Biological response of rats to resistant starch

Resposta biológica de ratos ao amido resistente

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ABSTRACT

Several investigations have been done on resistant starch (RS) due to its beneficial effects, such as reduction of dietary energy, increased fecal production and growth of beneficial microorganisms in intestinal tract, among others. The present study aimed at evaluating the influence of RS on some biological response parameters. Male Wistar rats (97.1±5.3g) were fed diets supplemented with 0, 3, 9 and 18% of RS. Animals were submitted to an adaptation period of five days. During the experimental period (15 days), data and samples were obtained to assess the following parameters: feed intake, body weight gain, apparent dry matter digestibility, apparent starch digestibility, wet and dry fecal production, fecal water content, pH and nitrogen. Addition of RS to the diets did not influence the feed intake, but it significantly reduced the animals body weight. Wet and dry fecal productions were significantly higher at adding 9 and 18% of RS. The consumption of RS also significantly increased the water content and nitrogen excretion in feces**,** and decreased the fecal pH. The effects observed in the present study might essentially resulted from reduced apparent starch digestibility**,** and from its fermentation by intestinal microflora, demonstrating the beneficial effects of RS on health maintenance.

Key Words. starch, undigestible carbohydrates, dietary fiber, intestinal function, fecal production, nitrogen excretion.

RESUMO

O amido resistente (AR) vem sendo intensamente pesquisado nos últimos anos em função de seus efeitos benéficos, como redução da energia da dieta, aumento da produção de fezes e desenvolvimento de microrganismos benéficos no trato intestinal, entre outros. O objetivo deste trabalho foi avaliar a influência do AR da dieta sobre alguns parâmetros de resposta biológica. Foram utilizados ratos machos Wistar (97,1±5,3g) alimentados com rações experimentais suplementadas com 0, 3, 9 e 18% de AR. Os animais foram submetidos a um período de adaptação de 5 dias e, durante o período experimental (15 dias), foram obtidos dados e amostras para a determinação do consumo, ganho de peso, digestibilidade aparente da matéria seca e do amido, produção de fezes úmidas e secas, umidade, pH e nitrogênio nas fezes. A adição de AR às rações não influenciou o consumo, mas diminuiu significativamente o peso dos animais. A produção de fezes úmidas e secas foi significativamente maior em níveis de 9 e 18% de AR. O consumo de AR também aumentou significativamente o teor de umidade e de nitrogênio nas fezes, bem como diminuiu o pH fecal. Os efeitos observados neste trabalho podem ser essencialmente atribuídos à menor digestibilidade aparente do amido e a sua fermentação pela microflora intestinal, o que demonstra seu efeito benéfico no auxílio à manutenção da saúde.

Palavras-Chave. amido, carboidratos indigestíveis, fibra dietética, função intestinal, produção de fezes, excreção de nitrogênio.

INTRODUCTION

Several modern diseases result from inadequate feeding, and some may be related to insufficient fiber intake. Although this fraction exerts important biological effects on health balance, its consumption is restricted, especially due to its sensorial properties, since the addition of traditional fiber sources causes pronounced organoleptic alterations (e.g. bread enriched with wheat bran)[1].

In this context, there is the option of using another indigestible carbohydrate, resistant starch (RS), defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals[2]. It has properties similar to fiber, but with less pronounced organoleptic alterations.

RS, not being digested by the enzymes in the human gastrointestinal tract, is considered a reductor of dietary energy, and may have significant effects on body weight[3]. It also increases fecal mass, helps prevent constipation and hemorrhoids, and dilutes toxic compounds[1]. This fraction has also been associated with reduced post-prandial glycemic and insulinemic responses, which may have beneficial implications for the management of diabetes[4]; and reduced blood levels of both cholesterol and triacylglycerols[5,6].

Behaving as a substrate for growth of beneficial microorganisms, RS may act as a potential prebiotic agent[7]. Besides, the products of starch fermentation reduce cecal and fecal pH[8,9], and help in the prevention of inflammatory intestinal diseases and certain types of cancer[10].

Considering this, the present research aimed at evaluating RS effects on feed intake, body weight gain, apparent dry matter digestibility, apparent starch digestibility, wet and dry fecal excretion, fecal water content, fecal pH and fecal nitrogen excretion in rats.

MATERIALS AND METHODS

Diets and treatments

Four diets (AIN 93G) were formulated (Table 1), according to the recommendations of the American Institute of Nutrition (AIN)[11], all of them containing equivalent contents of protein, fat, vitamins, minerals and total carbohydrates, but differing in RS content. The starch source was cornstarch containing less than 0.1% of RS. The RS source was a high amylose corn starch (Novelose 260®), containing 53.5% of RS type 2, obtained from National Starch & Chemical Industrial Ltda (Sao Paulo, Brazil). These diets formed the treatments:

- **Control:** diet not supplemented with RS;
- **3% RS:** diet where Novelose 260® substituted for part of the cornstarch in order to obtain 3% of RS in the total composition;
- **9% RS:** diet where Novelose 260® substituted for part of the cornstarch in order to obtain 9% of RS in the total composition;
- **18% RS:** diet where Novelose 260® substituted for part of the cornstarch in order to obtain 18% of RS in the total composition.

