ORIGINAL ARTICLE

Hypolipidemic effect of rice bran diet

Efeito hipolipidêmico da dieta contendo farelo de arroz

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ABSTRACT

Introduction: Hypercholesterolemia is a major risk factor established for coronary artery disease. The soluble fibers, polyunsaturated fatty acids and oryzanol contribute to the inhibition of cholesterol absorption and thus decrease the serum cholesterol levels.

Objective: In this study, the effect of rice bran diet offered to the offspring of rats after weaning was investigated regarding their growth and development, as well as concentration of glucose and total and fractionated cholesterol.

Materials and Methods: This study examined four different types of diets in 21-day-old Wistar rats, containing rice bran with or without lysine and with the addition of soybean protein, all diets compared with the standard diet. Blood levels were analyzed chemically for total cholesterol, HDL, LDL, glucose, protein and albumin. It was also measured the body and liver weights, as well as quantified the liver protein and DNA.

Results: Animals receiving rice bran diet with and without lysine had decreased plasma cholesterol ($p < 0.05$ and $p < 0.01$, respectively), when compared to the commercial standard. When soybeans were added to diets, there was a reduction in LDL cholesterol levels ($p < 0.05$) and soybeans with lysine led to a major reduction ($p < 0.01$). The blood glucose concentration was significantly lower only in the diet with rice bran without lysine ($p < 0.01$).

Conclusions: From this study we could conclude that the consumption of a diet with rice bran containing or not lysine had a hypocholesterolemic effect, but it was observed that the diet without addition of lysine affected the rats growth and development.

Keywords: anticholesteremic agents; hypoglycemic agents; animal nutritional physiological phenomena; diet.

RESUMO

Introdução: A hipercolesterolemia é um importante fator de risco estabelecido para doença arterial coronariana. As fibras solúveis, ácidos graxos poliinsaturados e orizanol contribuem para a inibição da absorção do colesterol e, consequentemente, diminuição dos níveis séricos de colesterol.

Objetivos: No presente estudo, foi investigado o efeito da dieta de farelo de arroz oferecido após o desmame de crias de ratos, em relação ao crescimento e desenvolvimento deles, bem como a concentração de glicose, colesterol total e fracionado.

Materiais e Métodos: Este estudo examinou quatro tipos diferentes de dietas em ratos Wistar de 21 dias, contendo farelo de arroz com e sem lisina, bem como a adição de proteína de soja, sendo todas dietas comparadas com a comercial. Foram analisados os níveis séricos de colesterol total, HDL, LDL, glicose e proteína. Também foi mensurado o peso corporal e do fígado, assim como quantificada a proteína e o DNA do fígado.

Resultados: A dieta contendo farelo de arroz com e sem lisina apresentaram uma redução nos níveis de colesterol plasmático (p<0,05 e p<0,01, respectivamente). Porém, a adição de proteína de soja reduziu o LDL colesterol (p<0,05), sendo que com a adição de lisina a diferença fica maior (p<0,01). A concentração de glicose sanguínea está significativamente menor apenas na dieta com farelo de arroz sem lisina (p<0,01).

Conclusão: Foi possível concluir que o consumo de uma dieta com farelo de arroz contendo lisina ou não tem um efeito hipocolesterolêmico, mas observou-se que a dieta sem adição de lisina afetou o crescimento e desenvolvimento dos ratos.

Palavras-Chave: anticolesterolemiantes; hipoglicemiantes; fenômenos fisiológicos da nutrição animal; dieta.

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INTRODUCTION

Hypercholesterolemia is a major risk factor established for coronary artery disease, being one of the most frequent causes of morbidity and mortality in Brazil (300 thousand per year), and it is responsible for the largest portion of hospital expenses in the Unified Health System (UHS)[1].

