Z Ernährungswiss 37: 13–17 (1998)

ORIGINAL CONTRIBUTION

M.-L. Heijnen
A. Beynen

Effect of consumption of uncooked (RS_2) and retrograded (RS_3) resistant starch on apparent absorption of magnesium, calcium, and phosphorus in pigs

Einfluß von ungekochter (RS_2) und retrogradieter (RS_3) resistenter Stärke auf das Ausmaß und den Ort der scheinbaren Absorption von Magnesium, Calcium und Phosphor bei Schweinen

Summary The aim of this investigation was to study the effect of uncooked (RS_2) and retrograded (RS_3) resistant starch on the size and site of the apparent absorption of magnesium, calcium, and phosphorus in swine. In an experiment with a parallel design, three groups of six piglets each consumed for two weeks a diet containing either glucose, RS_2 or RS_3. The piglets were cannulated at the end of the ileum which allowed estimation of the ileal and colonic mineral absorption. Urine, feces, and ileal digesta were collected for measurement of magnesium, calcium, and phosphorus. Dietary RS_2, but not RS_3, versus glucose reduced ($p < 0.05$) the total absorption of magnesium and calcium and the ileal absorption of phosphorus in the pig. Differences between species or in RS and/or mineral intake may explain why RS_2 reduced the apparent absorption of magnesium and calcium in pigs in this study and raised it in rats in earlier studies.

Zusammenfassung Das Ziel der Arbeit war es, den Einfluß von ungekochter „resistenter Stärke" (Resistant Starch type 2: RS_2) und von retrogradierter resistenter Stärke (Resistant Starch type 3: RS_3) auf das Ausmaß und den Ort der scheinbaren Absorption von Magnesium, Calcium und Phosphor bei jungen Schweinen zu untersuchen. In einem Versuch mit parallelem Aufbau erhielten drei Gruppen von sechs Schweinen entweder Glukose, RS_2 oder RS_3 für zwei Wochen. Die Schweine waren am Ende des Ileums mit einer T-Kanüle ausgerüstet, um die Mineralabsorption im Ileum und Dickdarm messen zu können. Harn, Stuhl und der Inhalt des Ileums wurden 3 Tage lang gesammelt und auf den Gehalt von Magnesium, Calcium und Phosphor bestimmt. RS_2, nicht aber RS_3, reduzierte die Gesamt-Absorption von Magnesium und Calcium, und die Absorption von Phosphor im Ileum der Schweine im Vergleich zum Glukose. Speziesunterschiede hinsichtlich der Qualität der resistenten Stärke, vielleicht auch die Mineralstoffaufnahme, dürften verantwortlich dafür sein, warum, im Gegensatz zu Angaben bei Ratte, RS_2 die scheinbare Absorption von Magnesium und Calcium in den Schweinen in dieser Studie verringerte.

Key words Resistant starch – uncooked starch – retrograded starch – magnesium absorption – calcium absorption – phosphorus absorption – pig

Schlüsselwörter Resistente Stärke – ungekochte Stärke – retrogradierte Stärke – Absorption von Magnesium – Absorption von Calcium – Absorption von Phosphor – Schweinchen

Received: 21 May 1997
Accepted: 03 September 1997

Supported by Unilever Research Laboratorium, Vlaardingen, The Netherlands, and conducted within the framework of the European Resistant Starch (EURESTA) research group working within the European Flair Concerted Action no. 11.

Marie-Louise Heijnen (✉)
Department of Human Nutrition
Wageningen Agricultural University
P.O.Box 8129
NL-6700 EV Wageningen
The Netherlands

A. Beynen
Department of Laboratory Animal Science
Utrecht University
P.O.Box 80166
3508 TD Utrecht
The Netherlands

Introduction

In rats, uncooked resistant starch (RS_2) compared with digestible starch raised apparent magnesium und calcium absorption (2, 23, 27, 31, 32). Uncooked (RS_2) versus retrograded resistant starch (RS_3) also raised apparent magnesium and calcium absorption in rats (14, 27). It has been proposed that RS_2, compared with either digestible starch or RS_3, raised apparent magnesium and calcium absorption by increasing ileal solubility of magnesium and calcium due to a reduction in pH (10, 12, 27, 32). In contrast to digestible starch, RS is not absorbed in the small intestine but may be fermented by the bacterial flora in the gut and some studies indicate that RS_2 is more fermentable than RS_3 (6, 22, 27). However, since RS_2 compared with RS_3 increased only apparent but not true magnesium absorption by decreasing endogenous magnesium loss with feces, the proposed mechanism seems to be incorrect (14).

