Benefits of fiber and antioxidant power of rice bran in Vitamin E deficient rats

Beneficios de la fibra dietética y poder antioxidante del salvado de arroz en ratas deficientes en Vitamina E

Beneficios da fibra alimentar e poder antioxidante do farelo de arroz em ratos deficientes em Vitamina E

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Abstract

Dietary fiber requirements are met by only a small fraction of the population. There is need for supplemented foods to fill this gap. Rice bran (RB) is high in fiber and has antioxidant properties. The effects of rice bran fiber on several metabolic indicators and the antioxidant capacity of rice bran in rats was reported. Rats were divided into 4 dietary groups: Vitamin E-sufficient with (+VitE/RB+) or without (+VitE/RB-) rice bran; Vitamin E-deficient with (-VitE/RB+) or without (-VitE/RB-) rice bran. Food intake, growth and feed efficiency were similar in all groups but wet and dry fecal mass of the RB+ groups were 3 times higher than the RB- groups. Blood hemoglobin and liver iron were also similar among all groups. However, the liver VitE concentration of the rats of (-VitE/RB-) group was 10x lower than the (+VitE/RB-) group. In contrast, liver VitE of the rats (-VitE/RB+) was only 2.6x lower. This effect of RB was also seen in erythrocytes since, catalase and glutathione reductase increased in the VitE deficient rats but RB prevented this increase. This study shows that dietary RB did not interfere with growth, feed efficiency and iron metabolism, it provided dietary fiber and laxation and partially prevented VitE deficiency.

Key words: rice bran * laxation * antioxidant * rat liver Vitamin E * erythrocyte catalase and glutathione reductase * dietary fiber gap

Resumen

Las recomendaciones de consumo de fibra no se cumplen y hay una necesidad por alimentos con fibra. El salvado de arroz (SA) tiene fibra y propiedades antioxidantes. Aquí se evaluaron estas propiedades en ratas suficientes (+) y deficientes (-) en VitE con o sin SA. Las ratas fueron divididas en cuatro grupos. Dos consumieron dietas +VitE y uno tenía RB. Los restantes consumieron dietas -VitE y uno tenía RB. El consumo de alimento, su eficiencia y el crecimiento, fueron similares entre los 4 grupos pero la masa fecal húmeda o seca fue 3 veces superior en los RB+. La hemoglobina en

sangre y el hierro hepático fueron similares entre grupos, pero en los grupos (SA-) la VitE hepática fue 10 veces menor en las ratas -VitE que en las +VitE. Sin embargo, en las ratas -VitE/SA+, la VitE hepática fue sólo 2,6 veces menor. Este efecto de SA también se detectó en los eritrocitos, ya que la catalasa y la glutatión reductasa aumentaron en el grupo -VitE/SA- pero no en el grupo -VitE/SA+. El estudio muestra que SA no interfirió con el crecimiento y el metabolismo del hierro, tuvo un efecto laxante y previno parcialmente la deficiencia de VitE.

Palabras clave: salvado de arroz * efecto laxante * antioxidante * Vitamina E hepática en ratas * catalasa y glutatión reductasa en eritrocitos * deficiencia de fibra dietética

Resumo

As recomendações de ingestão de fibras não são cumpridos e existe uma necessidade de alimentos ricos em fibras. Farelo de arroz (FA) tem fibra e propriedades antioxidantes. Aqui, estas propriedades foram avaliadas em ratos suficientes (+) e pobres (-) em Vitamina E (VitE) com ou sem FA. Os ratos foram divididos em 4 grupos. Dois consumiram dietas +VitE e um tinha FA. Os restantes consumiram dietas -ViteE e um tinha FA. O consumo de alimento, sua eficiência e crescimento foram semelhantes entre os 4 grupos, mas nos grupos (FA-) a VitE hepática foi 10 vezes menor nos ratos –VitE que nos +VitE. Entretanto, nos ratos –VitE/FA+, a VitE hepática foi apenas 2,6 vezes menor. Este efeito do FA também foi detectado nos eritrócitos, visto que catalase e glutationa redutase aumentaram no grupo -VitE/FA-, mas não no grupo -VitE/FA+. O estudo mostra que FA não interferiu no crescimento ou no metabolismo do ferro, porém teve um efeito laxante e impediu parcialmente a deficiência de VitE.

Palavras-chave: farelo de arroz * efeito laxante * antioxidante * vitamina E hepática em ratos * catalase e glutationa redutase em eritrócitos * deficiência de fibra alimentar.

