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Effect of amylose content of rice varieties on glycemic metabolism and biological responses in rats

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Abstract

The composition of rice grains can affect metabolic responses to rice consumption, mainly by the amylose:amylopectin ratio. Male Wistar rats were fed diets with grains of cooked rice of the cultivars BR-IRGA 409, Formosa and Mochi with high (TAH), intermediate (TAI) and low (TAL) amylose contents, respectively. During the experimental period, feed intake, body weight gain, apparent starch digestibility (ASD), wet and dry faecal production, faecal water content, faecal pH, faecal nitrogen excretion and postprandial blood glucose response were determined. Blood triglycerides and total cholesterol concentrations and liver weight were also determined. The animals in the TAH group presented lower ASD, higher wet and dry faecal production and faecal nitrogen excretion, reduced faecal pH, lower postprandial blood glucose response, serum triglyceride levels and liver weights, and higher serum total cholesterol levels. Amylose:amylopectin ratio significantly affects the rate and extension of rice starch digestion in the gastrointestinal tract, affecting some biologically relevant parameters.

Keywords: Rice grains; Metabolic responses; Amylose:amylopectin ratio; Rats

1. Introduction

Rice is one of the most important cereal crops worldwide, being widely used in human nutrition as a source of energy due to its high starch level (approximately 90% in polished white grains). However, the level of this constituent can vary among grains of different varieties due to genetic and environmental factors, as observed by Frei, Siddhuraju, and Becker (2003), who found values of total starch between 72% and 82% in brown rice grains of six cultivars grown in the Philippines. Besides, the rate and extension of starch digestion can be influenced by different factors, including variation in the amylose:amylopectin ratio, grain processing, physicochemical properties (particularly gelatinization characteristics), particle size and the presence of lipid–amylose complexes (Hu, Zhao, Duan, Linlin, & Wu, 2004).

These factors significantly affect some essential metabolic responses in the human organism, such as glycemic and insulinemic responses (Coffman & Juliano, 1987). Although rice is usually classified as a high glycemic response food when compared to other starchy foods, researchers report glycemic indices varying from 54% to 121%, for white rice (Brand, Nicholson, Thorburn, & Truswell, 1985; Brand-Miller, Pang, & Bramall, 1992; Jenkins, Wolever, & Taylor, 1981). This shows the importance of these factors in the metabolic responses to consumed rice.

The main differences in starch composition that influence physicochemical and metabolic properties of rice are caused by variation in the proportions of its two macromolecules, amylose and amylopectin (Zhou, Robards, Helliwell, & Blanchard, 2002). Amylose is essentially a

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linear molecule in which D-glucose units are linked by α-1,4 glucosidic bonds, while amylopectin, a branched polymer, contains both α-1,4 and α-1,6 bonds (FAO/WHO, 1997). Researches by Ong and Blanshard (1995) and Frei et al. (2003) report great variations in the amylose:amylopectin ratio in rice grains of different varieties, that allow their classification as waxy (1–2% amylose), very low amylose content (2–12%), low amylose content (12–20%), intermediate amylose content (20–25%) and high amylose content (25–33%) (Coffman & Juliano, 1987).

Considering the metabolic effects, Frei et al. (2003) report that starchy foods with high amylose levels are associated with lower blood glucose levels and slower emptying of the human gastrointestinal tract compared to those with low levels of this macromolecule. These conditions are relevant, especially in the formulation of diets for diabetics, because the slower digestion and absorption of carbohydrates help to maintain regular levels of glucose in the blood (FAO/WHO, 1997) and to reduce insulin response, probably due to increased time of intestinal transit (Lobo & Silva, 2001). This variation, associated with food processing, can result in different glycemic and insulinemic responses, and hormonal profiles (Kennedy & Burlingame, 2003).

Since the incidence of diabetes has been increasing in developing countries, and there are few studies that evaluate glycemic response to rice varieties from South America, the present research aimed at evaluating the effect of diets, with Brazilian cultivated rice grains of varieties with high, intermediate and low amylose contents, on glycemic and lipidic metabolism, as well as other biological responses in rats.

