E-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). The concentration of non-HDL-cholesterol was calculated by subtracting the concentration of HDL-cholesterol concentration from the total-C concentration.

The level of liver total lipids was determined gravimetrically after extraction by the method of Folch *et al.* [11]. The liver TAG and cholesterol concentrations were determined enzymatically as described elsewhere[12]. Steroids were extracted from the digestive contents (small intestine and caecum) and faeces by a mixture of chloroform–methanol (1:1, v/v) at 70°C for 60 h[13]. The concentrations of bile acids in caecal contents and faeces were determined enzymatically by the 3α-dehydrogenase assay method of Sheltaway & Losowsky[14] using taurocholic acid as standard. The concentrations of cholesterol and coprostanol in small intestinal contents and faeces were analysed by capillary GLC (Model HP5890A; Hewlett Packard, Palo Alto, CA, USA) equipped with a flame-ionization detector and a capillary column (30 m × 0·53 mm inner diameter) coated with DB-1 (J&W Scientific, Folsom, CA, USA)[15]. The oven temperature was 260°C and the flow rate of helium carrier gas was 16·9 ml/min. 5α-Cholestane (Nacalai Tesque Inc., Kyoto, Japan) was used as the initial standard for neutral sterol analysis. The concentration and composition of bile acid in the small intestinal contents was analysed by capillary GLC (Model HP5890A, Hewlett Packard) equipped with a flame-ionization detector and a capillary column (30 m × 0·25 mm inner diameter) coated with DB-210 (J&W Scientific)[16]. The oven temperature was programmed to increase from 60 to 235°C at rate of 10°C/min and the flow rate of helium carrier gas was 1·5 ml/min. Nordeoxycholic acid (Steraloid Inc., Wilton, NH, USA) was used as the initial standard for bile acid analysis. Standard bile acids, cholic acid (CA), deoxycholic acid, 12-oxo-chenodeoxycholic acid, 12-oxo-lithocholic acid, chenodeoxycholic acid (CDCA), α-muricholic acid, β-muricholic acid, ω-muricholic acid, lithocholic acid, hyodeoxycholic acid and ursodeoxycholic acid were purchased from Steraloid Inc. The levels of caecal organic acids were measured using HPLC (LC-6AL; Shimadzu, Kyoto, Japan) by the internal standard method[17].

RNA extraction from the liver and RT-PCR analysis of gene expression

Total RNA was extracted from frozen livers according to the method described by Chomczynski & Sacchi[18]. RNA integrity was verified by agarose gel electrophoresis using Oligotex-dT30 (Takara Bio, Shiga, Japan). mRNA (1 μg) was used for cDNA synthesis with 10 U RT (AMV; Takara Bio) and 2 μl oligo (dT) primer (Novagen Inc., Madison, WI, USA) according to manufacturers' instruction. Messenger RNA expressions of acyl-CoA cholesterol acyltransferase 1, acyl-CoA cholesterol acyltransferase 2, apoB, cholesterol 7α-hydroxylase (CYP7A1), cholesterol 27-hydroxylase, cholesterol 12α-hydroxylase (CYP8B1), farnesoid X receptor, hydroxymethylglutaryl-CoA reductase, LDL-receptor, liver X receptor, retinoid X receptor, microsomal TAG transfer protein, sterol regulatory element-binding protein (SREBP)-1a, SREPB-1c, SREBP-2 and β-actin, as a housekeeping gene for normalization, were determined by real-time monitoring of a PCR using a Light Cycler instrument (Roche Diagnostics, Mannheim, Germany). cDNA (2 μl) was amplified in a total volume of 20 μl using the 2 × QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany) and specific primers at 0·5 M each. After initial denaturation and activation of the polymerase at 95°C for 15 min, cycling was performed for fifty cycles with annealing at the temperatures shown in Table 2 for 25 s, synthesis at 72°C for 30 s and denaturation at 94°C for 15 s. Fluorescence was measured at the end of the elongation step at 72°C. The sequences of the gene-specific primers (Carl Roth, Karlsruhe, Germany) used in the study are listed in Table 2.

Statistical analyses

Data are expressed as means and standard deviations (n 6). Data were analysed by one-way ANOVA using the Super ANOVA statistical software package (Abacus Concepts, Berkeley, CA, USA), and the differences among groups were examined by Tukey's multiple range test using Super ANOVA when the F value was significant. $P<0·05$ was considered significant.