Introduction

In the first half of the 19th century the human diet changed (1). Due to new developments in the milling industry the ability to remove the germ and bran from the endosperm of cereal grains improved significantly. With this, the human population stopped eating whole cereal grains and began consuming refined cereals (1). This had profound effects on both the nutrition and health of mankind. The most important nutritional effects were observed in rice-consuming countries, where a significant increase in the incidence of beriberi was reported. In Japan, K.Takaki observed this outbreak and solved it by replacing part of the white rice ration offered to Japanese sailors with other foods (2). In Java, C. Eijkman showed that in birds as well as in human subjects, replacing white rice with brown rice prevented beriberi (3). Christian Eijkman was conferred the Nobel Prize in 1929 for his discovery of an antineuritic factor, present in rice bran (3). We now know that this factor is vitamin B1 (tiamine), whose concentration in whole rice and rice bran is almost 17 and 77 times higher than in polished rice respectively (4). The positive association between the consumption of refined grains and health was established by Dennis Burkitt, who, in the mid-1970s, proposed his theory of dietary fiber (5). This theory, based essentially on a comparison between the incidence of the so-called Western diseases between Africa and the US, proposed that the highest incidence of heart disease, appendicitis, diverticular disease, hemorrhoids, gallstones, hiatal hernia, colon cancer and obesity in the US and other Western countries was due to a low fiber intake.

In the late 20th century and early 21st century, knowledge in the area of cereal grains has come a long way. Now, whole cereal grains are not only considered good sources of essential nutrients and fiber but also, they provide resistant starches, oligosaccharides, antioxidants and many other compounds that may protect against chronic diseases (6). Some of these components, acting as prebiotics, may change the intestinal bacterial flora, improving the environment in the gastrointestinal tract and even acting on the immune system systemically, favoring a less inflammatory condition (6). These components are located mainly in bran and germ which make up the outer layers of whole grain cereals (7). The health benefits of whole grains and fiber touch a group of diseases among which cardiovascular disease, diabetes and insulin response, digestive diseases and cancer, as well as obesity, are the most important (8) (9). Thus, fiber and whole grains are identified as foods of public health priority and guidelines for their consumption have been established in many countries (8). However, these recommendations are not met by the vast majority of the world population and there is an urgent need for closing the so called “fiber gap” (10).

A possible way of doing this includes adding the fiber lost in processing back to refined cereal grains (10) and also incorporating fiber to foods that consumers are already eating, e.g., fast foods sandwich buns and pizza crust (11). For this purpose, cereal grains bran present a good choice, since they are rich in fiber, in essential nutrients as well as a variety of indigestible carbohydrates, phytochemicals and antioxidants (12) (13). In this regard, rice bran is an interesting product, since it is rich in antioxidants, like tocopherols, tocotrienols, oryzanols as well as other phytoesterols (12) (13). In addition, rice bran has high fiber content and it has been used to develop health promoting products which have hypolipidemic, anti-tumor, anti-oxidant, ergogenic and laxative properties (12) (13).

Venezuela is a rice producing country and rice bran is largely used for animal feeding. However, previous studies (14-16) have shown that rice bran obtained from Venezuelan rice variety Z-15 has a high concentration of dietary fiber as well as *in vitro* antioxidant activity. Also, these studies (14) showed that compared to brown rice, rice bran contained most of the antioxidants (tocopherols, tocotrienols, γ-oryzanol and total polyphenols) and had correspondingly higher values of *in vitro* antioxidant capacity measured using ferric reducing antioxidant power (FRAP), 2,2′-azinobis-3-ethylbenzotiazoline-6-sulphonic acid (ABTS), and oxygen radical absorbance capacity (ORAC) to measure antioxidant capacity. The data also showed that FRAP was sensitive to polyphenols and total tocotrienols, while ORAC was sensitive to polyphenols and total tocopherols. ABTS was the least sensitive of all assays tested. These observations indicated that, results from *in vitro* antioxidant capacity assays must be interpreted with caution particularly in complex systems containing various antioxidant components such us rice bran. These observations, together with new findings in the area of fiber and whole grains, suggest that their association with better health may be related to alterations of the microbiota (6) (7) and also to a potential effect of antioxidants on gene expression (17). This study reports the effect of rice bran on several aspects of the antioxidant system in rats fed with a VitE-sufficient or VitE-deficient diet (Liver VitE content, serum and liver thiobarbituric acid reactive substance concentration and the activities of antioxidant enzymes in erythrocytes), as well as its effect on food consumption, body weight, fecal mass production and iron metabolism.

