**Nutrient Requirements and Interactions**

**Consumption of Retrograded (RS₃) but Not Uncooked (RS₂) Resistant Starch Shifts Nitrogen Excretion from Urine to Feces in Cannulated Piglets¹²**

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**ABSTRACT** To study the effect of resistant starch (RS) on the route of nitrogen excretion, we fed three groups of six cannulated piglets each a diet containing either uncooked resistant starch (RS₂), retrograded resistant starch (RS₃), or glucose. The use of piglets with a cannula at the end of the ileum allowed measurement of the amount of nitrogen that entered the colon. Ileal digesta, urine and feces were collected quantitatively and weighed, and dry matter, starch and nitrogen content were determined. We hypothesized that RS₂ would lower colonic absorption of nitrogen when compared with RS₃, because RS₂ may be more fermentable than RS₃, thus trapping more nitrogen in bacteria. The piglets fed RS₂ had a significantly higher production of ileal digesta and feces than the piglets fed glucose or RS₃. In the piglets fed RS₂, 44% of the amount of RS fed was recovered in the ileal digesta; in the piglets fed RS₃, 71% was recovered. Thus, more fermentable material entered the colon in the RS₂-fed piglets than in the RS₃-fed piglets. Virtually no starch was recovered in the feces of any dietary group. Replacement of glucose by either RS₂ or RS₃ did not affect nitrogen retention but increased fecal nitrogen excretion. Compared with glucose, RS₃ but not RS₂ reduced urinary nitrogen excretion, mainly in the form of urea, and reduced the amount of nitrogen absorbed by the colon when expressed as a percentage of the amount of nitrogen entering the colon. This study provides evidence that RS₃, but not RS₂, shifts nitrogen excretion from urine to feces in cannulated piglets. J. Nutr. 127: 1828–1832, 1997

**KEY WORDS:** • resistant starch • uncooked starch • retrograded starch • nitrogen excretion • pigs

In rats, dietary uncooked resistant starch (RS₂)⁴ led to a shift of nitrogen excretion from urine to feces (Younes et al. 1995a). Dietary retrograded resistant starch (RS₃) increased fecal nitrogen excretion without affecting urinary nitrogen excretion in rats (Brunsgaard et al. 1995). In pigs, RS₂ in the form of raw potato products (Wünsche et al. 1987) and starch infusions into the terminal ileum (Gargallo and Zimmerman 1981, Misir and Sauer 1982, Mosenthin et al. 1992) also increased fecal nitrogen excretion, which was balanced by a reduction in urinary nitrogen output. A diet high in resistant starch (RS) was found to increase fecal nitrogen excretion in humans without a concomitant decrease in urinary nitrogen excretion (Birkett et al. 1996). If a shift of nitrogen excretion from urine to feces occurs after consumption of RS, it can be explained by increased bacterial protein synthesis and a subsequent decrease in colonic absorption of nitrogen in the form of ammonia. The indigestible fermentable carbohydrates that reach the colon supply energy for bacterial growth for which nitrogen also is required. Nitrogen is derived from ammonia produced by bacteria from dietary protein that escapes digestion and from endogenous proteins such as pancreatic and intestinal secretions and sloughed epithelial cells (Mason 1984) and blood urea after its diffusion into the gut (Rémésy and Demigné 1989, Younes et al. 1995a and 1995b). It is reported that 60–90% of fecal nitrogen is bacterial nitrogen (Ahrens and Kaufmann 1985, Mason 1984, Mosenthin et al. 1992, Stephen and Cummings 1980, Wünsche et al. 1987).

When higher amounts of substrates fermentable by bacteria are included in pig feed, the amount of soluble nitrogen in both feces and urine can be lowered, so that nitrate and ammonia generation from manure is also lowered, which in turn reduces environmental pollution (Hegedüs 1993, Kirchgessner and Roth 1993). In humans, an increase in fecal nitrogen excretion at the expense of renal excretion may be of interest for the dietary management of chronic renal disease, such as may occur in diabetic patients (Parillo et al. 1988, Rampton et al. 1984, Rivellese et al. 1985). Also, reduction of the absorption of ammonia from the gut, thereby decreasing urea production in the liver, may lessen the workload for the liver, which is beneficial for cirrhotic patients (Weber 1979, Weber et al. 1984, Rivellese et al. 1985).