Magnesium (11) and calcium (1, 4, 21, 28) may not only be absorbed from the small but also from the large intestine, especially from the cecum in the rat (10). Distal magnesium and calcium absorption may be stimulated by fermentable RS_2 by increasing the soluble pool of the mineral (27, 31, 32) through acidification of the cecal contents (2, 7, 27, 31, 32) and/or by hypertrophy of the cecal wall, for example, by increasing the surface area for absorption (7, 18, 31, 32). Scharrer and Lutz (25, 26) have proposed that the short-chain fatty acids (SCFA) produced during carbohydrate fermentation in the gut may enhance magnesium absorption by a Mg^{2+}/H^+ exchanger located in the apical membrane of the epithelium in the distal colon. SCFA may also stimulate colonic cell proliferation (20) which could increase the mineral absorption capacity.

To study the contribution of the small and the large intestine to the absorption of magnesium, calcium, and phosphorus, we fed piglets that were cannulated at the end of the ileum glucose, RS_2, and RS_3. Even though the pig is essentially a colon fermenter like man (9), fermentation also takes place in the distal third of the ileum of pigs (5). The latter may stimulate mineral absorption in the ileum. We hypothesized that, compared with glucose, RS_2 but not RS_3 would raise apparent magnesium and calcium absorption.

Methods

Diets

Three feeds were prepared containing glucose, RS_2 or RS_3. The exact composition of the feeds has been detailed elsewhere (13). The following RS preparations were used: uncooked high-amylose maize starch (Hylon VII; Cerestar, Vilvoorde, Belgium), containing 61.4 % RS_2 by wt. as measured *in vitro* according to the procudure of Englyst et al. (8); and retrograded high-amylose maize starch (extruded and retrograded Hylon VII; Cerestar, Vilvoorde, Belgium), containing 27.4 % RS_3 by wt. as measured in vitro according to the procedure of Englyst et al. (8). The feeds only differed in the type of carbohydrate used. The amount of glucose equivalents was equal for the three feeds. 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 equal the energy content of the feeds because there is no accurate estimate of the amount of energy that RS supplies.

Design

The experimental procedure has been detailed elsewhere (13). The experimental protocol was approved by an Animal Ethical Committee. Crossbred castrates (FL*NL)*GY aged 10 weeks and with an average body weight of 16 kg were used. At the age of 6 weeks they had been fitted with a post-valve T-cecum cannula (PVTC) allowing quantitative collection of the ileal digesta, as described by van Leeuwen et al. (30). No differences in growth performance, organ weights, nitrogen balance, mineral balances, and several blood variables were found between PVTC-pigs and intact pigs (15, 16). The piglets were housed individually in stainless-steel metabolic crates.

In an experiment with a parallel design, three groups of six animals each consumed either the glucose, RS_2 or RS_3 diet for 2 weeks. 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, i.e., an amount of feed that was equivalent to 2.6 times the maintenance requirement. Maintenance level was assumed to be 420 kJ per kg metabolic body weight. The feed was provided to the piglets in two meals of identical size, at 0800 and 2000 h. The piglets received tap water at a water:feed ratio of 2.35:1 (w/w). Food intake and initial and final body weights of the piglets did not differ significantly between the three diet groups. The piglets fed RS_2 or RS_3 consumed 114 g RS/d (13).

On days 9–11 feces was collected quantitatively from each animal and frozen at -20 °C until analysis. On days 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. 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. Feces and ileal digesta were pooled per animal per three days.

Measurements and calculations

In diet, feces, digesta and urine samples magnesium and calcium were analyzed by atomic absorption spectrophotometry and phosphorus was measured colorimetrically. Apparent total absorption of minerals was calculated as mineral intake minus fecal excretion and expressed as percentage of intake. Apparent ileal absorption was calculated as mineral intake minus ileal excretion and apparent colonic absorption was calculated as total absorption minus ileal absorption. Mineral intakes with tap water were less than 1 % of the intakes with the diet and were ignored when calculating mineral absorptions.