Recent evidence indicated that small dense LDL particles were prevalent in patients with atherosclerosis[2]. Lifestyle modification and low fat in the diet are crucial factors in the regulation of plasma cholesterol levels, as well as a form of treatment in the hypercholesterolemia. The increased soluble fiber in diet was shown to be an important component of serum cholesterol reduction, because the soluble fibers may increase the binding of bile acids in the intestinal lumen, which leads to a decreased enterohepatic circulation of bile acids, a subsequent increased hepatic conversion of cholesterol to bile and low-density lipoprotein (LDL) receptor upergulation, and enhanced LDL lipoprotein catabolism[3,4]. Another suggested mechanism is the increased viscosity of the food mass in the small intestine adjacent to the mucosa. The layer may act as a physical barrier to reduce the absorption of nutrients and bile acids. Furthermore, soluble fibers may reduce the rate of glucose absorption, leading to lower glycemic response and lower insulin concentrations. Compositional analysis in the rice bran reveals it is a good fiber source, because it has higher fat binding and emulsifying capacity, contributing for the low serum cholesterol[5].

Vegetable oils that are rich in polyunsaturated fatty acids, mainly linoleic acid, have also contributed in the hypocholesterolemic effect[6,3]. In some cases, the rice bran oil lowered plasma cholesterol more effectively than other commonly used vegetable oils rich in linoleic acid. This effect can be also associated with the occurrence of specific -oryzanol components (one group of ten sterol components). The effect in the -oryzanol can be attributed to the inhibition of cholesterol absorption causing a modest decreased serum cholesterol concentration[7-9].

Studies have demonstrated that tocotrienols exert powerful hypocholesterolemic effects in humans and animals[10,11]. The impact of pure tocotrienols isolated from rice bran contributes for the reduced cholesterol biosynthesis, specifically through the lowering of LDL and inhibition of 3-hidroxy-3-methylglutarylcoenzima A (HMGCoA) reductase, and it has been confirmed by a number of researchers[10,12-14].

The addition of multimixture, composed of rice bran, green leaves powder, seeds powder and eggshell powder, to the population diet has been encouraged in Brazil, as a mean to increase the production of breast milk and promote children's growth[15].

In this study the effect of rice bran diet offered to the offspring of rats after weaning was investigated regarding their growth and development, as well as concentration of glucose and total and fractionated cholesterol.

MATERIALS AND METHODS

Rice bran obtained from rice mills in *Camaquã*, state of *Rio Grande do Sul*, Brazil, was evaluated for its nutritive potential during three postnatal weeks in rats that were fed with maternal milk.

Animals

Lactating Wistar rats were obtained from a local breeding colony (Institute of Health Basic Sciences, Federal University of Rio Grande do Sul, Brazil). They were maintained on a 12-hour light-and-dark cycle in a ventilated room at 23ºC with free access to food and water. The litter size was adjusted to eight pups per mother on the first postpartum day. The protocols followed the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil, approval protocol number 03089/2004-1.

Diets

Rats were randomly assigned to five experimental groups in the postnatal period with their mothers, and each group received one of the following diets: Commercial Standard (C.S.) (Nuvilab-CR1, Nuvital, Curitiba, Brazil); Rice Bran (RB); Rice Bran with lysine 1% (RB + Lys); Rice Bran with soybean protein 12% (RB + SJ) and Rice Bran with soybean protein 12% and 1%

of lysine (RB + SJ + Lys). Every diet used defatted Rice Bran. All groups were killed by decapitation to collect blood. Additionally, the livers were rapidly removed and weighed.

Composition of the experimental diets given to rats and their macronutrients are described in tables 1 and 2.

Sample Preparation

After 21 days of treatment, all animals were killed by decapitation. Blood samples were collected in test tubes and immediately centrifuged at 3000 x g for 10 minutes to obtain serum samples (Eppendorf 5402; Hamburg, Germany). Livers were rapidly removed, weighed, and rinsed with NaCl 0.9% (1:5).

