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EFFECT OF MILLED RICE AMYLOSE CONTENT ON IN-VITRO DIGESTIBILITY AND GLUCOSE RESPONSES IN RATS

RIZK, LAILA F.[1], HANAA. A. DOSS[1], A. A. EL-HISSEWY[2] AND M. M. WASIF[3]

1 Food Technology Research Institute, ARC.

2 Rice Technology Training centre. ARC.

3 Nutrition Institute, Cairo.

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Abstract

Two rice varieties Giza 175 (high amylose) and Giza 181 (low amylose) were used in this investigation. The in vitro and in vivo digestibilities of raw, cooked milled rice and cooked parboiled rice of the two varieties were studied.

The hydrolysis rates with α amylase of high amylose rice (179.14-180.53) were lower than of those with low amylose rice (188.73-209.85).

Weight gains of rats differed significantly between groups. High amylose rice caused increase in feed efficiency in diabetic rats.

Rice with a higher amylose content, decreased serum glucose level from (321 to 213-226 mg/100ml than did low amylose rice (255-269 mg/100ml), the same trend was observed for serum cholesterol.

For cooked high amylose rice, it lowered the serum glucose to 213 against 269mg/100ml for cooked rice with low amylose, while in cooked parboiled rice, their values were 226 against 255 mg/100ml.

INTRODUCTION

Rice is the major staple food and source of calories for over half the world population. It is nutritious cereal grain whose variation in composition and form can be used to optimize carbohydrate absorption. Starchy endosperm is the single major constituent of rice grains (85-90%), varying ratios of amylose versus amylopectin have (recently) become an issue of nutritional concern.

Yoshizawa *et al.*, (1981) showed that digestibility of starch by amylose at an early stage of digestion was inclined to be higher in the starch containing less amylose. Gee and Johnson (1985) showed that the rate of hydrolysis of starch appears to be the most important factor governing the human glycemic response to food. Miller *et al.*, (1992) determined the glycemic (GI) and insulin index (II) values for 12 rice products using eight healthy subjects. The high amylose rice gave a lower GI and II than did the normal amylose and waxy rice varieties. They indicated that many varieties of rice whether white, brown, or parboiled should be classified as high GI food, only high amylose varieties gave low GI diets.

Oxue and Behall (1993) studied the effect of varying ratios of amylose versus amylopectin in foods. High amylose (HA) starches from corn and barley had been fed to chicks and rats and to human subjects in short and long term studies, the goals of these studies were investigating the effects of HA foods, to decrease blood insulin, glucose, cholesterol and triglycerides in normal subjects and those of higher than normal insulin response to a glucose tolerance test.

Eggum *et al.*, (1993) studied the digestible energy and protein content of raw and cooked milled rice and of cooked parboiled high amylose rice. In 5 rice varieties, differing in amylose content, the *in-vitro* and *in vivo* resistant starch levels were low values and were positively correlated with amylose content. High resistant starch levels were found in cooked and parboiled cooked rices than in raw rices. Values for undigestible energy were similar in raw rices but increased with processing, particularly in the higher amylose rices. The resistant starch content of cooked rice was increased by 1% with parboiling. Also, casiraghi *et al.*, (1993) found that percentage of starch digested were lower in parboiled and quick cooking parboiled rice than in polished rice.

Yokovame (1994) found that the rate of glucose absorption was affected by amylose content, form, milling and processing conditions. Rice varieties higher in amylose reported to produce relatively low serum glucose responses in human subjects.

Rani and Bhattacharya (1995) investigated microscopy of rice starch granules during cooking and found that low amylose rice starch showed total granules disintegration after 60min of cooking at 95°C, but high amylose granules showed only marginal disorganization. Also, low amylose starch granules are weak, fragile, so swell and easily disintegrated, while high amylose rice starch was relatively strong rigid and can resist swelling as well as disintegration.

Ong and Blanshard (1995) found that hard cooking rice had a higher amylose content (amylose/amylopectin ratio) and longer chain amylopectin than soft cooking rice. Longer chain amylopectin may encourage greater intra and/or inter molecular interaction with other rice grain components, giving firm texture. Also, different amylopectin structures may explain the different textural properties of rice with similar amylose content.

