EFFECT OF A SOYBEAN AND RICE BEVERAGE ON THE LIPID AND GLYCEMIC METABOLISMS IN HAMSTERS

Efeito da bebida de soja e arroz no metabolismo lipídico e glicêmico de hamsters

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ABSTRACT

There is a growing interest in functional foods that, apart from their nutritional function, bring health benefits, contributing to wellbeing and/or reducing the risk of certain diseases. The objective of this study was to evaluate the functional effect of a soybean and rice beverage on the ponderal and nutritional parameters and on the lipid and glycemic metabolisms of male hamsters. Four groups of 8 animals were used: Control (commercial feed), C (commercial feed with added cholesterol), B (commercial feed plus beverage) and CB (commercial feed plus cholesterol and beverage). The food efficiency coefficient was equivalent for all the groups. The weight of the liver was on average 28% heavier in groups C and CB than in the other groups, indicating cholesterol deposition in this organ. Although there was no significant difference, the concentrations of total cholesterol and of its fractions (LDL and HDL) and of the triglycerides, were lower in group CB than in group C, showing a possible benefic effect of the beverage. The blood glucose concentration did not differ significantly ($p<0.05$).

Index terms: HDL, LDL, triglycerides, glycemia, resistant starch.

RESUMO

É crescente o interesse por alimentos que, além da função de nutrir, trazem benefícios à saúde, contribuindo para o bem-estar e/ou reduzindo o risco de algumas doenças. Objetivou-se, neste estudo, avaliar o efeito funcional de bebida de soja e arroz nos parâmetros ponderais e nutricionais e no metabolismo lipídico e glicêmico de hamsters machos. Foram utilizados 4 grupos de 8 animais: Controle (ração comercial), C (ração comercial acrescida de colesterol), B (ração comercial e bebida) e CB (ração comercial acrescida de colesterol e bebida). O coeficiente de eficiência alimentar foi equivalente em todos os grupos. O peso do fígado dos grupos C e CB apresentou-se em média 28% maior que os demais, indicando deposição de colesterol nesse órgão. Embora não havendo diferença significativa, as concentrações de colesterol total e suas frações (LDL e HDL) e de triacilglicerídeos foram menores no grupo CB comparadas ao grupo C, mostrando possível efeito benéfico da bebida. A concentração de glicose no sangue não diferiu significativamente ($p<0,05$).

Termos para indexação: HDL, LDL, triglicerídeos, glicemia, amido resistente.

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INTRODUCTION

In recent decades there has been increasing interest in functional foods that optimize physiological functions, contributing to the well-being and good health and/or reduction in the risk of diseases. A food can be considered as functional if it can be demonstrated satisfactorily that it positively affects one or more functions of the organism, promoting the benefits cited above (JIMÉNEZ-COLMENERO et al., 2010).

Soy-based foods stand out in this context. Evidence has shown that the functional properties associated with the consumption of products derived from soybeans are attributed to proteins and phytochemicals, of which the following stand out: protease inhibitors, phytates, phytosterols, saponins, phenolic acids, phytic acid, lecithin, unsaturated fatty acids and isoflavones, as well as fibers and essential amino acids (HASLER, 1998; FRIEDMAN; BRANDON, 2001). The main scientific relevance associated with the action of soybean on health is its beneficial action on cardiovascular disease, with an emphasis on the reduction of cholesterol and inhibition of the formation of atherosclerotic plaque (KLEIJN et al., 2001, VILLANUEVA et al., 2011).

Studies have shown that the joint consumption of soybean and cereals, for example rice, results in protein mixtures with elevated biological value (WANG et al., 2000; HAGENIMANA et al., 2007). The association of this cereal with soybean could contribute to an increment of the bioactive substances in the food, considering that rice has been mentioned in various studies for presenting

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functional properties, due mainly to the presence of resistant starch (NAMRATHA et al., 2002; WALTER et al., 2005) and of antioxidants such as the orizanols (WILSON et al., 2000; BERGER et al., 2004).