Animals and experiment

Thirty-two male rats (*Rattus norvegicus*, Wistar albino F1, Biotério Central/Universidade Federal de Santa Maria, age 30 days, initial body weight 97.1±5.3g) were randomly

Table 1. Composition (g/kg) of the experimental diets (AIN 93G).

	Control	3% RS	9% RS	18% RS
Cornstarch	620.692	564.622	452.472	284.242
Casein	140	140	140	140
Sucrose	100	100	100	100
Soybean oil	40	40	40	40
Purified cellulose	50	50	50	50
Novelose 260®	0	56.07	168.22	336.45
Mineral and vitamin mix (1)	20	20	20	20
Sodium chloride	2.60	2.60	2.60	2.60
Bicalcium phosphate	15	15	15	15
Calcium carbonate	6	6	6	6
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5
TBHQ (2)	8	8	8	8

(1) Mineral and vitamin mix (g or mg/kg mix): K 102.86g; S 8.57g; Mg 14.48g; Fe 1.00g; Zn 0.86g; Si 0.14g; Mn 0.30g; Cu 0.17g; Cr 0.028g; B 14.26mg; F 28.73mg; Ni 14.31mg; Li 2.85mg; Se 4.28mg; I 5.93mg; Mo 4.32mg; V 2.87mg; nicotinic acid 3.00g; Ca pantothenate 1.60g; pyridoxine-HCl 0.70g; thiamin-HCl 0.60g; riboflavin 0.60g; folic acid 0.20g; biotin 0.02g; vitamin B12 2.50g; vitamin E 15.00g; vitamin A 0.80g; vitamin D3 0.25g; vitamin K1 0.075g.
(2) TBHQ: tert-butylhydroquinone.

distributed among the treatments (8 animals/treatment), and individually housed in metabolic cages, with free access to diet and water. The period of adaptation was 5 days, during which the animals were consuming the experimental diets. After that, the experimental period (15 days) began, when the determination of feed intake and the collection of the feces were made daily. The body weight of the animals was obtained every three days.

During adaptation and experimental period, temperature was maintained at 21±2ºC, and lighting was controlled by alternating periods of 12 hours of light and dark.

Analytical Methods

The determination of fecal water content (105ºC/12h) and fecal nitrogen (Micro-Kjeldahl) were carried out according to methods mentioned in AOAC[12]. Fecal pH was obtained from a solution of 1g of partly dried feces (50°C/48h) in 10ml of distilled water (method developed by the Núcleo Integrado de Desenvolvimento em Análises Laboratoriais, by orientation of Dr. Leila Picolli da Silva).

Starch content of the starch and RS sources and feces was determined by the AOAC[12] method 996.11, modified by Walter[13]. The samples (300mg) were incubated with thermostable α-amylase (100µl) in phosphate buffer pH 6.8 (3ml) at 95ºC for 5 minutes, protease (100µl) at 60ºC for 30 minutes, and amyloglucosidase (100µl) in acetate buffer 200mM pH 4.5 (4ml) at 50ºC for 30 minutes. After centrifugation, the supernatants were used to the quantification of digestible starch (DS). The residues were treated with dimethylsulfoxide (DMSO) (2ml) at 95ºC for 5 minutes, and then incubated with α-amylase (100µl) in phosphate buffer pH 6.8 (3ml) at 95ºC for 5 minutes, and amyloglucosidase (100µl) in acetate buffer 200mM pH 4.5 (4ml) at 50ºC for 30 minutes. After centrifugation, the supernatants were used to the quantification of RS. Glucose from DS and RS degradation was quantified by reaction with glucose oxidase-peroxidase reagent.

Apparent dry matter digestibility (ADMD) was calculated as the proportion of ingested dry matter that was not later recovered in the feces[14]. Apparent starch digestibility (ASD) was calculated as the proportion of starch ingested that was not later recovered in the feces[14].

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. The results were also submitted to correlation analysis at 5% of significance. Statistical analysis was performed using SPSS for Windows 8.0 (1997).

RESULTS AND DISCUSSION

Feed intake, body weight gain, apparent dry matter digestibility and apparent starch digestibility

The addition of RS to the experimental diets did not influence feed intake, but reflected significantly on body weight (Table 2). Some researches have demonstrated that increases in diluting compounds, even though they reduce digestible energy in the diet, do not cause significant effect on feed intake and, many times, on body weight gain of the animals[15]. Schulz et al.[16] and Younes et al.[17], using 24% of RS in the diet of young rats, also did not observe significant effects on feed intake and body weight of the animals. In studies with humans, the inclusion of 30g of RS per day in the diet did not affect the body weight, although the subjects reported increased satiety[18]. These results may be explained by the fact that, although the energy value of starch that was excreted intact in the feces is zero, fermented starch has a positive energy value, although significantly lower than that of starch which had been digested and absorbed in the form of glucose[19].