Table 1
Nutrient composition of the experimental diets (g/kg)

	TAH[A]	TAI[A]	TAL[A]
Rice	650.6	669.0	614.7
Casein	154.3	166.0	167.4
Sucrose	100.0	100.0	100.0
Soybean oil	68.6	68.5	68.6
Wheat fibre (isolated in neutral detergent)	34.1	36.2	34.6
Mineral mix[a]	35.0	35.0	35.0
Vitamin mix[b]	10.0	10.0	10.0
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
TBHQ[c]	0.014	0.014	0.014
Starch (%)	52.95	52.95	53.05
Protein (%)	20.0	20.0	20.0
Fat (%)	7.0	7.0	7.0
Crude energy (kcal)	3548	3548	3552

[A] TAH – treatment with high amylose content; TAI – treatment with intermediate amylose content; TAL – treatment with low amylose content.
[a] Mineral mix (g or mg/kg mix): Ca 142.94 g; P 44.61 g; K 102.81 g; Na 29.11 g; Cl 44.89 g; S 8.57 g; Mg 14.48 g; Fe 1.00 g; Zn 0.86 g; Si 0.14 g; Mn 0.30 g; Cu 0.17 g; Cr 0.03 g; B 14.26 mg; F 28.73 mg; Ni 14.31 mg; Li 2.85 mg; Se 4.28 mg; I 5.93 mg; Mo 4.32 mg; V 2.87 mg.
[b] Vitamin mix (g or mg/kg mix): nicotinic acid 3.00 g; Ca pantothenate 1.60 g; pyridoxine–HCl 0.70 g; thiamin–HCl 0.60 g; riboflavin 0.60 g; folic acid 0.20 g; biotin 0.02 g; vitamin B12 2.50 mg; vitamin E 15.00 g; vitamin A 0.80 g; vitamin D3 0.25 g; vitamin K1 0.075 g.
[c] TBHQ-tertiary-butylhydroquinone.

2. Materials and methods

2.1. Diets and treatments

Three diets were formulated according to the recommendations of the American Institute of Nutrition (AIN) (Reeves, Nielsen, & Fahey, 1993), by whole substitution of corn starch, and by partial substitution of casein and soybean oil for grains of cooked rice of the cultivars BR-IRGA 409, Formosa and Mochi, obtained from the Rice Experimental Station of IRGA (Cachoeirinha/RS), in the year of 2003. The mentioned diets were isocaloric, isoproteic and isoenergetic (Table 1). These three diets formed the treatments: the first, with high amylose content (TAH): diet formulated with cooked rice grains of the cultivar BR-IRGA 409, with 26.1% of amylose; the second, with intermediate amylose content (TAI): diet formulated with cooked rice grains of the cultivar Formosa, with 16.9% of amylose; the third, with low amylose content (TAL): diet formulated with cooked rice grains of the cultivar Mochi, with 2% of amylose.

2.2. Animals and experiment

The Ethics Committee for Laboratory Animals of Universidade Federal de Santa Maria (UFSM) agreed with the study protocol. Twenty-four male Wistar (F1) rats (*Rattus norvegicus* albino) (48.5 ± 1.6 g; age 21 days) were obtained from "Biotério Central" of UFSM and were randomly distributed among the treatments (8 animals/treatment), being individually housed in metabolic cages, with free access to feed and water. The period of adaptation to the experimental diets was 5 days. After that, the experimental period (16 days) began, when the determination of feed intake and the collection of the faeces were made daily. The body weight of the animals was obtained every three days. These data and samples were collected to determine feed intake, body weight gain, apparent starch digestibility, wet and dry faecal production, faecal water content, faecal pH and faecal nitrogen excretion. In the last three experimental days, all the animals were randomly selected in groups from 6 to 8 (2–3 animals/treatment) for analysis of the postprandial blood glucose response. After a 12 h overnight fast, the animals were fed 2 g of one of the test diets, totally consumed within 20 min. Blood samples of tail vein were taken at fasting (before the consumption of the meal) and 15, 30, 45, 60, 90 and 180 min after the meal, to measure serum glucose levels. In the last experimental day, after a 12 h overnight fast, the animals were weighed, anesthetized with ethyl ether and killed by cardiac puncture, and blood was collected for subsequent quantification of blood triglycerides and total cholesterol concentrations. On this occasion, the liver was immediately removed and weighed. During the adaptation and experimental period, temperature was maintained at 21 ± 1 °C, and lighting was controlled by an alternating 12-hour period of light and dark.