Evidence has suggested that the consumption of resistant starch is associated with favorable effects on the lipid profile and glycemic response due to the fermentation products produced and the delay in absorption of nutrients from the intestinal tract (GARCIA-ALONSO et al., 1999; WALTER et al., 2005; Zhang et al., 2009). In addition it is related to other physiological effects, such as an alteration in gastrointestinal transit and an increase in the sensation of satiety (KIM et al., 2003).

The objective of the present study was to evaluate the functional effect of a soybean and rice beverage on the ponderal and nutritional parameters ands on the lipid and glycemic metabolisms of male hamsters.

MATERIALS AND METHODS

Soybean and rice beverage

The preparation and physical-chemical analysis of beverage were performed according to Jaekel et al. (2010), and the beverage consisted of 70% soybean extract [*Glycine max* (L.) Merrill cv. BRS 213] and 30% rice extract [*Oryza sativa* L. cv. IRGA 417], with added sugar, showing the following physicochemical composition: protein (2.34%), lipids (0.72%), ash (0.35%), crude fiber (0.34%), carbohydrates (obtained by difference, 6.79%), resistant starch content (0.19%) and amino acid content (29.83g/16g N).

Biological assay

Thirty-two male Golden syrian/UFPel hamsters (*Mesocricetus auratus*), weaned after 21 days and with an initial weight between 49 and 74g, were maintained on a standard diet (commercial feed NuvilabCR1®) until completing 1 month of age. They were then weighed and divided at random into 4 groups (n=8): Control (commercial feed), C (commercial feed plus 0.2% cholesterol), B (commercial feed + 5mL beverage) and CB (commercial feed plus 0.2% cholesterol + 5mL beverage). The hypercholesterolemic diet was prepared weekly from dissolution of cholesterol powder (Eskisa S.A.) in ethanol at temperature of 60ºC, with immediate addition to the feed at a rate of 0.2%, followed by evaporation.

Each animal received 13g of commercial feed (BLAIR et al., 2002) and water "*ad libitum*". The beverage was administered daily to the respective groups during the morning in plastic drinking vessels, immediately after removal of the remaining feed and water. The same procedure was applied to the groups that did not receive the beverage (control and C) so that they suffered the same degree of stress. After ingesting the beverage each animal received feed and water. The experiment followed the norms of the Brazilian College of Animal Experimentation – COBEA, and was approved by the Ethics Commission of the Federal University of Pelotas, RS, Brazil (process nº 05/2006), and carried out in the Animal Experimentation Laboratory of the Food Science Department of UFPel for 68 days with the animals maintained in individual cages under controlled temperature (25 ± 2º C) conditions and a 12 hour photoperiod.

Ponderal and nutritional parameters

The animals were weighed every 7 days and the diet consumed monitored daily. These data were used to determine weight gain and the total ingestion of feed during the experiment.

Analysis of the liver

After euthanasia, the animals were laparotomized, the liver removed, washed with physiological serum and weighed.

Blood analysis

At the end of the experiment blood was collected by heart puncture after a 12-hour fasting period, the animals being anaesthetized by inhalation of ether in a balloon. The blood glucose level was immediately measured in a Accu-Chek Advantage II (Roche) equipment. The blood was centrifuged at 1000g for 15 minutes/4ºC to obtain the serum, and the total and LDL cholesterol levels determined using the enzymatic methods *Colestat Enzimático AA* and *LDL Colesterol Reactivo Precipitante*, respectively, both kits being from Wiener Laboratórios S.A.I.C. (Rosário, Argentina). HDL-cholesterol and triglycerides were also determined by enzymatic methods, using the kits *HDL LE* and *Triglicérides Liquiform* (Labtest Diagnóstica S.A, Minas Gerais, Brazil).

Statistical analyses

The data were submitted to an analysis of variance (ANOVA) and Tukey's test for the means comparison at a level of significance of 5%, using the software Statistica 6.0 (STATSOFT STATISTICA FOR WINDOWS, 2001).