The aim of this experiment was to study the effect of dietary RS₂ and RS₃ on nitrogen excretion in cannulated piglets. The
TABLE 1
Composition of the diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Glucose</th>
<th>RS2</th>
<th>RS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, g</td>
<td>680.5</td>
<td>376.9</td>
<td>275.1</td>
</tr>
<tr>
<td>RS2 preparation, g</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RS3 preparation, g</td>
<td>—</td>
<td>—</td>
<td>613.1</td>
</tr>
<tr>
<td>Constant components, g</td>
<td>320.0</td>
<td>320.0</td>
<td>320.0</td>
</tr>
<tr>
<td>Demineralized water, g</td>
<td>—</td>
<td>28.5</td>
<td>67.4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose equivalents, g/kg</td>
<td>619</td>
<td>619</td>
<td>619</td>
</tr>
<tr>
<td>Resistant starch, g/kg</td>
<td>169</td>
<td>169</td>
<td>168</td>
</tr>
</tbody>
</table>

1 Meritose (Cerestar, Vilvoorde, Belgium); dry weight 90.9%.
2 Uncooked high amylose cornstarch (Cerestar); dry weight 90.3%; 61.4 g resistant starch/100 g according to the procedure of Englyst et al. (1992).
3 Refined high amylose cornstarch (Cerestar); dry weight 90.8%; 27.4 g resistant starch/100 g according to the procedure of Englyst et al. (1992).
4 The constant components consisted of the following (g/kg diet): wheat gluten, 90; casein, 90; soybean oil, 20; cellulose, 50; CaCO₃, 12.5; CaHPO₄·2H₂O, 20; NaCl, 5; MgO, 2; KCl, 30; CaHPO₄, 20; NaCl, 5; MgO, 2; KHCO₃, 15; NaHCO₃, 2.5; premix, 12.5; Cr₂O₃, 3.0. The premix consisted of the following (mg): MnO₂, 70; FeSO₄·7H₂O, 100; CaSO₄·7H₂O, 400; ZnSO₄·7H₂O, 300; Na₂SeO₃·5H₂O, 0.2; KI, 0.5; CuSO₄·5H₂O, 100; CoSO₄·7H₂O, 2.5; thiamin, 2; riboflavin, 5; niacinamide, 30; d-calcium pantothenic acid, 12; pyridoxine, 3; cyanocobalamin, 0.04; folic acid, 1; biotin, 0.1; ascorbic acid, 50; choline chloride, 1000; menadione, 3; all-rac-α-tocopheryl acetate, 40; retinyl acetate and retinyl palmitate, 18 (2700 retinol equivalents); cholecalciferol, 0.045; comenial, 7962.615.

kg diet. Corrections were made for the different water contents of the carbohydrate preparations and for the water excluded during formation of glycosidic bonds. We did not try to equalize the energy content of the diets because there is no accurate estimate of the amount of energy that RS supplies; in any event, this energy is unlikely to greatly exceed 8.4 kJ/g (Livesey 1990). Because the piglets in the RS₂ and RS₃ groups ate 114 g RS/d, as determined by the in vitro method of Englyst et al. (1992), the energy intake may have been Rs approximately 958 kJ less in the RS groups than in the glucose group (i.e., 9% of the energy content of the glucose diet). The powdered diets were stored at 4°C until used for feeding.