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Table 2. Primer sequence, product size and annealing temperature

Gene	Primer sequence		Product size (bp)	Annealing temperature (°C)
	Sense	Anti-sense		
Acyl-CoA cholesterol acyltransferase 1 (ACAT1)	ATGTGGGAAGTAAATGAAGC	AAATACTAGCCAGACCGAAT	193	53
Acyl-CoA cholesterol acyltransferase 2 (ACAT2)	CGGTCATGCTGATCCTCTTT	GTGTCACCAGCTCCCAAAAT	204	55
apoB	TTGACACACTGAAGTTCCTA	ACATCAAACCCTGGTATTAG	124	60
Cholesterol 12α-hydroxylase (CYP8B1)	ATGAAGGCTGTGCGAGAG	TCTCTTCCATCACGCTGTC	127	59
Cholesterol 27-hydroxylase (CYP27)	CTCAAGAGACTGTCGGCAC	CTTCCTGGATCTCTGGGTT	114	57
Farnesoid X receptor (FXR)	CGTTCAGCGGAGATTTTCAA	ATTTTCAGCTCCCCGACACT	140	57
Hydroxamethylglutaryl-CoA reductase (HMG-CoA R)	GCTGGTGAGTTGTCCTTGAT	CTTCTTGGTGCATGTTCCCT	117	60
LDL-receptor (LDL-R)	CTTGCCCTGATGGTATGCTA	CTTGCGCTTCAGTGACACA	137	60
Liver X receptor (LXR)	TGCTAATGAAGCTGGTGA	AGAGGACACGGAGAAACAGT	144	57
Microsomal TAG transfer protein (MTP)	AGCGACATCACAGTGGACTC	GGCTCTCTTTTCTTCTCCGA	223	62
Retinoid X receptor (RXR)	CAAACATGGGGCTGAACC	AGCAGGATGACCTGGTCGT	148	57
Sterol regulatory element binding protein (SERBP-1a)	CCGAGATGTGCGAACTGGAC	TGTCTCCGTTCTCACCCCCA	129	55
Sterol regulatory element binding protein (SERBP-1c)	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAGGA	191	53
Sterol regulatory element binding protein (SERBP-2)	CACAATATCATTGAAAAGCGCTACG	TTTTTCTGATTGGCCAGCTTCAGCA	200	55
β-Actin	CTATGAGCTGCCTGACGGTC	AGTTTCATGGATGCACAGG	115	53

Results

Experiment 1

Faecal dry weight in rats fed the AL-27 diet was significantly lower than those in rats fed other test diets. The digestibility of starch and RS in the small intestine was as follows: AL-27 ⩾ AL-54 > AL-79, AL-62 > AL-86 > AL-76. The digestibility of starch in the small intestine decreased with increasing intakes of amylose and RS (Fig. 1; Table 3).

Experiment 2

Body weight gain in rats fed the AL-27 diet was significantly higher than that in rats fed the AL-62 diet, and tended to be higher than rats fed the AL-0, AL-54, AL-76, AL-79 and AL-86 diets (Table 4). Food intake was not affected by diet. Apparent starch digestibility was as follows: AL-0, AL-27 > AL-54, AL-62, AL-79, AL-86 > AL-76. Apparent starch digestibility and body weight gain decreased and tended to decrease with increasing RS intake (r −0·795, P=0·0327; r −0·748, P=0·0533). Plasma total-C concentration was the lowest in rats fed the AL-79 diet, and in rats fed the AL-62, AL-76 and AL-86 diets it was significantly lower than in rats fed AL-27. The concentration of plasma total-C decreased with increasing RS intake (r −0·835, P=0·0193). Plasma TAG concentrations in rats fed the AL-62 and AL-79 diets were significantly lower than those in rats fed the AL-0 and AL-27 diets, but those in rats fed the AL-54, AL-62 and AL-86 diets were not. Liver weight was as parallel as body weight gain. The concentration of liver total lipids in rats fed the AL-76 diet was significantly lower than that in rats fed the AL-27 diet, but those in rats

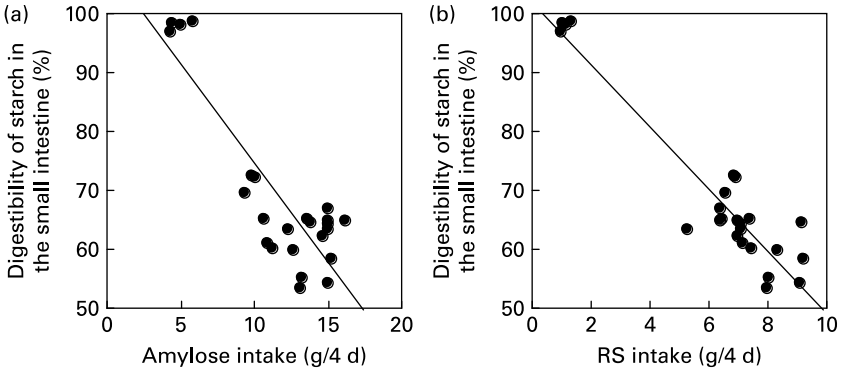

Fig. 1. Correlations between (a) digestibility of starch in the small intestine and amylose intake (r − 0·921, P=0·009) and (b) digestibility of starch in the small intestine and resistant starch (RS) intake (r − 0·970, P=0·001).