RESULTS AND DISCUSSION

Ponderal and nutritional evaluations

The different diets showed no evidence of influencing ($p<0.05$) the weight gain of the animals (Table 1), all showing the expected values for this species, as also observed by (SONG et al., 2003; VILLANUEVA et al., 2011).

Table 1 – Body weight, gain and final weights, daily feed consumption, feed ingestion, food efficiency coefficient (FEC), weight of liver and ratio of the liver to body weights for the hamsters submitted to the different experimental diets during 68 days.

Analysis*	DIETS***			
	Control	C	B	CB
Body weight (g)**	72.04±2.41[a]	71.21±1.84[a]	70.69±2.14[a]	75.31±1.99[a]
Weight gain (g)	44.19±2.80[a]	45.66±1.87[a]	44.53±1.95[a]	49.95±2.65[a]
Final weight (g)	116.23±3.20[a]	116.87±2.37[a]	115.22±3.49[a]	125.25±3.24[a]
Daily feed consumption (g)	7.54±0.06[b]	7.63±0.06[ab]	7.29±0.07[c]	7.97±0.06[a]
Feed ingestion (g)	4038.51	4088.71	3905.11	4270.58
FEC	0.01	0.01	0.01	0.01
Weight of liver (g)	3.41±0.15[b]	4.68±0.16[a]	3.52±0.10[b]	4.94±0.16[a]
Weight of liver (g)/ body (g)	0.029	0.040	0.030	0.039

*Means of 8 repetitions ± standard deviation; Means in the same line with different letters differ significantly according to Tukey's test ($p<0.05$); **After 7-day adaptation period fed on commercial feed; ***Control: commercial feed; C: commercial feed plus 0.2% cholesterol; B: commercial feed plus 5mL soybean and rice beverage; CB: commercial feed plus 0.2% cholesterol plus 5mL soybean and rice beverage.

The mean daily consumption of the animals fed on commercial feed plus the soybean and rice beverage (group B) was significantly ($p<0.05$) less than the others, although maintaining a mean ingestion above 7g day^{-1}. Similarly, other studies have shown that the daily consumption of feed by hampsters is never more than 9g in the adult phase (WILSON et al., 2007), without this interfering in their development.

The food efficiency coefficient (FEC) was equivalent for all groups, indicating similarity in the conversion of the food ingested. The smaller feed ingestion by group B indicates the influence of the beverage on ingestion, but did not influence the values for FEC, since these also accompanied the data for total weight gain.

Considering that the liver/body weight ratios of the animals maintained their proportionality, the animals in groups C and CB showed livers that were, on average, 28% heavier than the others, indicating cholesterol deposition in the organ. This was confirmed by the yellowish color, due to the hepatic steatosis of the animals that received the hypercholesterolemic diet (C and CB) as compared to the normal (intense red) color of those that received a diet without cholesterol (Control and B) (Figure 1). Kahlon et al., (1999) showed similar behavior with hypercholesterolemic diets.

Blood analysis

The serum lipid fractions are influenced by many factors such as the diet, individual characteristics, genotype and environmental conditions. The groups submitted to the hypercholesterolemic diet, with and without the soybean and rice beverage (C and CB) differed significantly ($p<0.05$) from those that received just commercial feed (control and B) with respect to total cholesterol and HDL (Table 2), which could be related to the greater gains in body and liver weight of these groups (C and CB).

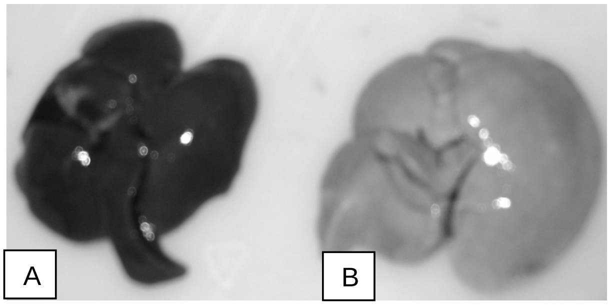

Figure 1 – Alterations in color of the livers of hamsters submitted to different experimental diets during 68 days: A – commercial feed (control group) and B – commercial feed plus 0.2% cholesterol (group C).