The addition of 18% of RS in the diet did not affect feed intake, but significantly reduced the body weight gain of the animals (Table 2). This was also observed in the results by Morand et al.[3], using the same level of RS in the diet of adult rats, demonstrating that this effect is independent of the

Table 2. Effect of increasing levels of resistant starch (RS) on animals' performance.

	Control	3% RS	9% RS	18% RS
Feed intake (g) (1)	104.06±11.15 a	101.91±10.27 a	103.91±3.31 a	99.95±9.18 a
Body weight gain (g) (2)	1.34±4.89 a	1.01±2.93 a	-0.75±2.23 a	-6.76±4.22 b
ADMD (%) (3)	91.91±0.53 a	91.35±0.61 a	88.95±0.99 b	86.83±1.27 c
ASD (%) (4)	99.29±0.11 a	98.82±0.37 b	98.26±0.35 c	97.86±0.31 d

(1) Feed consumed during the experimental period.
(2) Body weight gained during the experimental period.
(3) Apparent dry matter digestibility.
(4) Apparent starch digestibility.
Results expressed as mean value ± standard deviation.
Mean values followed by the same letter on the same line are not significantly different (Duncan's test at a level of 5% of significance).

development stage of the animal. Researches show that diets rich in RS reduce epididymal fat pads[5,20], probably due to the reduction of dietary energy caused by the substitution of DS by RS. Thus, it may reduce body fat and, consequently, body weight, just like the results obtained in the present research.

The dilution effect of RS is evidenced by the lower values of apparent dry matter digestibility (ADMD) in the present study, ascribed essentially to the lower apparent starch digestibility (ASD) (Table 2). However, it was expected a more pronounced reduction in the ASD, in accordance with the RS levels of the experimental diets. This fact was not observed, because part of the RS that escapes digestion in the small intestine is fermented by the colonic bacteria, reducing the amount of starch excreted in the feces. Andrieux e Sacquet[21] reported ASD values of 98% for conventional rats, compared to only 68% in germ free rats, demonstrating the effect of the microflora on RS fermentation. This increased bacterial activity may reflect significantly on quantitative and qualitative fecal characteristics, such as production and water content.

Wet and dry fecal production, and fecal water content

Wet and dry fecal production, although not affected by the addition of 3% of RS to the diet, was significantly increased with 9 and 18% of RS (Table 3). Fecal water content was significantly increased by RS addition, regardless of the level used (Table 3).

De Schirjver et al.[22], including 6% of RS to the diet of rats and pigs, also reported a significant increase in fecal production. Similarly, Faulks et al.[23], De Deckere et al.[5] and Verbeek et al.[24] observed increased wet and dry fecal production adding 10, 14 and 14% of RS to the diet of rats, respectively. In studies with humans, the consumption of 30 to 39g of RS per day, besides increasing fecal production, also facilitated defecation, but caused increased flatulence[18,25].

Other studies with humans also demonstrated that higher consumption of RS increases fecal production. Shetty e Kurpad[26], providing 100g per day of cornstarch rich in RS, reported an increase of 30% in fecal mass, without modifying the transit time. Scheppach et al.[27], by inhibiting starch digestion in the small intestine with acarbose, observed an increase of 68% in this parameter.

This increase of fecal production cannot be attributed only to the resistance of starch to digestion, since its apparent digestibility was higher than expected (Table 3), but also to its effects on bacteria present in the ceccum and large intestine, and on water retention. Eastwood[28] and Wenk[29] reported that the increased bacterial activity in the gastrointestinal tract, promoted by the higher content of indigestible carbohydrates, increases the excretion of bacterial constituents, which may represent a significant part of the fecal mass.

Regarding the higher fecal water content, De Schrijver et al.[22] and Gee et al.[19] reported results similar to those obtained in the present study, adding, respectively, 6 and 10% of RS to the diet of rats.

Stephen e Cummings[30] and Jeraci e Horvath[31] explain that the increase in fecal water content of animals fed diets rich in indigestible compounds may not be related only to its hydration capacity, but also to a higher production and excretion of bacterial mass (demonstrated by the increased fecal nitrogen excretion and the reduced fecal pH – Table 4), which also has a high water-holding capacity.

The increased fecal production is important to prevent constipation and hemorrhoids, as well as to provide a substrate for increased bacterial growth, which increases production and concentration of potentially protective by-products while diluting production and concentration of potentially toxic compounds[9].