Serum Analysis

The serum samples were incubated at 37º C for 10 min for biochemical analysis that were performed in a Multi-test Analyzer (Mega; Merck, Darmstadt, Germany), using specific kits supplied by Merck as follows: total protein (protein-SMT, 1.19703.0001, biuret method); albumin (albumin-SMT, 1.19722.0001, bromocresol method); glucose (GLUC-DH 1.07116.0001); cholesterol (cholesterol-SMT, 1.19738.0001, CHOD-PAP method); and LDL cholesterol (1.14992.0001, CHOD-PAP method). HDL cholesterol was determined using a kit (HDL cholesterol direct FS) from DiaSys (Diagnostic Systems International, Holzheim, Germany)[16].

Liver Analysis

We have measured levels of protein and hepatic DNA. Liver protein was measured following the method of Lowry et al.[17] and DNA was measured following the method of Burton[18].

Statistical Analysis

Statistical differences were assessed by analysis of variance (ANOVA) followed by the Tukey HSD (Post Hoc) multiple-range test whenever values were initially significant. Significance level was set a $p<0.05$ and $p<0.01$. All analyses were done with SPSS 8.0 (SPSS, Chicago, USA).

RESULTS

Body and Hepatic Weight

Table 3 shows there was statistically significant differences in body and hepatic weight between groups fed with C.S. and RB+SJ (with and without lys). Rats fed with RB+SJ (with and without lys) diet had weight gains greater than C.S. A significant reduction in body weight of animals that were fed with rice bran without lysine was also observed in comparison to the standard diet. The hepatic weight had the same statistical difference than body weight. The results demonstrate that the rats body weight is higher in comparison to the standard diet when soybean protein is added to the RB diet.

Liver Protein and DNA Concentration

Table 3 shows there was statistically significant differences only in the liver DNA of the RB diet with and without supplementation of lysine in comparison to the standard diet. However, there was no difference on the liver protein data.

Serum Analysis

Table 4 shows that rats fed with RB without lysine have decreased glucose and all values of serum cholesterol and its fractions compared to the control group. However, the RB with lysine decreased only the LDL and cholesterol levels, without changing the HDL, albumin and protein levels. Rats fed with RB+SJ with or without lysine only had a decrease in the LDL cholesterol levels compared to control group.

DISCUSSION

Nutritionally adequate diets are critical because they allow sufficient milk production for the mother survival and growth while maintaining the litter. Nutrient needs in the lactating period are largely determined by demand for milk production created by the suckling litter. The mothers seem to have ability to mobilize sufficient energy from tissue stores to support lactation[19]. Insufficient protein or amino acid supplies can decrease milk production and reduce the litter growth[20].

A number of studies have demonstrated that low dietary lysine intake during lactation affects the metabolic state and tends to increase muscle protein degradation, suggesting that dietary protein was insufficient to support milk production[21]. This study showed that the RB group without lysine had a reduction in all parameters, including body mass, liver and blood

TABLE 1 - Composition of the experimental diets given to rats (g/100 g).

Component	RB	RB + Lys	RB + SJ	RB + SJ + Lys
Rice Bran (*)	92.18	91.18	56.80	55.80
Starch	--	--	23.10	23.10
Soybean Protein (**)	--	--	12.00	12.00
Rice Oil	3.86	3.86	4.20	4.20
Lysine	--	1	--	1.00
Salt Mix (+)	3.68	3.68	3.60	3.60
Vitamin Mix (++)	0.28	0.28	0.30	0.30