Although there were no significant differences, the concentrations of total cholesterol and its fractions (LDL and HDL) and of triacylglycerols were lower in the animals that received the hypercholesterolemic diet with the beverage (CB) as compared to the animals that received the feed plus cholesterol (C). The greatest effect evidenced by the beverage referred to the values for total cholesterol

Table 2 – Concentrations (mmol L^{-1}) of total cholesterol, LDL, HDL, triglycerides and glycemia of the hamsters submitted to different experimental diets for 68 days.

Biochemical evaluations*	DIETS**			
	Control	C	B	CB
Total cholesterol	3.24±0.51^b	7.08±0.82^a	3.35±0.49^b	6.20±0.50^a
LDL	2.08±0.42^a	4.35±0.92^a	2.47±0.59^a	3.60±0.59^a
HDL	1.81±0.19^b	2.95±0.14^a	1.79±0.07^b	2.76±0.10^a
LDL/HDL	1.15	1.47	1.38	1.78
Triglycerides	1.26±0.07^a	1.59±0.33^a	1.20±0.17^a	1.55±0.17^a
Glycemia	7.00±0.39^a	7.14±0.58^a	5.66±0.39^a	7.26±0.36^a

*Means in the same line with different letters differ significantly according to Tukey's test ($p<0.05$); **Control: commercial feed; C: commercial feed plus 0.2% cholesterol; B: commercial feed plus 5mL soybean and rice beverage; CB: commercial feed plus 0.2% cholesterol plus 5mL soybean and rice beverage.

and LDL-cholesterol. The group CB showed values that were 12.4% and 17.2% inferior, respectively, as compared to those of group C. Such an effect is in agreement with numerous citations reporting a possible influence of products derived from soybean in the prevention of an increase and/or a reduction in cholesterol (HO et al., 2003; SONG et al., 2003; VILLANUEVA et al., 2011). In the same way, the rice could have contributed to this effect, considering that much research has indicated its influence in the control of lipid metabolism due to the fermentation products produced and the delay in absorption of nutrients from the intestinal tract (GARCIA-ALONSO et al., 1999; WALTER et al., 2005; MITRA et al., 2007; ZHANG et al., 2009).

The serum cholesterol levels of the groups control and B were very close to each other and well below those of the hypercholesterolemic groups (C and CB) (Table 2), indicating that the beverage did not increase these levels. The group CB showed a cholesterol concentration below that of group C, indicating the benefic effect of the soybean and rice beverage in the diet. These data agree with those of Wong et al. (1998), in which the results suggested that the reducing effect of soybean protein on cholesterol occurred especially in hypercholesterolemic individuals.

Although not as effective as the soybean, rice also contributed to a reduction in cholesterol mainly due to the resistant starch, an effect also demonstrated by Mitra et al. (2007). In the present study this effect could have been reduced due to the small amount of rice in the beverage, which contained 30% rice extract obtained from 15% of parboiled whole grains. However one must consider that the effect of rice was added to that of the soybean, resulting in a lower concentration of cholesterol in group CB as compared to group C.

With respect to the LDL concentration, the groups did not differ ($p<0.05$). However in the group fed on the hypercholesterolemic diet, this concentration was 21% higher as compared to the group that received the soybean and rice beverage in addition to the feed (CB). The benefic effect of soybean on LDL was also demonstrated by Ho et al. (2003), Song et al. (2003), Wang et al. (2004) and Wilson et al. (2007). A study on parboiled rice containing resistant starch also demonstrated a reduction in the LDL levels (Mitra et al., 2007).

The reduction in the LDL levels is important in hypercholesterolemic individuals, since elevated plasmatic LDL concentrations are highly associated with the occurrence of coronary arterial diseases such as atherosclerosis (Vilela, 2007).