Table 3. Digestibilities of starch and resistant starch (RS) in the small intestine*
(Mean values and standard deviations)

	Dietary groups											
	AL-27		AL-54		AL-62		AL-76		AL-79		AL-86	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g/14 d)	33	4	29	4	29	4	26	6	28	4	25	9
Food intake (g/14 d)	205	14	212	16	218	208	208	11	210	11	204	6
Food intake (g/last 4 d)												
Total	59·7	7·8	61·1	2·7	65·3	6·5	61·8	4·2	61·2	2·4	56·5	5·5
RS	1·1	0·1	6·9	0·3	8·0	0·8	8·5	0·6	6·9	0·3	6·2	0·6
Dry weight of ileorectostomy effluents (g/last 4 d)	6·0^{a}	0·6	11·7^{b}	1·4	13·9^{b}	2·0	13·8^{b}	1·0	12·8^{b}	0·9	11·8^{b}	0·7
Starch digestibility (%)†	98·2^{d}	0·6	70·3^{c}	3·0	61·8^{b}	2·4	55·5^{a}	1·9	63·8^{b}	1·1	65·3^{bc}	2·8
RS digestibility (%)‡	80·2^{a}	10·6	27·5^{c}	8·3	11·1^{b}	3·0	6·0^{a}	1·0	11·4^{b}	2·1	9·5^{ab}	2·0

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see the Materials and methods section and Table 1. Rats were fed one of the test diets for 28 d (n 6).
† (Starch intake − starch in ileorectostomy effluents)/starch intake.
‡ (RS intake − RS in ileorectostomy effluents)/RS intake.

fed the AL-54, AL-62, AL79 and AL-86 diets were not. The concentration of liver total lipids decreased with increasing RS intake (r −0·845, P=0·0167). The concentrations of liver total-C and TAG were not affected by diet.

The dry weights of small intestinal content in rats fed the AL-54, AL-62 and AL-76 diets were significantly heavier than those in rats fed the AL-0 and AL-27 diets, but those in rats fed the AL-79 and AL-86 diets were not (Table 5). The dry weight of small intestinal contents exponentially increased with increasing RS intake (r 0·949, P=0·0011). The amounts of bile acids in small intestinal contents in rats fed the AL-54, AL-62, AL-76, AL-79 and AL-86 diets were significantly higher than in rats fed the AL-0 diet, but not in rats fed the AL-27 diet. The amount of bile acids in small intestinal contents increased as the intake of RS increased (r 0·910, P=0·0044).

The ratios of the CA group/CDCA group in rats fed the AL-62, AL-76, AL-79 and AL-86 diets were significantly higher than those in rats fed the AL-0 and AL-27 diets.

The CA group/CDCA group ratio of bile acids in the small intestinal contents increased with increasing RS intake (r 0·840, P=0·0180). The CA group/CDCA group ratio for bile acids in the small intestinal contents increased with increasing level of CYP8B1 mRNA (r 0·879, P=0·0091).

The caecal wall weights in rats fed the AL-62 and AL-76 diets were significantly heavier than those in rats fed the AL-0, AL-27 and AL-86 diets (Table 6). That in rats fed the AL-79 diet was significantly heavier than those in rats fed the AL-0 and AL-86 diets. The moisture of caecal content was not affected by diet. The dry weights of caecal content in rats fed the AL-54, AL-62, AL-76, AL-79 and AL-86 diets were significantly heavier than those in rats fed the AL-0 and AL-27 diet. The amounts of bile acids in caecal content were significantly higher in rats fed the AL-54, AL-62 and AL-76 diets than in rats fed the AL-0 and AL-27 diets.

The amount of total SCFA in caecal contents increased logarithmically with increasing dry weight of caecal contents (r 0·908, P=0·0047). The amount of succinic acid in caecal contents was higher in rats fed the AL-76 diet than in rats fed other diets. The amounts of lactic acid in caecal content in rats fed the AL-62, AL-76 and AL-79 diets were significantly higher than those in rats fed the AL-0 and AL-27 diets, but those in rats fed the AL-54 and AL-86 diets were not. The amounts of acetic acid in caecal content in rats fed the AL-54, AL-62, AL-76, AL-79 and AL-86 diets were significantly higher than those in rats fed the AL-0 and AL-27 diets, and that in rats fed the AL-76 diet was significantly higher than that in rats fed the AL-86 diet. The amounts of propionic and n-butyric acid in caecal content were significantly higher in rats fed the AL-54, AL-62, AL-76 and AL-86 diets than in rats fed the AL-0 and AL-27 diets, but was not significantly higher in rats fed the AL-79 diet than in rats fed the AL-27 diet. The amount of succinic acid + lactic acid in caecal contents exponentially increased with increasing RS intake (P=0·0003) and the dry weight of caecal contents (r 0·997, $P<0·0001$).