Statistical analysis

Differences between group means for each variable were evaluated by analysis of variance with the GLM (General Linear Model) procedure of SAS (release 6.09; Statistical Analysis Systems Institute Inc., Cary, NC, USA). The model contained 'feed' as a fixed factor. When the analysis of variance indicated a significant effect of 'feed' ($p < 0.05$), Tukey's Studentized Range test was used for pair-wise comparison of the group means. This method encompasses a downward adjustment of the significance limit for multiple testing.

Results

Magnesium

Total apparent magnesium absorption was approximately 62 % of intake (Table 1). Total magnesium absorption was lower ($p < 0.05$) in the RS_2 than in the glucose group (Table 1). Relatively less magnesium seemed to be absorbed from the colon and more from the ileum in the RS_2 and RS_3 groups compared with the glucose group.

Calcium

Calcium intake differed slightly between the three dietary groups (Table 1) due to small differences in calcium content measured in the diets. Total apparent calcium absorption was approximately 73 % of intake (Table 1). Total absorption of calcium was lower ($p < 0.05$) in the RS_2 than in the glucose group (Table 1). No significant differences were found in ileal and colonic calcium absorption.

Phosphorus

Phosphorus intake differed slightly between the three dietary groups (Table 1) due to small differences in phosphorus content measured in the diets. Total apparent phosphorus absorption was approximately 73 % of intake (Table 1). Total absorption of phosphorus was similar during glucose, RS_2 and RS_3 consumption (Table 1). Phosphorus was absorbed mainly from the ileum. Ileal absorption was lower ($p < 0.05$) in the RS_2 than in the glucose group (Table 1). No differences were found in colonic phosphorus absorption. Relatively more phosphorus seemed to be absorbed from the colon and less from the ileum in the RS_2 and RS_3 groups compared with the glucose group.

Discussion

In the present study, dietary RS_2, but not RS_3, versus glucose reduced the apparent total absorption of magnesium and calcium and the apparent ileal absorption of phosphorus in the pig.

In contrast to the present findings in piglets, RS_2 enhanced the apparent absorption of magnesium and calcium in rats (2, 14, 23, 27, 31, 32). This discrepancy may be due to species differences in, e.g., hormonal control; bacterial flora, anatomy or physiology of the digestive tract; or in eating pattern over the day. Moreover, the piglets had a much higher intake of RS and minerals per MJ of energy intake than the rats. The values for total, colonic and ileal absorption of magnesium, calcium, and phosphorus are similar to those found by van der Heijden et al. (29) but higher than those in the study of Larsen and Sandström (17). However, in the latter study the pigs were older and the magnesium and phosphorus intakes were higher than in our study.

Magnesium absorption tended to be shifted from colon to ileum to some extent in the piglets fed RS_2 and RS_3 when compared with those fed glucose. This may be connected with the findings that RS seems to be fermented already in the ileum, RS_2 to a greater extent than RS_3 (13). Fermentation in the ileum of the pig is possible as especially the distal third of the ileum contains a significant amount of bacteria (3, 5, 19), even though the pig is essentially a colon fermenter, like man (9). The bacteria found in the ileum are part of the normal ileal flora, and are not airborne microorganisms that came into the gut when the piglet was operated (5). In the study from van der Heijden et al. (29) magnesium was absorbed mainly from the colon, in contrast to our findings. Both in our study and that from van der Heijden et al. (29) calcium and phosphorus were absorbed mainly from the ileum. Larsen and Sandström (17) found that minerals were absorbed from the small intestine and excreted in the large intestine.

Because in the pig RS is fermented both in the ileum and the colon and in man probably almost exclusively in the colon, and mineral absorption may be affected by fermentation in the gut, the pig does not seem to be a good model for man to estimate the contribution of the various parts of the digestive tract to mineral absorption.