Before the experiment, the piglets were fed a cornstarch-based diet with the following guaranteed analysis (g/kg diet): moisture, 10; crude protein, 165; crude fat, 34; crude fiber, 66; crude ash, 61. The piglets were divided into three groups of six animals each so that body weight distributions of the groups were similar. Each group of piglets was randomly assigned to the glucose, RS₂, or RS₃ diet. Within a 4-d period, the ration gradually changed from the commercial diet to each of the three experimental diets. After that, the experimental diets were given for another 10 d. It was considered important to standardize the intake of glucose equivalents and the nutritional status of the animals because ileal digesta were to be collected. Therefore, the piglets were fed on a restricted basis. The piglets were given an amount of feed that was equivalent to 2.6 times the maintenance requirement; this feeding regimen had already been established prior to the start of the experiment. Maintenance level was assumed to be 420 kJ/kg metabolic wt. The feed was provided to the pigs in two meals of identical size, at 0800 and 1600 h during the adaptation period and at 0800 and 2000 h during the collection period, starting 2 d in advance. The piglets received tap water at a water/feed ratio of 2.35:1 (wt/wt). Body weights were measured at the beginning and the end of the study.

Collection of feces, urine and ileal digesta. On d 9–11, urine and feces were collected quantitatively from each animal. Urine was collected in a bucket that was placed under the tray with a funnel that was present under the tenderfoot mesh floor of the cages. Feces were removed from the cage floor and the tray. Urine and feces collections were frozen at −20°C until analysis.

On d 12–14, ileal digesta were collected quantitatively for 12-h periods, starting 15 min before the morning meal and ending 15 min before the evening meal. One hour before the collection period, the PVTC cannula was opened to adapt the animals and the digesta flow. During this hour, the position of the valve changed, and instead of protruding into the intestinal lumen, it protruded into the lumen of the cannula. Digesta flowed through the cannula into a small plastic bag attached to the cannula with a self-tightening nylon strap. Every hour, the bags were replaced, weighed and frozen at −20°C.

Chemical analyses. The feces and ileal digesta were thawed, pooled per animal per 3 d, homogenized in demineralized water with a blender (Braun Multimix MX32; Braun, Frankfurt/Main, Germany) and then freeze-dried overnight. Dry matter content was determined as the weight difference before and after freeze-drying. Starch was measured in fecal and ileal samples as the difference between total glucose and free glucose, adapted from the method from Björck et al. (1987). The urine was thawed and pooled per animal per 3 d. Creatinine was measured in lightly acidified urine with the use of a commercial test kit (Creatinine, MA-KIT 10 ROCHE; Roche Diagnostics, Basel, Switzerland) and a COBASBIO auto-analyzer (Hoffmann-La Roche B.V., Mijdrecht, The Netherlands). Urea was measured in nonacidified urine by the urease method with the use of a commercial test kit (Urea UV, MA-KIT 10 ROCHE; Roche Diagnostics) and the auto-analyzer.

Nitrogen in feed, fecal, ileal and urine samples was measured by the Kjeldahl method. Nitrogen balance was calculated as nitrogen intake minus nitrogen excretion via feces and urine. Colonic nitrogen absorption was calculated as nitrogen in ileal digesta minus nitrogen in feces.

Statistical analysis. The experiment was designed with three parallel groups of six piglets each. Each group consumed a different diet containing glucose, RS₂, or RS₃. Differences between group means for each outcome variable were evaluated by one-way ANOVA with the GLM (General Linear Model) procedure of SAS (release 6.09,
Urine of the ileal digesta was higher in the RS 2 group than in the glucose group, and higher in the RS 3 than in the RS 2 and 3 groups. Absorption of nitrogen was calculated as a percentage of intake. In piglets fed the glucose diet, mean (+SEM) nitrogen absorption was 96.3 ± 0.4%. Feeding the RS 2 and RS 3 diets resulted in percentage nitrogen absorptions of 92.5 ± 0.4% and 91.2 ± 0.9%, respectively. Both the RS 2 diet (P < 0.05) and the RS 3 diet (P < 0.05) significantly reduced nitrogen absorption compared with the glucose diet.

### RESULTS

**Body weight and food intake.** Both initial and final body weights did not differ among the dietary groups. Food intakes did not differ among the three groups (Table 2).