Dry weight of faeces extracted per day was not affected by diet. Bile acids extracted in faeces per day was as follows: AL-76, AL-79, AL-86 > AL-54, AL-62 > AL-0, AL-27 dietary group (Table 6). Faecal excretion of bile acids increased with increasing RS intakes (r 0·866, P=0·0117). The amounts of coprostanol and total neutral sterol were significantly higher in rats fed the AL-62, AL-76, AL-79 and AL-86 diets than in rats fed the AL-0 and AL-27 diets, but that in rats fed the AL-54 diet was not.

The mRNA levels of farnesoid X receptor and hydroxymethylglutaryl-CoA reductase were not affected by diet (Table 7). The mRNA levels of acyl-CoA cholesterol acyltransferase 2, apoB, cholesterol 27-hydroxylase, liver X receptor, microsomal TAG transfer protein and SREBP-1a were also not affected by diet (data not shown). The mRNA level of acyl-CoA cholesterol acyltransferase 1 in rats fed the AL-0 diet was significantly higher than those in rats fed the AL-76, AL-79 and AL-86 diets. The mRNA level of CYP7A1 in rats fed the AL-65 diet was significantly higher than those in rats fed the AL-0 and AL-27 diets, but those in rats fed the AL-54, AL-62, AL-79 and AL86 diets were not. The expression of CYP7A1 mRNA increased exponentially as the intake of RS increased (r 0·811, P=0·0267). The mRNA levels of CYP8B1 in rats fed the AL-76 and AL-79 diets were significantly higher in rats fed the AL-0 and AL-27 diets, but those in rats fed the AL-54 and AL-62

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Table 4. Effects of amylose contents in maize starch on body weight, body weight gain, food intake and food efficiency in ovariectomized rats*

(Mean values and standard deviations)

	Dietary groups													
	AL-0		AL-27		AL-54		AL-62		AL-76		AL-79		AL-86	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g/3 weeks)	30^{ab}	5	35^{b}	5	27^{ab}	11	20^{a}	7	27^{ab}	13	23^{ab}	5	25^{ab}	7
Food intake (g/3 weeks)														
Total	302	13	329	65	300	34	271	15	280	41	287	12	275	20
Amylose	0	0	26	5	48	5	50	3	64	9	68	3	71	5
Resistant starch	1	0	6	1	34	4	33	2	40	2	31	5	30	2
Apparent starch digestibility (%)	$96{\cdot}9^{cd}$	0·1	$97{\cdot}3^{d}$	0·3	$96{\cdot}0^{bcd}$	0·6	$95{\cdot}1^{b}$	1·2	$92{\cdot}8^{a}$	1·5	$95{\cdot}5^{bc}$	0·6	$95{\cdot}0^{b}$	0·9
Apparent energy intake (kJ/3 weeks)†	4999^{ab}	215	5444^{b}	1076	4773^{ab}	541	3792^{a}	210	3785^{a}	554	4054^{a}	170	3898^{a}	283
Plasma lipids (mmol/l)														
Cholesterol														
Total	$3{\cdot}46^{bc}$	0·30	$3{\cdot}84^{c}$	0·54	$3{\cdot}21^{bc}$	0·32	$3{\cdot}05^{ab}$	0·53	$2{\cdot}88^{a}$	0·19	$2{\cdot}45^{a}$	0·19	$2{\cdot}88^{ab}$	0·19
HDL	$2{\cdot}58^{bc}$	0·18	$2{\cdot}67^{c}$	0·32	$2{\cdot}16^{ab}$	0·25	$2{\cdot}12^{a}$	0·30	$2{\cdot}03^{a}$	0·28	$1{\cdot}79^{a}$	0·16	$2{\cdot}00^{ab}$	0·19
Non-HDL‡	$0{\cdot}88^{ab}$	0·26	$1{\cdot}17^{b}$	0·31	$1{\cdot}05^{ab}$	0·16	$0{\cdot}93^{ab}$	0·29	$0{\cdot}85^{ab}$	0·23	$0{\cdot}66^{a}$	0·16	$0{\cdot}81^{ab}$	0·26
TAG	$1{\cdot}21^{b}$	0·41	$1{\cdot}20^{b}$	0·49	$0{\cdot}70^{ab}$	0·21	$0{\cdot}57^{a}$	0·23	$0{\cdot}84^{ab}$	0·26	$0{\cdot}55^{a}$	0·16	$1{\cdot}03^{ab}$	0·40
Phospholipids	$3{\cdot}38^{b}$	0·31	$3{\cdot}55^{b}$	0·29	$2{\cdot}66^{a}$	0·36	$2{\cdot}59^{a}$	0·38	$2{\cdot}71^{a}$	0·52	$2{\cdot}27^{a}$	0·14	$2{\cdot}55^{a}$	0·27
Liver weight (g)	$6{\cdot}91^{ab}$	0·53	$7{\cdot}34^{b}$	0·43	$6{\cdot}70^{ab}$	0·82	$5{\cdot}97^{a}$	0·51	$5{\cdot}94^{a}$	0·53	$6{\cdot}42^{ab}$	1·02	$6{\cdot}54^{ab}$	0·50

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* For details of procedures and diets, see the Materials and methods section and Table 1. Rats were fed one of the test diets for 28 d (n 6).