Legend of diets: Rice Bran (RB); Rice Bran with 1% of lysine (RB + Lys); Rice Bran with 12% of soybean protein (RB + SJ) and Rice Bran with 12% of soybean protein and 1% of lysine (RB + SJ + Lys).
Commercial non-purified diet from Nuvilab–CR1: 22.0% protein, 41.3% carbohydrate and 4.0% lipids.
(*) Rice Bran from *Helmut Tessmann Ind. e Com. de Óleos Vegetais Ltda*. (Rod. Br 116, Km 388, Camaquã/RS - Brazil).
(**) Soybean protein: Samprosoy (The Solae Company, Esteio/RS - Brazil).
(+) Mineral mixture (mg/100 diet): 557 NaCl; 3.20 KI; 1556 KH2PO4; 229 MgSO4; 1526 Ca CO3; 108 FeSO4-7H2O; 16 MnSO4-H2O; 2.20 ZnSO4-7 H2O; 1.90 CuSO4- 5 H2O and 0.09 CoCl2 - 6 H2O.
(++) Vitamin Mix (Roche, São Paulo, Brazil; mg/100 g diet): 4 Vitamin A; 0.5 Vitamin D; 10 Vitamin E; 0.5 Menadione; 200 Choline; 10 PABA, 10 Inositol; 4 Niacin; 4 Panthothenic Acid; 0.8 Riboflavin; 0.5 Thiamin; 0.5 Pyridoxine; 0.20 Folic Acid; 0.04 Biotin and 0.003 Vitamin B12.

concentrations, except the liver protein, characterizing malnutrition.

Epidemiological studies have suggested that reduced growth in fetal and neonatal life is related to the development of glucose intolerance and diabetes in adult life. This association is, at least in part, a consequence of fetal and early postnatal malnutrition, possibly due to maternal deprivation. Malnutrition in both humans and experimental animals is associated with reduced insulin secretion and decompensated glucose homeostasis[22].

The glucose level in the blood of rats fed with RB showed a trend towards reduction when compared to other diets. However, the results of the present study showed that a reduced level of plasma glucose suggests being a consequence of malnutrition in the rats fed with RB without lysine. This data shows that lys and soybean protein diets can be related with the better development of the rats. The hypoglycemic effect of fibers in the prevention of type II diabetes mellitus may be seen as a

trend in diets with rice bran and soybean protein, suggesting that this effect could be observed if these animals were evaluated later.

Since the middle of the 1970s, the role of dietary fiber in health and nutrition has stimulated a wide range of research activities and caught public attention. Accumulating evidence favors the view that increased intake of dietary fiber can have beneficial effects against chronic diseases, such as cardiovascular diseases and diabetes[5].

A study on rats with rice bran and phytic acid supplementation reduced the serum glucose level, and also the hepatic glycosidase enzyme activity was significantly higher compared to the control group. An increase in the expression of hepatic glucokinase could cause an increase in the utilization of blood glucose for energy production or glycogen storage in the liver[23].

The data of the present study shows that the rice bran diet with lysine contributed in reducing serum cholesterol and LDL, maintaining the HDL levels, suggesting that

TABLE 2 - Macronutrients of diets (g/100 g).

Composition	C.S.	RB	RB + Lys	RB + SJ	RB + SJ + Lys
Carbohydrates	41.30	40.00	39.57	44.69	44.30
Total Fat	4.00	4.29	4.28	4.31	4.31
Protein	22.00	16.22	17.05	21.04	21.04

Legend of diets: Rice Bran (RB); Rice Bran with 1% of lysine (RB + Lys); Rice Bran with 12% of soybean protein (RB + SJ); Rice Bran with 12% of soybean protein and 1% of lysine (RB + SJ + Lys) and Commercial Standard (C.S).

TABLE 3 – Body and liver weight.

Group	Body weight (g)	Liver weight (g)	Liver Protein (mg%)	Liver DNA (mg%)
C.S.	31.09±0.88	1.06±0.01	20.62±0.52	354.22±6.76
RB	27.42±0.63*	0.97±0.02*	20.43±1.11	306.40±7,71*
RB + lys	32.42±0.38	1.11±0.02	20.25±0.43	309.84±16.02*
RB + SJ	39.41±0.45**	1.38±0.03**	18.73±0.07	346.88±10.80
RB + SJ + lys	39.15±0.37**	1.24±0.03**	21.98±0.27	346.94±12.07

Legend of diets: Rice Bran (RB); Rice Bran with 1% of lysine (RB + Lys); Rice Bran with 12% of soybean protein (RB + SJ); Rice Bran with 12% of soybean protein and 1% of lysine (RB + SJ + Lys) and Commercial Standard (C.S.).
Values are expressed as mean ± standard error of the mean (n=8-12). Data was analysed by ANOVA followed by the Tukey HSD (Post Hoc) multiple-range test whenever values were initially significant. *p<0.05 and **p<0.01 compared to the control group (C.S.).

this effects may be related to the fact that the rice bran is an excellent soluble fiber and tocotrienol source[5,24].