Crosby (1998) studied the properties of high amylose corn starch, the amylose molecule can form strong intra and inter molecular network and was the predominant gel forming polymer, producing stronger, more thermally stable gels.

The aim of this study was to clarify the response of high amylose rice to plasma glucose and cholesterol.

MATERIALS AND METHODS

Raw and cooked milled rice and cooked parboiled milled rice of two varieties (high amylose and low amylose) were used in this study to investigate the effect of varying ratio of amylose versus amylopectin in rice to decrease blood glucose *in-vitro* and *in-vivo* studies.

The milled rice varieties Giza 175 (high amylose) and Giza 181 (low amylose) were obtained from the rice Breeding section, field crops Research Institute. The same two varieties of white rice after parboiling were obtained from Rice Technology Training Center, Alexandria, Egypt (RTTC).

Processing :

Rice were tested in three forms:

1. Raw rice (uncooked white rice)
2. Cooked rice
3. Cooked parboiled rice

Cooking:

Each sample was cooked for the predetermined length of time in excess water, then the sample was semidried at room temp., followed by drying in an air oven.

Chemical analysis

A portion of each sample was ground using universal type laboratory rice mill. The contents of moisture, protein and carbohydrates were determined as reported in A.O.A.C (1990). The procedure of Juliano (1971) was used for the determination of amylose content in milled rice.

In-vitro Digestion

The rice was tested *in-vitro* (raw, cooked and cooked parboiled rice) to investigate their susceptibility to (-maylase using the method of Michalinos, (1962), then ferricyanide number was determined according to the procedure given by Kerr and Severson (1943).

In vivo study:

In the present study, the effect of high amylose rice as antidiabetic agent was investigated. Male albino rats Sprague Dawley Strain (forty eight) weighing between 140 and 185g. provided from Nutrition Institute, were housed individually in wire cages with screen bottom. The experimental animals were fed for one week on standard diet.

The standard diet was consisted of Casein 10%, cotton seed oil 10%, cellulose 5%. Salt mixture 4% (Hegested *et al.*, 1941), vitamin mixture 1% (Campbell 1961), corn starch 69.8% and cholin chloride 0.2%. After that period rats were divided into two groups, the first group of 6 rats was fed on standard diet and was considered as control A.

The second group (42 rats) was injected with 150mg/kg body weight of re-crystallized alloxan to induce hyperglycemia after fasting over night according to Lazzarow and Palay, (1954).

To ensure occurrence of diabetes in rats, blood sample was withdrawn 48 hour after alloxan injection and glucose (mg/100) was determined according to Asatorr and King (1954).

One group (6 rats) from diabetic rats were fed on standard diet and kept as positive control (B). The other diabetic groups were divided into six groups, (6 rats each) and fed on experimental diet.

The first group was fed with raw rice, the second with cooked rice and the third with cooked parboiled rice all contain high amylose. The three other groups were fed on the rice variety (Giza 181) low amylose.

Each rat was weighed every two days and its food consumption was determined.

Blood samples were obtained from each rat from orbital vinous plexuses each 7^{th} day after feeding on different rice varieties.

The experiment was terminated when the glucose level in blood showed constant values. At the end of the experimental period, animals were fasted overnight and scarified under ether anaesthesia. Blood was collected from hepatic portal, serum was separated and kept in plastic vial at –20°C until analysis.

Total cholesterol (Abell *et al.*, 1985) and glucose were determined in blood serum of the experimental rats.

RESULTS AND DISCUSSION

The chemical composition of two rice varieties Giza 175 and Giza 181 is reported in (Table 1). The results show that, the average protein content was 9.30 and 7.84%; Lipid 0.526 and 0.436%, ash 0.55 and 0.72%; total carbohydrates 85.35 and 86.96%. The amylose content 28.1 and 19.5 for Giza 175 and Giza 181 resp.

In-vitro study

The in vitro digestibilities of low and high amylose rice varieties are shown in (Table 2). The results showed that the hydrolysis rates of high amylose rice variety (179.14-180.53) were lower than those of low amylose rice variety (189.73-209.85).