† Food intake × energy value (kJ/g).

‡ Total − HDL.

Table 5. Effects of amylose content in diet on dry weight and bile acids in the small intestinal contents in ovariectomized rats*

(Mean values and standard deviations)

	Dietary groups													
	AL-0		AL-27		AL-54		AL-62		AL-76		AL-79		AL-86	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Small intestinal contents														
Dry weight (g)	0·49^{a}	0·08	0·51^{a}	0·08	0·72^{b}	0·13	0·71^{b}	0·13	0·74^{b}	0·04	0·66^{ab}	0·11	0·68^{ab}	0·09
Bile acids (μmol/contents)	68·9^{a}	9·3	81·1^{ab}	10·1	98·9^{b}	12·0	103·4^{b}	18·4	95·7^{b}	9·1	94·7^{b}	11·0	93·9^{b}	6·4
Composition of bile acid														
CA group (%)														
CA	26·0^{a}	8·4	27·9^{a}	4·5	28·1^{a}	3·9	35·0^{a}	8·9	42·4^{b}	6·9	35·4^{ab}	4·6	36·2^{ab}	6·2
Deoxycholic acid	1·0	0·3	0·7	0·2	1·1	0·3	1·2	0·3	1·3	0·4	1·3	0·5	0·9	0·2
12-Oxo-chenodeoxycholic acid	1·6^{ab}	1·2	1·7^{b}	0·3	1·0^{ab}	0·3	0·6^{a}	0·3	0·8^{ab}	0·2	1·2^{ab}	0·4	0·7^{ab}	0·5
12-Oxo-lithocholic acid	27·1	0·2	25·3	0·3	33·0	0·3	29·8	0·7	29·1	4·2	29·5	0·4	31·0	0·4
CDCA group (%)														
CACD	3·4^{a}	0·7	3·6^{a}	0·3	3·6^{a}	0·5	3·2^{a}	0·6	7·3^{b}	1·4	5·1^{b}	0·2	5·2^{b}	0·2
α-Muricholic acid	6·6^{a}	1·3	6·4^{a}	1·4	4·6^{a}	1·1	8·5^{ab}	3·2	12·8^{b}	4·5	7·7^{ab}	0·5	6·8^{a}	2·2
β-Muricholic acid	0·2	0·1	0·4	0·2	0·3	0·1	0·2	0·1	0·2	0·1	0·2	0·0	0·2	0·1
ω-Muricholic acid	0·4	0·1	0·4	0·2	0·3	0·1	0·3	0·1	0·5	0·2	0·3	0·1	0·3	0·1
Lithocholic acid	3·0	1·0	2·5	0·4	2·9	1·0	3·3	0·6	3·0	0·6	3·9	1·2	2·8	0·4
Hyodeoxycholic acid + ursodeoxycholic acid	30·1^{c}	5·8	31·1^{c}	2·3	25·2^{bc}	4·6	18·0^{bc}	4·4	2·6^{a}	1·4	15·5^{b}	5·4	16·0^{b}	3·6
CA group/CACD group ratio	1·26^{a}	0·36	1·25^{a}	0·27	1·72^{ab}	0·32	1·98^{b}	0·32	2·79^{c}	0·31	2·06^{b}	0·33	2·20^{bc}	0·24

CA, cholic acid; CACD, chenodeoxycholic acid.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

* For details of procedures and diets, see the Materials and methods section and Table 1. Rats were fed one of the test diets for 28 d (n 6).

Table 6. Effects of amylose content in diet on caecal tissue weight, and dry weight, bile acids and organic acids in the caecal contents, and faecal excretion in ovariectomized rats*
(Mean values and standard deviations)

