Test Diets

The native starch used to formulate diet A (Table II) contained only 0.5% RS, a value similar to that reported by Berry (1986). Treated starch contained 11.47% RS, almost all (97–100%) measured as insoluble fiber (Prosky et al 1988). Native starch and treated starch constituted 74.9% of the diets; other diet components added to meet the rats' nutrient requirements (NRC 1987) accounted for the remaining 25.1% (Table II). Antibiotics, where used, were added to the drinking water and included streptomycin sulfate (5 mg/ml), neomycin sulfate (4 mg/ml), bacitracin (4 mg/ml), and amphotericin B (0.1 mg/ml); the levels used were as prescribed by Srivastava et al (1976). Water containing antibiotics was freshly prepared on a regular basis.

Animals

Three groups of male, weanling rats (10 rats per diet) of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in mesh-bottom, stainless steel cages in a controlled environment. Each rat was allowed to consume an adequate, and identical, amount of total diet during the four-week test period. Deionized water was offered ad libitum. Body weight records were maintained.

Fecal Collection

For each rat, feces were collected quantitatively throughout the four-week test period, pooled, air-dried, weighed, and stored under refrigeration. A few freshly voided fecal pellets were also collected at frequent intervals, analyzed for moisture content, and then added to the pool collection.

Analytical

RS in starch samples was determined by the enzymatic-gravimetric method of Prosky et al (1988); this method measures insoluble fiber and soluble fiber separately. Moisture in freshly voided fecal samples was determined by air-drying the feces at room temperature. Calculated factors (Table III) were used to convert dry fecal weight (air-dried feces) to wet fecal weight. Fecal volume was measured in a long-stem graduated cylinder using fine sand as the embedding medium (Table III). Feces recovered from the sand were finely ground and analyzed for RS again using the method of Prosky et al (1988).

Statistical

Mean comparisons were made with Duncan's multiple-range test using the Statistical Analysis System (SAS 1982).

RESULTS AND DISCUSSION

Formation of Resistant Starch

A positive correlation is reported to exist (Berry 1986, Sievert and Pomeranz 1989) between amylose content of the starch and RS formation. Changes in processing parameters such as autoclaving and cooling cycles and freeze-drying steps also affect RS formation. Autoclaving and cooling cycles appear to favor RS formation more than autoclave temperature (above 100°C) or the freeze-drying step (Bjorck et al 1987, Table I).

In isolated wheat starch, Bjorck et al (1987) reported RS formation of 8%, a value similar to that reported by Sievert and Pomeranz (1989). Berry (1986), however, reported a yield of up to 15% RS after five repeated cycles of autoclaving and cooling. In the present study, yields of 11.47% (bulk quantity) or 11.95% RS (preliminary studies, Table I) were obtained when native starch was subjected to five repeated cycles of autoclaving and cooling. These various studies suggest that a maximum of about half of the 20–25% amylose present in wheat (Ring et al 1988) can be converted to RS; amylomaize starch, which contains 70% amylose, has shown much higher yields.

Resistant Starch in Diets

At the level used, the native starch diet provided 0.4% RS (diet A), and treated starch (diets B and C) provided 8.6% RS (Table II). For the entire four-week period, each rat consumed a total of 0.7 g (diet A) or 16.3 g (diets B and C) of RS.

Growth Response

Because all diets contained the same level (12%) and source of protein (casein) and were fed to the rats in identical amounts (Table II), the growth response of groups of rats fed these diets differed minimally (Table III).

TABLE I
Effect of Processing on the Content of Resistant Starch in Isolated Wheat Starch

Sample	Number of Times Starch is Autoclaved[a]	Cooled[a]	Freeze-dried	RS[b] (%)
1	0	0	0	0.46
2	1	1	1	4.35
3	1	1	1	5.17
4	1	2	1	8.89
5	2	2	1	5.56
6	2	2	2	6.15
7	3	2	1	5.05
8	3	3	1	6.53
9	3	3	1	8.98
10	3	3	2	6.59
11	4	4	1	7.48
12	5	1	1	9.49
13	5	5	1	11.95

[a] In a 24-hr period.
[b] Resistant starch.