This agreed with Yoshizawa *et al.*, (1981) who found that digestibility of starch by amylase was higher in starch containing low amylose. Also, Bjorek *et al.*, (1990) reported that with increasing amylose content, a decrease occurred *in-vitro* digestibility. Digestibility of starch was negatively correlated with amylose content. Table 3, shows that a significant decrease of average (gain) in body weight for rats injected with alloxan. Diabetic rats fed on low amylose rice gave non significant increase in body weight gain (18 Vs 19-24) while a significant increase was found on feeding with high amylose rice (18 Vs 28-32).

It could be said that, high amylose rice showed glucose lowering effect when compared with low amylose rice, consequently, weight gains of rats differed significantly ($p<0.05$ between groups). This may attributed to the differences observed in blood glucose responses.

Regarding feed efficiency (Table 3), it is decreased from 0.1997 in healthy rats to 0.037 in diabetic rats. Feeding rice with low amylose to diabetic rats increased the feed efficiency (from 0.042 to 0.048) while feeding rice with high amylose increase it (from 0.0587 to 0.0666). This observation means that high amylose rice caused an increase in feed efficiency in diabetic rats.

The results in (Table 4) reveal that rice with a higher amylose content decreased serum glucose level significantly in diabetic rats from 321 control Vs 213-227mg/100ml. than did the low amylose rice variety 321 control Vs 255-269mg/100ml. These results agree with Goddard *et al.*, (1984) who tested rices of different amylose contents (0%, 14-17 and 25%) and found that rice with the highest amylose content elicited the lowest glycemic and insulinemic responses. These authors attributed the observed effects to the formation (during cooking) of amylose complexes based on the constitutive lipids. Amylose lipid complexes were reported to be resistant to α-amylase *in-vitro,* Eliasson and Krog (1985) as well as *in-vivo,* Holm *et al.*, (1983).

Also, (Table 4) shows that the cholesterol in alloxanized rats was highly increased comparing with healthy rats, the results revealed that rice with a higher amylose content decreased cholesterol level in diabetic rats (349 Vs 287-303 mg/100ml) than did the low amylose rice variety (349 Vs 308-329 mg/100ml). Similar results have been reported by Amelsvoort and Weststrate (1992) who found that high amylose maize starch showed positive lowering effect on blood glucose and insulin levels, as well as triglycerides and cholesterol in humans. Goddard *et al.*, (1984) observed similar responses from high amylose rice. Behall *et al.*, (1989) concluded that long term intake of high amylose starch may benefit individuals with elevated glucose and insulin levels and apparent insulin resistance.

For cooked rice, it was found that high amylose (28% amylose) lowered the serum glucose to 213 against 269 mg/100ml for cooked low amylose, while in cooked parboiled rice it was 226 against 255mg/100ml.

Also, the same trend was observed for the serum cholesterol. Akerberg *et al.*, (1998) investigated boiled rice made from varieties with different amylose/amylopectin ratios, they found that the high amylose rice (28% amylose) gave significantly lower GI=64 than ordinary GI=83 and GI=87 for (20% amylose) and GI=88 for waxy (0-2% amylose).

The results indicate that, only varieties of rice with a high amylose content (parboiled or not) said to be potentially useful with diabetes, wether where they decreased availability of glucose or lowered the rate of digestion. These varieties provide slow source of carbohydrate for modulation of postprandial glucose and insulin plasma level.

High amylose rice is a relative newcomer to cereal science investigation, applications and may have future industrial applications.

Table 1. Chemical composition of rice varieties.

Rice variety	Amylose %	Protein %	Lipid %	Ash %	Total carbohydrates
Giza 181	19.5	7.84	0.436	0.72	86.96
Giza 175	28.1	9.3	0.526	0.55	85.35

Table 2. Ferricyanide Number for rice varieties before and after hydrolysis with α-Amylase.

Sample	Ferricyanide Number		Increase
	Before hydrolysis with α-Amylase	After hydrolysis with α-Amylase	
Giza 181			
Raw Rice	5.52	215.37	209.85
Cooked Rice	6.58	204.07	197.49
Cooked parboiled rice	6.01	195.74	189.73
Giza 175			
Raw Rice	4.93	185.33	180.40
Cooked Rice	7.64	186.78	179.14
Cooked parboiled Rice	7.61	188.14	180.53

Giza 175 = high amylose.

Giza 181 = low amylose.