2.3. Analytical methods

The determinations of faecal water content (105 °C/12 h) and faecal nitrogen (Micro-Kjeldahl) were carried out according to methods mentioned in AOAC (1995). Faecal pH was obtained from a solution of 1 g of partly dried faeces (60 °C/48 h) in 10 ml of distilled water. Starch content of the experimental diets and faeces was determined by the AOAC method 996.11 (1998), modified by Walter, Silva, and Perdomo (2005). In this method, after the degradation of digestible starch by the combined use of α-amylase, protease and amyloglucosidase, resistant starch is solubilized with dimethylsulfoxide and hydrolyzed with α-amylase and amyloglucosidase, and the resulting glucose is determined by glucose oxidase-peroxidase reagent. Apparent starch digestibility (ASD) was calculated as the proportion of starch ingested that was not later recovered in the faeces. Serum glucose of the animals was determined using Accu-Chek Active® (Roche) monitoring kit. Serum triglycerides and total cholesterol in the blood were determined with the kits Enzymatic Triglycerides Liquid and Cholesterol 250, respectively, from Doles. The weight of the liver was calculated as g/100 g of animal weight.

2.4. Experimental design and statistical analysis

The experiment was carried out in a completely random design. The results obtained were submitted to analysis of variance, with the means compared by Duncan's test at 5% of significance. Statistical analyses were performed using SPSS 8.0 for Windows.

3. Results and discussion

3.1. Feed intake, body weight gain and apparent starch digestibility

The different amylose contents of the experimental diets did not affect feed intake and body weight of the animals (Table 2), as also observed by other researchers. Kabir et al. (1998), when supplying diets containing bean starch with 32% of amylose and waxy corn starch with 0.5% of amylose to normal and diabetic rats, did not observe significant effects on animals' feed intake and body weights. Similarly, studies with humans reported no influence of amylose content on consumption or satiety, as observed by Goddard, Young, and Marcus (1984), when studying the effect of diets with different rice cultivars (23–25%, 14–17% and 0% of amylose) on blood glucose and insulin responses.

Whereas apparent starch digestibility (ASD) results presented a similarity among treatments (Table 2), treatments with intermediate (TAI) and low (TAL) amylose contents showed higher values. Although digestibility studies based on analyses of starch in the faeces have demonstrated an almost complete digestion (99.9%) of starch in cooked and raw waxy and non-waxy rice cultivars in rats and humans (Eggum, Juliano, Perez, & Acedo, 1993), the influence of the molecular structure of the respective polymers of starch on the digestive metabolism should be considered. The hypothesis is that amylose, having an essentially linear and packed chain, is more compact in the granule, which makes the access of digestive enzymes difficult. It differs from the amylopectin molecule which, having a branched chain, allows greater access of these enzymes. Thus, amylose might not be totally digested by the enzymes in the gastrointestinal tract, being partly excreted in the faeces (Goddard et al., 1984; Behall, Scholfield, & Canary, 1988). This can explain lower ASD observed in this study for the treatment with high amylose content (TAH).