Fecal pH and nitrogen excretion

The increase of RS levels in the experimental diets significantly influenced fecal pH ($r = -0.90$; $p<0.01$) and fecal nitrogen excretion ($r = 0.84$; $p<0.01$) (Figure 1, Table 4).

The decreased pH with RS consumption as well as the increased fecal concentration of short chain fatty acids was also observed in studies with humans[8,25]. Similarly, Hillman et al.[32] reported a reduction in fecal pH with increasing levels of cellulose in diets for humans. This effect may be explained by the increase of indigestible carbohydrates available for fermentation, which, reaching the colon, are fermented by the microflora, resulting in the production of organic acids. Part of these acids is used by the organism, and part is excreted in the

Table 3. Effect of increasing levels of resistant starch (RS) on fecal production and water content.

	Control	3% RS	9% RS	18% RS
WFP (g) [(1)]	10.68±1.07 [c]	11.97±2.50 [c]	15.64±1.18 [b]	18.72±2.85 [a]
DFP (g) [(2)]	8.70±0.83 [c]	8.82±1.10 [c]	11.48±1.00 [b]	12.70±1.62 [a]
Fecal water content (%)	18.47±1.22 [b]	25.21±6.33 [a]	26.42±6.27 [a]	30.06±7.86 [a]

[(1)] Wet fecal production during the experimental period.

[(2)] Dry fecal production during the experimental period.

Results expressed as mean value ± standard deviation.

Mean values followed by the same letter on the same line are not significantly different (Duncan's test at a level of 5% of significance).

feces, resulting in lower pH, which is desirable for the maintenance of a healthy intestinal microflora.

The increase in fecal nitrogen excretion is also an indication of increased fermentative activity in the cecocolic region of animals submitted to diets with higher levels of RS (Table 4). De Schrijver et al.[22] and Younes et al.[17] reported that the addition of 6 and 25% of RS to the diet of rats, respectively, significantly increased fecal nitrogen excretion. Likewise, the consumption of diets rich in fiber also increases fecal nitrogen excretion, which is normally associated with considerable development of ceccum microflora[33].

Probably, the results obtained in these studies, as those obtained in the present study, are due to the accelerated growth of cecocolic microorganisms, since the breakdown of high amounts of carbohydrates increases nitrogen incorporation in bacterial proteins[34,17].

The nitrogen required for optimal bacterial growth is provided by proteins escaping small intestine breakdown, endogenous proteins (pancreatic and intestinal secretions, sloughed epithelial cells), or blood urea diffusing into digestive contents[17]. Therefore, the increase in fecal nitrogen excretion could correspond to an increased fecal excretion of bacterial proteins and to a shift of nitrogen excretion from urine to the feces[35].

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. So the presence of fermentable carbohydrates in the colon, neutralizing these metabolites, reduces the risk of certain kinds of cancer[10]. Besides, the shift of nitrogen excretion from urine to feces may help the management of chronic renal disease[17].

Through the results obtained in the present study, we may conclude that, except for feed intake, RS type 2 significantly affected all other evaluated parameters. These effects are essentially attributed to the lower apparent starch digestibility and its fermentation by the intestinal microflora, which demonstrate its beneficial effect on health maintenance.

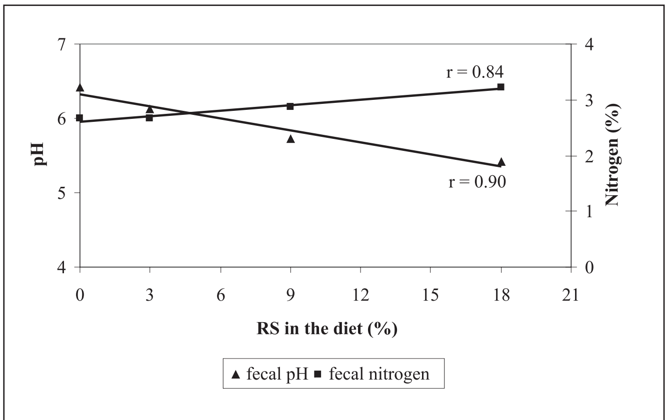

Figure 1. Effect of different levels of resistant starch (RS) in the diet on fecal pH and nitrogen excretion in rats.

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Table 4. Resistant starch (RS) effects on fecal pH and nitrogen excretion.

	Control	3% RS	9% RS	18% RS
Fecal pH	6.42±0.08 ^a^	6.12±0.15 ^b^	5.73±0.21 ^c^	5.41±0.22 ^d^
Fecal nitrogen excretion (%)	2.66±0.12 ^c^	2.66±0.12 ^c^	2.88±0.04 ^b^	3.22±0.22 ^a^

Results expressed as mean value ± standard deviation.
Mean values followed by the same letter on the same line are not significantly different (Duncan’s test at a level of 5% of significance).

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