Studies have repeatedly shown that rice bran gives interesting health benefits in both humans and experimental animals. These benefits include prevention of cardiovascular disease and diabetes in humans. Hamsters, rats, chicks and pigs fed with rice bran also have decreased serum cholesterol[25-30]. A number of researchers reported that biological effects might be due to the synergistic effects of the multiple bioactive microcomponents present in the rice bran. These microcomponents include -oryzanol and tocotrienols and might be able to maintain glucose levels by exerting effects on glucose absorption, utilization and excretion. These compounds are free radical scavengers and can improve some diabetes complications such as atherosclerosis and hyperlipidemia[24].

Study in rats that were fed for 8 weeks with cycloartenol in amounts identical to that found in rice bran oil unsaponifiable matter obtained a significant reduction in serum cholesterol and triglycerides. The authors explained this by the fact that cycloartenol can be related to increased cholesterol excretion, as well as inhibition of its endogenous production[31].

Dietary protein have been shown to affect plasma cholesterol concentration. It is well established that in different experimental species, soybean protein isolate versus casein in the diet decreases serum cholesterol concentration. The cholesterol-lowering effect might be attributed to the presence of large amounts of non-protein components in the soybean. Soybean protein consumption by rats has been shown to enhance fecal steroid excretion and activate plasma LCAT (lecithin-cholesterol acyltransferase) activity[32-34].

A study with the addition of 20% of soy lecithin for 14 days in the diet of rats showed an increase in the production of bile salts and a stimulus to the cholesterol secretion into the bile, providing a reduction in serum cholesterol levels[35]. The present study demonstrated that the groups fed with RB+SJ with and without lysine presented decreased LDL levels, which was also found in other studies [32-35].

A study on diets containing addition of soybean protein showed that, after 11 weeks, there was a hypocholesterolemic effect[36]. In our study, we could demonstrate that there was a significant reduction in the LDL levels in the diets with rice bran and soybean, as well as described in a study with humans[2].

TABLE 4 – Serum concentration (mg/100 mL serum).

Group	Cholesterol	HDL	LDL	Glucose	Protein	Albumin
C.S.	101.92± 6.92	44.92±2.50	21.00±2.04	120±4.60	3.94±0.10	2.10±0.07
RB	69.17±2.27**	25.83±1.07**	7.25±0.85**	77.25±2.13**	2.53±0.06**	1.46±0.04**
RB with lys	85.75±3.56*	43.5±1.12	10.83±0.46**	115.67±3.86	3.88±0.13	1.91±0.03
RB + SJ	95.00±4.41	49.75±2.21	15.75±1.54*	113.42±4.44	4.18±0.10	2.24±0.05
RB + SJ + lys	89.67±4.04	48.75±2.53	14.08±1.51**	114.08±4.69	4.27±0.13	2.16±0.10

Legend of diets: Rice Bran (RB); Rice Bran with 1% of lysine (RB + Lys); Rice Bran with 12% of soybean protein (RB + SJ); Rice Bran with 12% of soybean protein and 1% of lysine (RB + SJ + Lys) and Comercial Standard (C.S).
Values are expressed as mean ± standard error of the mean (n=8-12). Data was analysed by ANOVA followed by the Tukey HSD (Post Hoc) multiple-range test whenever values were initially significant. *p<0.05 and **p<0.01 compared to the control group (C.S.).

Taken together, the results of present study indicate that the consumption of a diet with rice bran containing or not lysine has a hypocholesterolemic effect. On the other hand, it was observed that thediet without addition of lysine affected the growth and the development of the rats, as well as induced a considerable reduction in plasma glucose.

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