**Ileal digesta and feces.** The piglets fed RS 3 had a markedly higher production of ileal digesta and feces (Table 2) than the piglets fed glucose or RS 2 (P < 0.05). The dry matter content of the ileal digesta was higher in the RS 2 group than in the glucose group, and higher in the RS 3 than in the RS 2 and glucose groups (P < 0.05). The dry matter content of the feces was approximately twice as high (P < 0.05) in the RS 2 and RS 3 groups as in the glucose group (Table 2). A considerable amount of starch was recovered in the ileal digesta from the piglets fed 114 g of RS 2 or RS 3 per day: 50 and 81 g/d, respectively, compared with only 1 g/d in the glucose group (Table 2). The amounts of starch in ileal digesta were significantly different among all dietary groups (P < 0.05). In the feces, very little starch was recovered in any of the dietary groups (Table 2).

**Urine.** Urine production and urinary creatinine excretion were similar in the dietary groups (Table 2). Urinary urea excretion was lower in the RS 3 group than in the glucose and RS 2 groups (P < 0.05).

**Nitrogen balance.** The nitrogen intake was similar in the dietary groups (Table 3). The nitrogen content of the ileal digesta was significantly higher in the RS 3 group than in the glucose group (P < 0.05). Of the amount of nitrogen entering the colon (i.e., the nitrogen content of the ileal digesta, since these were collected at the end of the ileum), about 1 g/d was absorbed by the colon in the glucose and RS 2 groups and only 0.6 g/d in the RS 3 group. The latter was significantly (P < 0.05) lower than in the glucose group when expressed as a proportion of the amount of nitrogen entering the colon, i.e., the amount of nitrogen in the ileal digesta. In the RS 2 and RS 3 groups, nitrogen excretion via the feces was 100 and 150% higher, respectively, than in the glucose group (P < 0.05). Nitrogen excretion via the urine, mainly as urea, was 14% lower in the RS 3 (P < 0.05) group compared with the glucose group. The nitrogen balance of ~11 g/d was not different in the three dietary groups (Table 3).

**Apparent nitrogen absorption.** Apparent mouth-to-anus absorption of nitrogen was calculated as a percentage of intake. In piglets fed the glucose diet, mean (+SEM) nitrogen absorption was 96.3 ± 0.4%. Feeding the RS 2 and RS 3 diets resulted in percentage nitrogen absorptions of 92.5 ± 0.4% and 91.2 ± 0.9%, respectively. Both the RS 2 diet (P < 0.05) and the RS 3 diet (P < 0.05) significantly reduced nitrogen absorption compared with the glucose diet.

### DISCUSSION

Dietary RS 2 and RS 3, in contrast to glucose, increased fecal nitrogen excretion, resulting in a reduced apparent nitrogen absorption. The increase in fecal nitrogen after RS consumption was probably due to the combination of a decrease in ileal nitrogen absorption and an increase in bacterial nitrogen through stimulation of bacterial growth in the gut by fermentation of undigested RS. The latter is confirmed by the recovery of virtually no starch in the feces of the piglets fed RS 2 and RS 3, indicating that overall both RS 2 and RS 3 were extensively fermented. Wünsche et al. (1987) also recovered no starch in the feces of pigs that consumed RS 2 from raw potatoes. In humans, 1% (Van Munster et al. 1994) to approximately 20% (Heijnen, M.-L., unpublished results, Phillips et al. 1995, Van Munster et al. 1994) and rats (Heijnen et al. 1996, Schulz et al. 1993). Thus the pig seems a good RS fermenter.