Table 3. Food efficiency and gain in body weight for alloxanized rats given (low and high amylose rice varieties).

Groups	Gain in body wt (g)	Food intake (g)	Food efficiency
Control A (negative)	91.8±7.9	464.00±38.9	0.1997±0.031
Control B (positive)	18.0±7.7	478.17±31.4	0.0370±0.016
Raw low amylose rice	19.8±6.6	460.20±33.7	0.0420±0.012
Cooked low amylose rice.	20.5±7.5	477.80±35.1	0.0425±0.015
Cooked parbioled low amylose rice.	24.5±11.8	495.70±41.0	0.0480±0.020
Raw high amylose rice	29.8±13.3	455.30±26.5	0.0660±0.073
Cooked high amylose rice.	32.0±9.0	472.60±43.0	0.0666±0.012
Cooked parbioled high amylose rice.	28.5±8.3	477.20±44.9	0.0587±0.013

Table 4. Means of serum glucose and cholesterol for alloxanized rats fed low and high amylose varieties.

Groups	Serum Glucose mg/100ml	Serum cholesterol mg/100ml
Control A (negative)	99.0±5.1	173.3±22.7
Control B (positive)	321.7±34.5	349.7±35.6
Raw low amylose rice	264.0±37.4	329.5±34.3
Cooked low amylose rice.	269.2±26.9	316.5±34.4
Cooked parbioled low amylose rice.	255.0±22.8	308.5±23.0
Raw high amylose rice	227.0±20.9	303.0±29.0
Cooked high amylose rice.	213.3±22.7	287.7±14.0
Cooked parbioled high amylose rice.	226.7±19.7	298.0±20.8

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تأثير محتوي الأميلوز في الأرز الأبيض علي مستوي الهضم معملياً وعلي مستوي الجلوكوز في دم الفئران

ليلي فهمي رزق[1] ، هناء عزيز دوس[1] ، أحمد الحصيوي[2] ، ماهر مرجان[3]

١ معهد بحوث تكنولوجيا الأغذية
٢ مركز تدريب تكنولوجيا الأرز بالأسكندريه
٣ معهد التغذية

أستخدم في هذا البحث صنفين من الأرز الأبيض، جيزة ١٧٥ (عالي الأميلوز)، جيزة ١٨١ (منخفض الأميلوز)، تم تقدير نسبة الهضم بواسطة α-أميليز وأيضاً تم تقدير نسبة الجلوكوز في الدم لفئران التجارب لكل من الصنفين (أرز غير مطهي - أرز مطهي - أرز بعد القلي).

وكان معدل الهضم بواسطة α-أميليز للصنف جيزة ١٧٥ (عالي الأميلوز) يتراوح (١٧٩,١٤-١٨٠,٥٣) بينما للصنف المنخفض في الأميلوز يتراوح (١٨٨,١٣-٢١٠,٤٤).

في تجربة التغذية وجد فروق معنوية بين مجاميع فئران التجارب في الزيادة في الوزن بالنسبة للأرز المرتفع في نسبة الأميلوز وأيضاً زيادة في كفاءة الغذاء بالمقارنة بالفئران المصابة بمرض السكر.

أيضاً الأرز المرتفع في محتوي الأميلوز أدي إلي انخفاض مستوي الجلوكوز في الدم من ٣٢١ إلي ٢١٣-٢٢٦ مجم/١٠٠مل عنه في الأرز المنخفض في الأميلوز من ٣٢١ إلي ٢٥٥-٢٦٩ مجم/١٠٠مل). وأيضاً نفس الاتجاه بالنسبة إلي الكوليسترول في الدم.

بالنسبة للأرز المطهي العالي في نسبة الأميلوز أدي إلي انخفاض نسبة السكر إلى ٢١٣ مجم/١٠٠مل بالمقارنة بالأرز المطهي المنخفض في نسبة الأميلوز ٢٦٩ ملجم/١٠٠ مل.

بينما في الأرز المغلي المطهي المرتفع في نسبة الأميلوز أدي إلي انخفاض ٢٢٦ بالمقارنة المنخفض في نسبة الأميلوز ٢٥٥ مجم/١٠٠ مل ونفس الاتجاه بالنسبة للكوليسترول.