Table 1 Apparent absorption of magnesium, calcium, and phosphorus in cannulated piglets fed diets with glucose, uncooked resistant starch (RS_2), or retrograded resistant starch (RS_3)[1]

Mineral	Diet		
	Glucose	RS_2	RS_3
Magnesium			
Intake (mmol/d)	30 ± 0.5	30 ± 0.7	30 ± 0.5
Ileal absorption (% of intake)	33 ± 6.0	33 ± 5.2	37 ± 2.8
Colonic absorption (% of intake)	34 ± 3.9	21 ± 3.8	26 ± 4.2
Total absorption (% of intake)	67 ± 3.7[b]	54 ± 3.6[a]	63 ± 3.3[a,b]
Calcium			
Intake (mmol/d)	151 ± 2[b]	140 ± 3[a]	147 ± 2[a,b]
Ileal absorption (% of intake)	58 ± 1.9	51 ± 3.7	55 ± 1.9
Colonic absorption (% of intake)	21 ± 1.2	16 ± 2.7	20 ± 2.9
Total absorption (% of intake)	78 ± 2.5[b]	67 ± 2.5[a]	75 ± 2.2[a,b]
Phosphorus			
Intake (mmol/d)	107 ± 2[b]	100 ± 2[a]	101 ± 2[a,b]
Ileal absorption (% of intake)	69 ± 2.0[b]	56 ± 3.8[a]	63 ± 1.5[a,b]
Colonic absorption (% of intake)	7 ± 5.3	13 ± 2.6	12 ± 2.1
Total absorption (% of intake)	75 ± 6.8	69 ± 2.4	75 ± 2.3

[1]Values are means ± SEM for 6 piglets per dietary group. Values in the same row with different superscripts are significantly different.

Furthermore, the ileal starch digestibility in piglets and man may be different (24). Therefore, the amount of starch that was truly resistant in the piglets was most probably not the intended amount fed since the latter is based on *in vitro* RS analysis that is validated in ileostomy patients (8).

In conclusion, RS_2, but not RS_3, versus glucose reduced the total apparent absorption of magnesium and calcium in pigs in the present study, in contrast to the increase found in rats in earlier studies. This discrepancy may be explained by differences between species and/or by differences in RS and/or mineral intake.

Acknowledgments We thank Kasper Deuring and Anne Hoek (Institute for Animal Nutrition and Physiology ILOB/TNO, Wageningen) for their help in conducting the experiment; Piet Roeleveld (Institute for Animal Nutrition and Physiology ILOB/TNO, Wageningen) for preparation of the feeds; Inez Lemmens (Department of Laboratory Animal Science, Utrecht University) for laboratory analyses; and Jan Burema (Department of Human Nutrition, Wageningen Agricultural University) for statistical advice.

References

1. Ammann P, Rizzoli R, Fleish H (1986) Calcium absorption in rat large intestine *in vivo*: availability of dietary calcium. Am J Physiol 251:G14–G18
2. Andrieux C, Sacquet E (1986) Effects of amylomaize starch on mineral metabolism in the adult rat: role of the microflora. J Nutr 116:991–998
3. Bach Knudsen KE, Jensen JJ, Hansen I (1993) Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in β-D-glucan. Br J Nutr 70:537–556
4. Bronner F, Pansu D, Stein WD (1986) An analysis of intestinal calcium transport across the rat intestine. Am J Physiol 250:G561–G569
5. Chesson A, Richardson AJ, Robertson JA (1985) Fibre digestion and bacteriology of the digestive tract of pigs fed cereal and vegetable fibre. In: Just A, Jørgensen H, Fernández JA (eds) Digestive Physiology in the Pig. Report 580. National Institute of Animal Science, Copenhagen, pp 272–275
6. Cummings JH, Edwards C, Gee JM, Nagengast FM, Mathers JC (1995) Physiological effects of resistant starch in the large bowel. In: Asp N-G, van Amelsvoort JMM, Hautvast JGAJ (eds) Proceedings of the Concluding Plenary Meeting of EURESTA. CIP-data Koninklijke Bibliotheek, The Hague, pp 38–55, ISBN 90-9008390-1
7. Demigné C, Levrat M-A, Rémésy C (1989) Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. J Nutr 119:1625–1630
8. Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch

fractions. Eur J Clin Nutr 46 (Suppl 2):S33–S50
9. Graham H, Åman P (1986) The pig as model in dietary fibre digestion studies. Scand J Gastroenterol 22: 55–61
10. Hara H, Nagata M, Ohta A, Kasai T (1996) Increases in calcium absorption with ingestion of soluble dietary fibre, guar-gum hydrolysate, depend on the caecum in partially nephrectomized and normal rats. Br J Nutr 76:773–784
11. Hardwick LL, Jones MR, Brautbar N, Lee DBN (1991) Magnesium absorption: mechanisms and influence of vitamin D, calcium, and phosphate. J Nutr 121:13–23
12. Heijnen AMP, Brink EJ, Lemmens AG, Beynen AC (1993) Ileal pH and apparent absorption of magnesium in rats fed on diets containing either lactose or lactulose. Br J Nutr 70:747–756
13. Heijnen MLA, Beynen AC (1997) Consumption of retrograded (RS_3) but not of uncooked (RS_2) resistant starch shifts nitrogen excretion from urine to feces in cannulated piglets. J Nutr 127: 1828–1832
14. Heijnen MLA, van den Berg GJ, Beynen AC (1996) Dietary raw versus retrograded resistant starch enhances apparent but not true magnesium absorption in rats. J Nutr 126:2253–2259
15. Köhler T, Mosenthin R, Verstegen MWA, Huisman J, den Hartog LA, Ahrens F (1992) Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. 1. Growth performance, N-balance and intestinal adaptation. Br J Nutr 68:293–303
16. Köhler T, Verstegen MWA, Mosenthin R, Wensing T, den Hartog LA, Huisman J (1992) Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. 2. Blood variables and mineral balances. Br J Nutr 68:305–315
17. Larsen T, Sandström B (1993) Effect of dietary calcium level on mineral and trace element utilization from a rapeseed (*Brassica napus* L.) diet fed to ileum-fistulated pigs. Br J Nutr 69: 211–224
18. Levrat M-A, Rémésy C, Demigné C (1991) Very acidic fermentations in the rat cecum during adaptation to a diet rich in amylase-resistant starch (crude potato starch). J Nutr Biochem 2:31–36
19. Liu YF, Fadden K, Latymer E, Low AG, Hill MJ (1985) The use of the cannulated pig to study the effect of dietary fibre supplements on the bacterial flora of the porcine hindgut. In: Just A, Jørgensen H, Fernández JA (eds) Digestive Physiology in the Pig. Report 580. National Institute of Animal Science, Copenhagen, pp 300–303
20. Lupton JR, Kurtz PP (1993) Relationship of colonic luminal short-chain fatty acids and pH to *in vivo* cell proliferation in rats. J Nutr 123: 1522–1530
21. Nellans HN, Goldsmith RS (1981) Transepithelial calcium transport by the rat cecum: high-efficiency absorptive site. Am J Physiol 240:G424–G431
22. Olesen M, Rumessen JJ, Gudmand-Høyer E (1994) Intestinal transport and fermentation of resistant starch evaluated by the hydrogen breath test. Eur J Clin Nutr 48:692–701
23. Rayssiguier Y, Rémésy C (1977) Magnesium absorption in the caecum of rats related to volatile fatty acids production. Ann Rech Vét 8:105–110
24. Roe M, Brown J, Faulks R, Livesey G (1996) Is the rat a suitable model for humans on studies of cereal digestion? Eur J Clin Nutr 50:710–712
25. Scharrer E, Lutz T (1990) Effects of shortchain fatty acids and K on absorption of Mg and other cations by the colon and caecum. Z Ernährungswiss 29:162–168
26. Scharrer E, Lutz T (1992) Relationship between volatile fatty acids and magnesium absorption in mono- and polygastric species. Magnesium Res 5:53–59
27. Schulz AGM, van Amelsvoort JMM, Beynen AC (1993) Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. J Nutr 123:1724–1731
28. Trinidad TP, Wolever TMS, Thompson LU (1996) Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans. Am J Clin Nutr 63:574–578
29. van der Heijden A, Lemmens AG, Beynen AC (1995) Effect of dietary fructose versus glucose on apparent magnesium absorption in pigs and rats. J Anim Physiol Anim Nutr 74:123–130
30. van Leeuwen P, van Kleef DJ, van Kempen GJM, Huisman J, Verstegen MWA (1991) The post valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. J Anim Physiol Anim Nutr 65:183–193
31. Younes H, Levrat M-A, Demigné C, Rémésy C (1993) Relationship between fermentations and calcium in the cecum of rats fed digestible or resistant starch. Ann Nutr Metab 37:311–319
32. Younes H, Demigné C, Rémésy C (1996) Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. Br J Nutr 75:301–314