	Dietary groups													
	AL-0		AL-27		AL-54		AL-62		AL-76		AL-79		AL-86	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Caecum														
Caecal tissue weight (g)	0·59^{a}	0·08	0·63^{ab}	0·15	0·98^{abc}	0·21	1·08^{c}	0·14	1·38^{c}	0·21	1·06^{bc}	0·08	0·52^{a}	0·42
Caecal content														
Moisture (g/100 g)	76·0	1·8	76·7	1·1	77·0	1·7	77·1	1·7	77·0	0·6	78·2	1·6	77·0	1·2
Dry weight (g)	0·52^{a}	0·1	0·60^{a}	0·06	1·26^{b}	0·24	1·56^{b}	0·28	2·49	0·20^{c}	1·48^{b}	0·19	1·23^{b}	0·15
Bile acids (mmol/content)	8·15^{a}	2·38	9·40^{a}	2·39	26·16^{d}	9·48	24·71^{cd}	9·16	20·08^{bcd}	5·68	14·14^{abc}	3·84	10·56^{ab}	2·05
Organic acids (mmol/content)														
Succinic acid	1·4^{a}	0·2	1·7^{a}	0·3	6·8^{a}	2·7	8·1^{a}	4·1	28·0^{b}	12·5	10·0^{a}	4·3	7·6^{a}	7·0
Lactic acid	7·6^{a}	4·2	7·8^{a}	2·3	31·8^{ab}	15·9	38·7^{b}	17·6	58·3^{b}	14·6	35·5^{b}	19·6	32·3^{ab}	11·3
Acetic acid	114^{a}	19	129^{a}	35	388^{bc}	97	499^{bc}	122	553^{c}	100	447^{bc}	115	359^{b}	78
Propionic acid	32^{a}	9	34^{ab}	8	164^{c}	37	157^{c}	43	123^{c}	28	103^{bc}	40	123^{c}	56
n-Butyric acid	18^{a}	6	15^{a}	5	216^{c}	55	218^{c}	93	105^{b}	14	78^{ab}	17	181^{c}	56
Total SCFA†	164^{a}	28	178^{a}	46	767^{bc}	133	874^{c}	194	780^{bc}	120	628^{b}	102	663^{bc}	89
Faecal excretion‡														
Dry weight (g/d)	1·12	0·03	1·12	0·08	1·32	0·19	1·40	0·32	1·25	0·20	1·32	0·25	1·45	0·66
Bile acids (mmol/d)	19·2^{a}	0·9	19·3^{a}	0·9	22·5^{ab}	3·0	24·2^{ab}	3·8	27·7^{b}	3·6	26·4^{b}	4·0	27·7^{b}	2·2
Neutral sterol (mmol/d)														
Coprostanol	1·06	0·09	1·09	0·20	1·44	0·37	1·37	0·26	1·19	0·34	1·44	0·21	1·42	0·20
Cholesterol	0·27^{a}	0·08	0·36^{a}	0·08	0·63^{ab}	0·17	1·07^{bc}	0·27	1·18^{c}	0·44	0·82^{bc}	0·29	1·10^{c}	0·23
Total§	1·33^{a}	0·13	1·45^{a}	0·28	2·07^{ab}	0·45	2·44^{b}	0·34	2·37^{b}	0·77	2·26^{b}	0·48	2·52^{b}	0·42

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see the Materials and methods section and Table 1. Rats were fed one of the test diets for 28 d (n 6).
† Acetic + propionic + butyric acids.
‡ Faeces were collected on the last 3 d of the experimental period.
§ Coprostanol + cholesterol.

Table 7. Effects of amylose content in diet on mRNA level of genes on cholesterol metabolism in ovariectomized rats (arbitary units)*
(Mean values and standard deviations)

	Dietary groups													
	AL-0		AL-27		AL-54		AL-62		AL-76		AL-79		AL-86	
Gene†	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ACAT1	0·90^{ab}	0·20	1·30^{b}	0·22	0·92^{ab}	0·28	1·13^{ab}	0·34	0·83^{a}	0·17	0·82^{a}	0·22	0·79^{a}	0·18
CYP7A1	0·93^{a}	0·30	0·99^{a}	0·22	1·13^{ab}	0·36	1·08^{ab}	0·38	1·65^{b}	0·46	1·45^{ab}	0·27	1·34^{ab}	0·23
CYP8B1	1·49^{a}	0·28	2·15^{ab}	0·50	2·84^{abc}	0·89	3·01^{abc}	1·06	4·25^{c}	1·26	4·29^{c}	0·88	3·25^{bc}	0·76
FXR	1·07	0·13	1·42	0·24	1·23	0·23	1·24	0·31	1·08	0·19	1·15	0·25	1·08	0·19
HMG-CoA R	1·03	0·28	1·08	0·25	1·09	0·44	1·07	0·25	1·33	0·34	1·01	0·31	1·04	0·25
LDL-R	0·96	0·14	0·71	0·12	0·82	0·23	0·67	0·18	0·75	0·21	0·64	0·10	0·75	0·24
SREBP-1c	1·26^{c}	0·44	1·13^{bc}	0·39	0·55^{a}	0·09	0·44^{a}	0·17	0·50^{a}	0·18	0·72^{ab}	0·13	0·71^{ab}	0·21
SREBP-2	1·61^{ab}	0·48	2·22^{b}	0·34	1·43^{a}	0·45	1·23^{a}	0·30	1·25^{a}	0·16	1·23^{a}	0·22	1·47^{a}	0·43

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see the Materials and methods section and Table 1. Rats were fed one of the test diets for 28 d (n 6).
† For details of genes, see Table 2.

diets were not. The mRNA level of SREBP-1c in rats fed the AL-54, AL-62, AL-76 diets were significantly lower than those in rats fed the AL-0 and AL-27 diets. The mRNA level of SREBP-2 in rats fed the AL-54, AL-62, AL-76, AL-79 and AL-86 diets were significantly lower than that in rats fed the AL-27 diet, but were not significantly lower than that in rats fed the AL-0 diet.