### TABLE 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>Glucose</th>
<th>RS 2</th>
<th>RS 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>16.6 ± 0.4</td>
<td>16.4 ± 0.6</td>
<td>16.7 ± 0.4</td>
</tr>
<tr>
<td>Final</td>
<td>21.1 ± 0.5</td>
<td>20.9 ± 0.7</td>
<td>21.2 ± 0.5</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>682 ± 11</td>
<td>678 ± 15</td>
<td>683 ± 11</td>
</tr>
<tr>
<td>Ileal digesta, g/d</td>
<td>577 ± 92</td>
<td>780 ± 57</td>
<td>1159 ± 46b</td>
</tr>
<tr>
<td>Production</td>
<td>69 ± 3a</td>
<td>149 ± 8b</td>
<td>201 ± 6c</td>
</tr>
<tr>
<td>Dry matter</td>
<td>1 ± 1a</td>
<td>50 ± 2b</td>
<td>81 ± 3c</td>
</tr>
<tr>
<td>Starch</td>
<td>55 ± 8a</td>
<td>91 ± 7a</td>
<td>142 ± 15b</td>
</tr>
<tr>
<td>Dry matter</td>
<td>27 ± 4a</td>
<td>52 ± 2b</td>
<td>52 ± 4b</td>
</tr>
<tr>
<td>Starch, mg/d</td>
<td>46 ± 9</td>
<td>129 ± 39</td>
<td>141 ± 57</td>
</tr>
</tbody>
</table>

**Production, mL/d**: 971 ± 34 | 915 ± 29 | 862 ± 53

**Creatinine, mmol/d**: 7.0 ± 0.4 | 7.2 ± 0.3 | 6.9 ± 0.4

**Urea, mmol/d**: 168 ± 4b | 160 ± 6b | 141 ± 5a

1 Values are means ± SEM for six piglets per dietary group. Within a row, values with different superscripts are significantly different (P < 0.05). Note that starch is expressed in g/d for the ileal digesta and in mg/d for the feces.

### TABLE 3

**Nitrogen balance and apparent colonic nitrogen absorption in piglets fed diets containing glucose, uncooked (RS 2) or retrograded resistant starch (RS 3)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Glucose</th>
<th>RS 2</th>
<th>RS 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake, g/d</td>
<td>16.4 ± 0.3</td>
<td>16.7 ± 0.4</td>
<td>17.1 ± 0.3</td>
</tr>
<tr>
<td>N in ileal digesta, g/d</td>
<td>1.7 ± 0.1a</td>
<td>2.3 ± 0.2b</td>
<td>2.1 ± 0.1ab</td>
</tr>
<tr>
<td>N in feces, g/d</td>
<td>0.6 ± 0.1a</td>
<td>1.2 ± 0.1b</td>
<td>1.5 ± 0.2b</td>
</tr>
<tr>
<td>N in urine, g/d</td>
<td>4.9 ± 0.1b</td>
<td>4.6 ± 0.2ab</td>
<td>4.2 ± 0.1a</td>
</tr>
<tr>
<td>Balance, g/d</td>
<td>10.9 ± 0.1</td>
<td>10.9 ± 0.2</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>N absorbed by colon3</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>% of N in ileal digesta</td>
<td>62 ± 6b</td>
<td>43 ± 5ab</td>
<td>30 ± 5a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM for six piglets per dietary group. Within a row, values with different superscripts are significantly different (P < 0.05).

2 Calculated as N intake minus N in feces and urine.

3 Calculated as N in ileal digesta minus N in feces.
Fermentation of RS in the colon probably induced a lower colonic pH (not measured). A lower pH enhances the conversion of ammonia (NH$_3$) into ammonium (NH$_4^+$). Ammonium is less well absorbed by the colon than ammonia and will be excreted in the feces. This process may also have contributed to the observed increase in fecal nitrogen excretion. Further, consumption of RS has been shown to induce high rates of urea transfer from the blood into the cecum in rats (Younes et al. 1995a). Because ureolytic bacteria degrade urea to ammonia, which can be incorporated into bacterial protein, a RS-induced urea diffusion into the cecum may have contributed to the observed increase in fecal nitrogen excretion, too. Such an effect would cause an underestimation of the calculated amounts of nitrogen absorbed by the colon of the two RS groups.