TABLE II
Composition of Test Diets

Components (%)	Native Starch A	Treated Starch: No Antibiotics B	Treated Starch: Antibiotics[a] C
Starch	74.9	74.9	74.9
Mineral mix[b]	4.7	4.7	4.7
Vitamin mix[c]	1.0	1.0	1.0
Casein[d]	14.4	14.4	14.4
Soybean oil	5.0	5.0	5.0
Resistant starch, %	0.4	8.6	8.6

[a] Added to drinking water.
[b] Contained (in sucrose base) Ca, P, Fe, Mg, K, Na, Cr, Cu, I, Mn, Se, and Zn to meet rats' requirements (NRC 1987).
[c] AIN vitamin mixture 76 obtained from ICN Biochemicals, Cleveland, OH.
[d] Contained 83.4% protein.

TABLE III
Intestinal Responses in Rats[a]

Variable	Diet A	Diet B	Diet C
Starch	Native	Treated	Treated
Antibiotics	No	No	Yes
Diet intake, g	190 ± 0 a	190 ± 0 a	190 ± 1 a
Body weight gain,[b] g	50 ± 4 a	48 ± 2 a	49 ± 6 a
Fecal measurements			
Wet weight,[c] g	5.1 ± 0.4 a	37.2 ± 7.5 b	95.8 ± 4.4 c
Dry weight, g	2.5 ± 0.2 a	16.2 ± 3.3 b	29.0 ± 1.3 c
Volume, ml	2.3 ± 0.3 a	13.8 ± 2.3 b	21.8 ± 1.2 c
Digestibility of RS[d]			
RS consumed, g	0.7 ± 0.0 a	16.3 ± 0.0 b	16.3 ± 0.1 b
RS excreted, g	0.4 ± 0.0 a	9.4 ± 2.7 b	14.0 ± 0.6 c
RS digested, %	49.4 ± 5.8 a	37.1 ± 12.9 b	14.3 ± 3.7 c

[a] Values are averages ± SD (8–10 rats per diet). Means in a row not followed by the same letter are significantly different ($P < 0.05$).
[b] Initial body weights averaged 52 g for each group.
[c] Factors used to convert dry fecal weight to wet fecal weight: diet A, 2.1; diet B, 2.3; diet C, 3.3.
[d] Resistant starch.

Fecal Bulking Effect

Human epidemiological studies show a lower incidence of colorectal cancer in population groups that consume diets high in fiber (Trowell et al 1985). Various hypotheses are put forward to explain this, including one postulating that a higher fecal bulk would result in the dilution of potential carcinogens in the intestinal lumen.

Fecal bulking capacity of fiber sources varies. Sources that are high in insoluble fiber such as wheat bran provide more fecal bulk than those that are high in soluble fiber, a fraction highly fermentable in the colon (Nyman and Asp 1982, Nyman 1985).

Including treated starch in the diet increased the wet fecal weight over sixfold in comparison with native starch (diet B vs. A); the increase in dry fecal weight was of a similar magnitude (Table III). When the hind-gut bacterial population was effectively, if not completely, suppressed by antibiotics, the increase in wet fecal weight was even greater, nearly 18-fold (diet C vs. A). Increases in fecal volume paralleled increases in fecal weight; volume increases were fivefold for diet B and over eightfold for diet C compared with diet A (Table III). Thus, fecal weight and volume measurements suggested that the fecal bulking capacity of RS (with or without antibiotics) was substantial.

Digestibility of Resistant Starch

Measurement of RS digestibility was based on intake and fecal output data (Table III). RS is viewed by some (Berry 1986, Bjorck et al 1987) as a component like soluble fiber that is easily fermented by bacteria. It is, however, measured as essentially all insoluble fiber and expected to ferment less readily than soluble fiber. In the group of rats fed treated starch but not antibiotics, about one-third of the RS consumed was fermented (Table III). This contrasts with the high degree (80–92%) of metabolism reported by Bjorck et al (1987), who incorporated heat-treated starch to provide up to 5% RS in the diet. In the present study, when hind-gut fermentation was suppressed (by antibiotics), only 14.3% of the RS consumed was digested (diet C). This suggests that RS is highly resistant to mammalian amylolytic enzymes. Extending this observation to a human situation may mean that RS obtained through processed foods or added to foods can be an effective fecal bulking agent and, thus, should be properly classified as a fiber component as suggested earlier by Berry (1986).

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