Discussion

The content of RS in test starches increased with increasing amylose content (r 0·907, $P=0·0048$), which was in agreement with the result of Brown *et al.* [19]. However, when amylose content further increased from 75·7 to 79·6 and 85·8 %, RS content decreased. Particle size of starch granules decreased with increasing amylose content[20,21]. Particle surface area of starch granules increase as the particle size of starch granules decrease. Digestibility of starch can be attributed to particle size and surface area[22–24]. Therefore, decreased RS content of AL-79 and AL-86 compared to AL-76 would be due to the increased surface area of starch granules.

Low amylose starches are more digestible than high amylose starches[25,26]. The higher the amylose content of starch the greater its resistance to digestion because it forms tightly packed granules in cells. Starches with high amylose/low amylopectin content tend to be of the type-B structure, while those with low amylose/high amylopectin content are of either the type-A or intermediate type-C form[27]. The study on the X-ray diffraction of maize starches across a series of differing amylose contents (0–84 %) showed the changes from A to B via C with an increase in amylose content, the transition occurring at about 40 % amylose[20]. Type-A and type-C starches are more digestible than type-B starches[25,28]. These show that the digestibility of starch in the small intestine is affected by not only particle size and surface area of starch granule but also structure of the starch.

It is well known that the deficiency of total energy intake immediately leads to the lowering of plasma cholesterol level[29]. In the present study, the reduction of energy intake led to the decrease of plasma total-C concentration. Therefore, the decreased concentration of plasma total-C would depend on the reduction of the energy intake. On the other hand, the following is also considered as a factor of hypocholesterolaemic effect of RS: (1) increased faecal excretion of bile acids; (2) increased biliary production of bile acids; or (3) increased synthesis of fermentation products that affect hepatic cholesterol synthesis[30]. Undigested starches can bind bile acids[31]. Therefore, an increased faecal excretion of bile acid through a bile acid binding by undigested starches would cause an increased hepatic synthesis of bile acid synthesis, consequently leading to the decrease of plasma total-C concentration. An increased synthesis of bile acid lowers the hepatic cholesterol pool, and this in turn leads to an up-regulation of gene expression and proteolytic activation of SREBP-2. As a consequence of this, SREBP-2 target genes like LDL-receptor and hydroxamethylglutaryl-CoA reductase would be up-regulated, however, they are not. We do not have an answer to this inconsistency now. The last factor is considered controversial, as *in vivo* propionate concentrations might not be high enough to decrease the activity of hydroxymethylglutaryl-CoA reductase[32,33]. In the present study, the expression of hydroxymethylglutaryl-CoA reductase mRNA was not affected by diet. In addition, there is no correlation between the amount of propionic acid in caecal content and the concentration of plasma total-C. Sacquet *et al.* [34] reported that HAMS lowered the plasma cholesterol concentration in germ-free rats. Therefore, the last factor would hardly take part in the hypocholesterolaemic effect of RS.

Several studies have shown that HAMS reduces plasma TAG concentrations in rats[36–38]. A relatively low insulinaemia has been observed in rats fed HAMS[36,39]. Feeding a diet rich in HAMS might produce a lower glycaemic response, consequently leading to declined lipogenesis in liver and adipose tissue[35,36]. Because newly synthesized fatty acids are preferentially channelled into VLDL, the lipogenic activity of the liver is a key factor in hepatic VLDL-TAG output[40,41]. It has been reported that propionate inhibits fatty acid synthesis[42,43] and decreases fatty acid synthetase mRNA level in cultured hepatocytes[44]. In the present study, the concentration of plasma TAG and the amount of propionic acid in the caecal content decreased, and increased as the dietary level of RS increased (r −0·843, $P=0·0173$; r 0·853, $P=0·0146$). Therefore, feeding a diet rich in RS

might produce lower lipogenesis in liver and lower VLDL secretion, consequently leading to a decreased plasma TAG concentration. However, the key experimental data are lacking that allows assessment of the quantitative contributions of propionic acid to the synthesis and regulation of lipid *in vivo*. On the other hand, the concentration of plasma TAG is also controlled by the amount of TAG absorbed from the small intestine. The digestion of high amylose starch may be slower than low amylose starch[35]. The slower digestion of starch rich in RS might make the digestion of TAG slow, which might result in a lower concentration of plasma TAG in rats fed a diet rich in RS.