Dietary RS$_1$, but not RS$_2$, reduced urinary nitrogen excretion, mainly by reducing urinary urea excretion, which can be explained by the observed reduced colonic nitrogen absorption. A priori, we expected the effects of RS$_2$ and RS$_3$ to be just the other way around, because we assumed that RS$_2$ is fermented to a greater extent than RS$_3$ (Cummings et al. 1995, Olesen et al. 1994, Schulz et al. 1993). The discrepancy between the results and our expectations can be explained if the observed lesser amount of starch in the ileal digesta after RS$_2$ feeding, instead of RS$_3$ feeding, points to RS$_2$ being fermented in the small intestine to a greater extent than RS$_3$. The correctness of this interpretation depends on the validity of the definition of RS for pigs. We cannot exclude the possibility that starch that is resistant in humans is digestible in pigs. The measurement of RS by the procedure of Englyst et al. (1992) is validated in human ileostomists and not in pigs. If the amount of RS consumed was indeed indigestible for the pigs, then less fermentable substrate would have entered the colon in the RS$_2$-fed pigs than in the RS$_3$-fed pigs. Thus, RS$_2$ and RS$_3$ differed as to the site of fermentation, although their overall digestibility was equal. RS$_2$ was fermented 56% in the ileum and 44% in the colon, whereas RS$_3$ was fermented 29% in the ileum and 71% in the colon. Fermentation in the ileum of pigs is possible, because the distal third of the ileum contains a significant amount of bacteria, i.e., $\sim 10^8$–$10^9$ viable counts/g digesta (Bach Knudsen et al. 1993, Chesson et al. 1985, Liu et al. 1985). It is unlikely that our results are affected by use of PVTC-cannulated pigs, because the bacteria found in their ileum are part of the normal ileal flora and are not airborne microorganisms that came into the gut during surgery (Chesson et al. 1985). The occurrence of ileal fermentation of RS corresponds with the observed increase in the amounts of ileal nitrogen and digesta. However, the increase in ileal nitrogen after RS feeding may also reflect a decrease in protein digestion and absorption.

The feeding of RS$_3$, but not of RS$_2$ ($P = 0.35$), significantly reduced urinary nitrogen excretion when compared with the feeding of glucose. Possibly, RS$_2$ would also reduce urinary nitrogen excretion at dietary protein concentrations lower than that of 180 g/kg used in this study. At lower protein intakes urinary nitrogen excretion is lower, so that any change is more easily detectable. However, in this study as in other studies using high protein diets, RS has been shown to diminish urinary nitrogen excretion. Younes et al. (1995a) found, in rats fed diets containing 260 g protein/kg, that consumption of RS significantly reduced urinary nitrogen excretion. Gargallo and Zimmerman (1981) found, in pigs fed a diet with 185 g protein/kg, that starch infusion into the terminal ileum increased fecal nitrogen excretion, which was balanced by a reduction in urinary nitrogen output.

The effect of RS$_3$ on the routes of nitrogen excretion as found in the present study agrees with studies in which starch was infused into the terminal ileum of pigs (Gargallo and Zimmerman 1981, Misir and Sauer 1982, Mosenthin et al. 1992). In those studies, fermentation also took place mainly in the colon, and the increase in fecal starch excretion was balanced by a decrease in urinary nitrogen excretion, as in our study after RS$_3$ feeding. However, we have no explanation for the discrepancy between the effect of RS$_2$ on the routes of nitrogen excretion in the present study and in the study by Wünsche et al. (1987). In the latter study, the increase in fecal nitrogen excretion was balanced by a decrease in urinary nitrogen excretion after pigs were fed RS$_2$, whereas in the present study this balancing did not occur after RS$_2$ feeding but only after RS$_3$ feeding. In conclusion, this study provides evidence that RS$_3$, but not RS$_2$, shifts nitrogen excretion from urine to feces in cannulated pigs. It is important to stress that RS$_3$ and RS$_2$ were compared on the basis of identical amounts of RS in the feeds as determined by the method of Englyst et al. (1992). If these results can be extrapolated to humans, consumption of RS$_3$ instead of digestible carbohydrate may lower the workload for the kidneys and the liver and may therefore be beneficial for patients with kidney or liver malfunction (Parillo et al. 1988, Rampton et al. 1984, Rivellese et al. 1985, Weber 1979, Weber et al. 1985).

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**LITERATURE CITED**


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