Table 2
Effects of different amylose:amylopectin ratios on animals' performance (age 26–42 days)

	TAH[A]	TAI[A]	TAL[A]
Feed intake (g/day)	15.85 ± 1.19[NS]	15.90 ± 1.07[NS]	15.97 ± 1.29[NS]
Body weight gain (g/day)	78.21 ± 7.04[NS]	77.80 ± 10.94 [NS]	74.55 ± 12.03[NS]
ASD[a] (%)	99.94 ± 0.024[b]	99.97 ± 0.016[a]	99.98 ± 0.008[a]

[A] TAH – treatment with high amylose content; TAI – treatment with intermediate amylose content; TAL – treatment with low amylose content.
[a] Apparent starch digestibility Results expressed as mean values ± standard deviation Mean values followed by different letter on the same line are significantly different (Duncan's test at a level of 5% of significance).

3.2. Wet and dry faecal production, faecal water content, faecal pH and nitrogen excretion

Wet and dry faecal productions were significantly higher in the treatment with high amylose content (TAH), while faecal water content was not affected (Table 3). The increased faecal production in the treatment with high amylose content (TAH), as well as the result of ASD, confirms the lower digestibility of amylose by the enzymes in the gastrointestinal tract. However, this fact cannot be attributed only to amylose excretion. Other studies have demonstrated that a higher consumption of slowly digestible and/or indigestible carbohydrates results in increased microbial activity in the gastrointestinal tract, especially of the acid bacteria species, increasing the excretion of microbial mass, which may represent a significant percentage of the faecal production (Wenk, 2001). The products generated by these microbial populations are potentially protective of the gastrointestinal tract, and can also increase immunoglobulin production (Morita et al., 1998). Besides, the increased faecal production also has an important effect on constipation and haemorrhoids prevention (Baghurst, Baghurst, & Record, 1996). This increase in the microbial activity is also demonstrated by the reduction in faecal pH and increased faecal nitrogen excretion observed in the treatment with high amylose content (Table 4).

Table 3
Effects of different amylose:amylopectin ratios on mean faecal production and faecal water content (age 26–42 days)

	TAH[A]	TAI[A]	TAL[A]
WFP[a] (g/day)	0.97 ± 0.10^{a}	0.83 ± 0.11^{b}	0.87 ± 0.06^{b}
DFP (g/day)[b]	0.71 ± 0.06^{a}	0.64 ± 0.07^{b}	0.65 ± 0.06^{ab}
Faecal water content (%)	26.24 ± 3.47^{NS}	25.22 ± 4.51^{NS}	24.80 ± 4.72^{NS}

[A] TAH – treatment with high amylose content; TAI – treatment with intermediate amylose content; TAL – treatment with low amylose content.
[a] Wet faecal production.
[b] Dry faecal production results expressed as mean values ± standard deviation. Mean values followed by different letter on the same line are significantly different (Duncan's test at a level of 5% of significance).

Table 4
Effects of different amylose:amylopectin ratios on faecal pH and nitrogen excretion (age 26–42 days)

	TAH[A]	TAI[A]	TAL[A]
Faecal pH	6.39 ± 0.19^{b}	6.56 ± 0.13^{ab}	6.64 ± 0.20^{a}
Faecal nitrogen excretion (%)	5.27 ± 0.11^{a}	4.17 ± 0.25^{b}	3.94 ± 0.23^{c}

Results expressed as mean values ± standard deviation Means values followed by different letter on the same line are significantly different (Duncan's test at a level of 5% of significance).
[A] TAH – treatment with high amylose content; TAI – treatment with intermediate amylose content; TAL – treatment with low amylose content.

From the results, it can be hypothesized that, with a higher amylose content in the diet, more substrate becomes available for fermentation, which, when reaching the colon, is fermented by the bacterial flora, resulting in the production of organic acids. Part of these acids is used by the organism, with beneficial effects on health, and part is excreted in the faeces, resulting in lower pH, observed in the treatment with high amylose content (TAH), which is desirable for the maintenance of the intestinal microflora. The increase in faecal nitrogen excretion is also an indication of increased fermentative activity, as observed in the present study regarding the treatment with high amylose content (TAH) (Table 4). A similar result was observed by Younes, Demigné, Behr, and Rémésy (1995) who, adding non-digestive fractions of starch to the diet of rats, observed a significant increase in faecal nitrogen excretion, which is normally associated with considerable development of caecal microflora (Eggum, Beames, Wolstrup, & Bach Knudsen, 1984), since the breakdown of high amounts of carbohydrates increases nitrogen incorporation in bacterial proteins (Younes et al., 1995).