The concentration of liver total lipids increased with increasing concentration of liver TAG (r 0·895, P=0·0064), suggesting that the change in total liver lipids is due to the change in liver TAG. SREBP-1c plays a significant role in the nutritional regulation of hepatic fatty acid synthesis[45]. There was significant positive correlation between the level of SREBP-1c mRNA and concentration of liver TAG (r −0·832, P=0·020). Reduced hepatic lipogenesis, as suggested by a lower hepatic gene level of SREBP-1c, may account for the lower liver lipids concentration.

Two pathways of bile acid synthesis have been identified in the rat model for hepatic bile acid synthesis: classic (neutral) and alternative (acidic). In rats, the classic pathway is the only pathway in which cholesterol is utilized in CA synthesis[54]. CYP8B1 is required for the synthesis of CA[46]. The percentage of CA in bile acids in the small intestinal contents increased with increasing level of CYP8B1 mRNA (r 0·867, P=0·0115). CDCA was a stronger suppressor of the mRNA expression of CYP7A1 than CA[55,56]. Farnesoid X receptor negatively regulates bile acid production by inhibiting transcription of the CYP7A1 gene[49]. Farnesoid X receptor is activated by bile acids, such as CDCA[50]. Though the level of farnesoid X receptor mRNA was not affected by diet, faecal excretion of bile acids increased exponentially as RS intake increased (r 0·886, P=0·0078). CDCA inhibited cholesterol synthesis[51]. Therefore, the higher CA compared with CDCA in the small intestinal contents may be to promote the synthesis of bile acid in order to compensate bile acids excreted into the faeces.

The caecal tissue weight increased with increasing dry weight of caecal contents and amount of succinic acid in caecal contents (r 0·808, P=0·0279; r 0·873, P=0·0104). There was a positive correlation between caecal contents and caecal tissue weight in rats fed diets containing retrograded starch[52]. The greater pool size of succinic acid is likely to play a role in stimulating the growth of caecal tissue[9]. Therefore, the increase in caecal contents and succinic acid pool size may contribute to the heavier caecal tissue weight.

Succinic acid and lactic acid are intermediates of global fermentation in the gut and are finally metabolized to SCFA by stable gut fermentation[53]. Therefore, succinic acid and lactic acid are rarely detected in the hindgut digesta of rats under normal conditions[54]. However, the excess accumulation of these acids has been reported in rats fed on indigestible oligosaccharide or HAMS[9,55]. The amount of succinic acid in the caecum increased as the dietary level of HAMS increased[56]. In the present study, the higher amount of succinic acid or lactic acid in caecal contents was found in rats fed the diet with the high levels of RS. Under nitrogen-limited conditions, the excess amounts of fermentable carbohydrate in the caecum should lead to production of lactic acid or succinic acid[9,57]. Unlike SCFA, these acids are not well absorbed by the large intestine[58]. Therefore, the excess accumulation of these acids in the caecum would depend on an excess production by an imbalance in carbohydrate/protein ratio of caecal content and a very slowly absorption.

Coprostanol is a metabolite of cholesterol, formed by the action of gut microflora, and its presence may indicate fermentation activity in the large intestine. The caecum is the site of vigorous microbial activity and of the microbiological reduction of cholesterol to coprostanol in the rat[59]. The coprostanol/cholesterol ratio of neutral sterol in faeces decreased with increasing total amount of organic acids in caecal contents (r −0·892, P=0·0069). The microbial transformation of cholesterol to coprostanol has been related, so far, to only a few species, namely *Eubacterium lentum* and *E. coprostanoligenes*[60,61]. Therefore, a decreased coprostanol/cholesterol ratio of neutral sterol in faeces may be due to the suppression of the multiplication of the microorganism to reduce cholesterol into coprostanol by an increased amount of total organic acids in the caecal contents.

The faecal excretion of total neutral sterol increased linearly with increasing RS intake (r 0·918, P=0·0036). The bile flow from the liver to the intestine in rats fed the HAMS diet were significantly greater than those in rats fed the normal maize starch diet[62]. Therefore, an increased faecal excretion of neutral sterol with increasing RS intake would be due to an increased bile flow.

In conclusion, the amount of RS in test starches increased with increasing amylose content in starch. Food intake was not significantly different among the groups, but the small intestinal digestibility of test starches in ileorectostomized rats decreased with increasing intakes of amylose and RS, and the concentrations of plasma TAG and total-C in ovariectomized rats decreased with increasing intakes of RS. The amount of bile acids in the small intestinal contents and the excretions of bile acids and neutral steroids in faeces increased with increasing RS intake. Starch rich in RS is less digestible and more effective in preventing ovarian hormone deficiency-induced hyperlipidaemia.

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