The HDL-cholesterol concentrations were similar in the groups control and B. The groups fed a diet with no cholesterol presented HDL concentrations about 37% lower than those of the groups fed a hypercholesterolemic diet (C and CB). The latter two groups presented similar behavior, although a lower concentration was observed for group CB. Other studies with soybean based hypercholesterolemic diets indicated similar values for HDL (Song et al., 2003; Yang et al., 2007). High blood HDL-cholesterol levels indicate a low probability of developing cardiovascular diseases, since the HDL lipoproteins are responsible for the transport of endogenous cholesterol back to the liver, removing it from the arterial walls (Vilela, 2007).

The LDL/HDL ratio is commonly calculated to evaluate the risk of coronary diseases, based on evidences mentioned by Ho et al. (2003) that an elevated LDL-cholesterol concentration is atherogenic, whilst a high level of HDL-cholesterol has a cardio-protective effect. The reduction of the LDL/HDL ratio in group B indicated the influence of the soybean and rice beverage in normocholesterolemic animals. Lower values, varying from 0.72 to 1.72 were observed by Ho et al. (2003) and Song et al. (2003).

With respect to the triacylglycerides, although presenting no statistical difference ($p<0.05$), the lowest concentrations were found for the groups control and B. The groups fed on the hypercholesterolemic diet showed very similar concentrations, but it is possible to perceive a probable effect of the soybean/rice beverage, since group CB showed a lower mean value. Similar values were observed by Fukui et al. (2004) and Wang et al. (2004).

The influence of soybean on the concentration of triacylglycerides has been demonstrated by previous studies (SONG et al., 2003; LEE, 2006; SHUKLA et al., 2007). Rice was also shown to reduce the triacylglycerides in a study by Mitra el al. (2007) and Zhang et al. (2009).

The groups did not differ with respect to the blood glucose concentration. Numerically the highest concentrations were found for groups C and CB and the lowest mean for group B, indicating a possible influence of the soybean/rice beverage in normocholesterolemic hamsters. The effect of rice was indicated by Marett and Slavin (2004), where at the end of the study, the authors observed that individuals fed on a rice starch-based diet presented lower mean values for glucose. The influence of resistant starch from different sources showed a lower concentration of plasmatic glucose for the group that received rice starch (KIM et al., 2003).

A decrease in the contents of HDL-cholesterol, triacylglycerides and glycemia was verified in the normocholesterolemic animals submitted to the diet containing the beverage. However the effect was not significant in the animals of this group or in the hypercholesterolemic ones. This fact could be attributed to the amount of beverage administered to the animals, the concentration of the substances in the beverage alleged to be functional, the duration of the experiment and the cholesterol concentration (0.2%) in the diet.

With respect to the blood glycemic levels, although there was no evidence of a significant influence ($p<0.05$) of the soybean/rice beverage, the normocholesterolemic group that received the beverage presented a much lower glucose concentration than the other animals. This effect could be related principally to the resistant starch contained in the rice extract, as mentioned by other authors (WALTER et al., 2005). The opposite behavior was observed in the group that received both the beverage and the hypercholesterolemic diet, indicating a possible influence of cholesterol in the action of the resistant starch. Obviously in the evaluation of this effect, one should consider the resistant starch content in the beverage and cholesterol content in the diet.

The effect obtained from different soybean and rice products, and principally from their concentrated or isolated components (soybean protein and resistant starch), which is considerably different from affirming that the soybean or rice present that effect, with this study.

CONCLUSIONS

The soybean/rice beverage did not alter the ponderal parameters or the FEC. Nevertheless the hypercholesterolemic diets, with and without the beverage, caused an increase in the weight of the liver and the presence of hepatic steatosis. The increase in weight and the degree of damage to the liver were lower than for the group that received the soybean/rice beverage, indicating a possible benefic influence of the beverage.

The soybean/rice beverage showed an influence on the lipid profile of hypercholesterolemic hamsters, reducing the concentrations of serum cholesterol and its fractions, LDL and HDL, and the triacylglycerols and glycemic levels in normocholesterolemic hamsters.

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