The nitrogen required for optimal bacterial growth is provided by proteins escaping small intestinal breakdown, endogenous proteins (pancreatic and intestinal secretions, sloughed epithelial cells), or blood urea diffusing into digestive contents (Younes et al., 1995). Therefore, the increase in faecal nitrogen excretion could correspond to an increased faecal excretion of bacterial proteins and the change of nitrogen excretion from urine to the faeces (Demigné & Rémésy, 1982). This may help the management of chronic renal disease (Younes et al., 1995). Besides, several sources of nitrogen used for rapid bacterial growth are metabolites of protein (phenol, cresol, indoles, amines and ammonia) that may have deleterious effects on the organism, such as development of skin, bladder and bowel cancer. Therefore, the presence of fermentable carbohydrates in the colon, neutralizing these metabolites, reduces the risk of certain kinds of cancer (Tharanathan, 2002).

3.3. Serum glucose, triglyceride and cholesterol in the blood and liver weight

The animals undergoing treatment with low amylose content (TAL) presented higher postprandial blood glucose responses than did the animals undergoing treatments with intermediate and high amylose contents (Fig. 1). Similar to the results observed in this work, Kabir et al. (1998), evaluating the effect of amylose content on glycemic response in rats, observed that the consumption of a diet rich in amylose for three weeks reduced the postprandial blood glucose response, glucose incorporation into lipids and epididymal fat pads of the animals. Similarly, Goddard et al. (1984) and Brand-Miller et al. (1992), evaluating the effect of increased contents of amylose of rice cultivars on the glycemic response in humans, also observed that amylose contents are directly related to glycemic and insulinemic responses. The higher postprandial blood glucose response in the animals undergoing treatment with low amylose content (Fig. 1) is explained by the lower digestibility of this polymer when compared to amylopectin. Being more slowly digested and absorbed by the organism, diets with high amylose content result in lower blood glucose concentration, as observed in Fig. 1. The lower glycemic response observed for the treatments with high amylose content is especially important for patients with diabetes, helping to maintain regular levels of blood glucose.

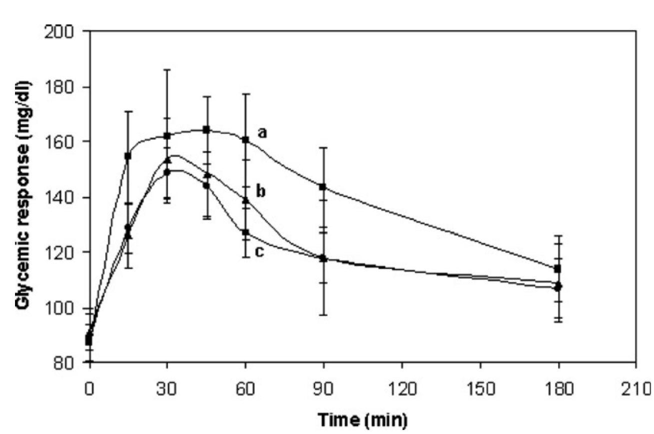

Fig. 1. Postprandial blood glucose response in rats consuming feeds with different amylose:amylopectin ratios. (-■-) treatment with low amylose content (TAL), (-▲-) treatment with intermediate amylose content (TAI), (-●-) treatment with high amylose content (TAH).

However, according to Panlasigui et al. (1991), the amylose content of rice alone should not be considered a decisive factor for starch digestibility and glycemic response to this cereal, since rice cultivars with similar amylose contents can differ in physicochemical properties, especially gelatinization. Studying the rate of *in vitro* starch digestion and the *in vivo* blood glucose and insulin responses to three varieties of long-grain rice with similar amylose contents (26.7–27%), the mentioned researchers observed lower responses to glucose and insulin in diets composed of grains of rice cultivars that presented technological characteristics of high gelatinization temperature, high minimum cooking time, low amylograph consistency and low expansion volume upon cooking. Some researchers report high blood glucose concentrations as being the main determining factor for high serum total cholesterol and triglyceride concentrations, influencing the progression of coronary diseases and non-insulin-dependent diabetes (Zavarini, Bonora, & Pagliara, 1989). In the present work, the serum triglyceride levels were higher in the treatments with higher glycemic response (TAL and TAI). However, as opposed to expected findings, total cholesterol level in serum increased in the treatment with lower glycemic response (TAH) (Table 5). Similarly, Behall and Howe (1995), evaluating the effect of diets with 30% and 70% of amylose in humans, observed significant decrease in the serum triglycerides, insulin and total cholesterol levels after the consumption of a diet rich in amylose compared to a diet rich in amylopectin.

The results also demonstrate that the higher the amylose content, the lower is the liver weight (Table 5). This fact can be explained by the relationship between starch digestibility and its effect on hepatic metabolism of glucose. Reaching the liver, the glucose originating from starch degradation follows three main pathways: (a) transport to the blood, in order to maintain its concentration sufficiently high to supply energy to the brain and other tissues; (b) conversion into glycogen, and storage in the liver and muscles; (c) conversion into fatty acids, and transport by the triglycerides (Lehninger, Nelson, & Cox, 1995). Amylopectin, being more easily degraded, provides a higher glucose flow to the liver than does amylose, within the same period of time. This higher flow produces excess of glucose in the liver, where it will be metabolized into fatty acids, increasing serum triglyceride concentration. This higher flow of glucose to the liver also results in increased hepatic metabolism, promoting hypertrophy and/or higher fat accumulation in this organ, which can explain the increased liver weight observed in the animals submitted to the treatments with lower amylose contents (TAL and TAI). We emphasize, however, that the responses to the different amylose levels were not linear, which indicates that the molecular interaction between starch types is differentiated according to changes in their proportions as well as to genotype (BR-IRGA 409, *indica* type; Formosa and Mochi, *japonica* type). This could influence the spatial distribution of the molecules in the grain, directly reflecting the individual's metabolic response.

Table 5
Fasting serum triglycerides and total cholesterol concentrations and liver weights in rats consuming feeds with different amylose:amylopectin ratios

	TAH[A]	TAI[A]	TAL[A]
Triglycerides (mg/dl)	15.4 ± 1.27^{b}	29.5 ± 5.60^{a}	20.5 ± 9.75^{ab}
Total cholesterol (mg/dl)	112 ± 12.68^{a}	79.4 ± 6.42^{b}	87.5 ± 8.96^{b}
Liver weight (g/100 g of animal weight)	3.81 ± 0.23^{b}	4.18 ± 0.32^{a}	4.26 ± 0.34^{a}

Results expressed as mean values ± standard deviation.
Means values followed by different letter on the same line are significantly different (Duncan's test at a level of 5% of significance).
[A] TAH – treatment with high amylose content; TAI – treatment with intermediate amylose content; TAL – treatment with low amylose content.

4. Conclusion

The results observed in this work allow one to conclude that the amylose:amylopectin ratio significantly affects the rate and extension of rice starch digestion in the gastrointestinal tract, influencing faecal excretion and constitution, postprandial blood glucose response, serum triglycerides and total cholesterol concentration, and liver weight. Thus, amylose content, normally used to evaluate some properties of product consumption (cohesion, softness, cooking water consumption, cooking time and cooking gravimetric and volumetric yields), can help in the choice of the grain to be used in the diet and to aid the control of some biologically relevant parameters, such as blood glucose and triglyceride concentrations.

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