Materials and Methods

RICE BRAN PREPARATION

The sample of rice bran used in this study was prepared from paddy rice Zeta-15 (Z-15), obtained from the "Asociación Venezolana de Productores de Arroz (Acarigua, Estado Portuguesa. Venezuela)". Paddy rice Z-15 with a water content of 12±1% was husked into brown rice in a Mini Tester Husker (Rimac MTH-35A from Rice Machinery Supply Corp., Hialeah Gardens. Fl. USA). The bran was obtained by polishing the brown rice during 90 seconds in a rice polisher (Grainman 60-115-60-2H5, from Douglas International Corp. Coral Gables Fl.USA). The polished rice was kept for other studies and the bran recovered was sieved using a 60 mesh ASTM sieve in order to have a uniform particle size. The lipases were inactivated in a tray dryer (30 min at 100 °C), as recommended by Champagne 1994 (18). This Z-15 rice bran was used to prepare the diets shown in Table I. The diet was formulated essentially as AIN-93-G diet (19) but the dietary carbohydrates used were corn starch with no fiber or antioxidant Ter-butylhydroquinone were added. These ingredients were excluded since they could interfere with the objective of the experiment, which was to assess the effects of fiber and the antioxidant capacity of rice bran Z-15. For the same reason, the VitE present in the soybean oil incorporated in the diet was eliminated. For the elimination of VitE from soybean oil, 50 g of the oil, 50 g of hexane, 4 g of activated charcoal and a magnetic bar were placed in a 250 mL Erlenmayer flask. The mixture was stirred for 5 h at room temperature. Then the mixture was filtered under vacuum to remove the charcoal and the solution of oil in hexane was kept under vacuum overnight to remove the hexane (20).

EXPERIMENTAL DESIGN

Twenty eight Sprague Dawley rats, weighing 78.5±9 g, obtained from the Experimental Animals Facility of the Universidad Simón Bolívar, were placed in individual stainless steel hanging wire cages and conditioned during 7 days with a control diet (Table I). They were then randomly assigned to 4 groups, 7 rats per group. The experimental groups and their corresponding diets are shown in Table I.

The rats were fed the experimental diets *ad libitum* for 15 days. The animals had free access to drinking water. Food intake and body weight were monitored every other day, using an Ohaus 1600 series Dial-O-Gram balance provided with an animal container.

During the last four days of the experiment, feces were collected and weighed and they were dried in a convection oven at 80 °C to constant weight. At the end of the experiment, all rats were killed by decapitation under ether anesthesia, blood was collected in heparinized tubes and hemoglobin was determined immediately. The blood samples were centrifuged (2500 rpm, 10 min, 4 °C). The plasma was collected and stored at -80 °C until use. The buffy coat was removed and the remaining erythrocytes were collected, washed and centrifuged 3 times with 3 mL physiological saline. Then, the washed packed red cells were hemolysed using 2

volumes of H_2O, centrifuged to eliminate debris. The washed, hemolysed red cells were stored at -80 °C until used for enzyme activity determinations. The livers were dissected, weighed and kept at -80 °C until use. The animal care and handling were carried out according to the guidelines issued by the National Research Council (22).

Table I. *Composition of the diets[1] offered to the four groups[2] of rats included in the study.*

Experimental groups				
Vitamin E (VitE)	+	+	-	-
Rice Bran (RB)	-	+	-	+
(g/100 g Diet)				
Vit free Casein[3]	20.0	18.6*	20.0	18.6*
VitE free Soy Oil[4]	7.0	5.3*	7.0	5.3*
Min Mix[3,5]	3.5	3.5	3.5	3.5
VitMix[3,6]	1.0	1.0	-	-
VitE free Vit Mix[3,6]	-	-	1.0	1.0
Choline Bitartrate[3]	0.2	0.2	0.2	0.2
L-Methionine[3]	0.3	0.3	0.3	0.3
Z-15 Rice Bran (RB)[7]	-	12.8	-	12.8
Corn Starch[8]	68	58.3	68	58.3

1. The diets were formulated as recommended by the American Institute of Nutrition (AIN-93-G) (19). 2. Groups were: I) Control diet. With VitE, without rice bran (+VitE/RB-). II) With VitE, with rice bran (+VitE/RB+). III) Without VitE, without rice bran (-VitE/RB-). IV) Without VitE, with rice bran (-VitE/RB+). 3. Purchased from Harlan-Teklad. Madison, Wi USA. 4. Purchased locally and treated with hexane and activated charcoal to eliminate VitE (20). 5. The mineral mix was AIN-93G-MX. (19). 6. The vitamin mixes were prepared with individual vitamins, using the formulation (AIN-93-VX) (19), Vitamin E acetate was omitted in the VitE free Vit Mix. 7. Prepared as described in the text and incorporated in the diet at the expense of the corn starch. 8. Purchased locally from Pandock C.A. Caracas-Venezuela. * 12.8 g rice bran provided 1.39 g rice protein and 1.67 g rice oil (21).

ANALYTICAL DETERMINATIONS

All chemical reactants were analytical grade and determinations were run in duplicates.

ERYTHROCYTE ANTIOXIDANT ENZYME ACTIVITIES

Catalase activity

The enzyme activity was determined in a dilute sample (1:10 in H_2O) of the red cell hemolysate. The reaction mixture was the same as that described for rat mesencephalic cell cultures or rat liver homogenates described by Cohen *et al.* (23) which uses the ability of the H_2O_2 remaining after stopping the reaction to oxidize reduce iron and allow the ferric iron to react with thiocyanate (KSCN) with the formation of the red complex ferrithiocyanate (FeSCN). The catalase activity of the hemolysate samples, corresponding to each of the 28 rats included in the study was determined from the absorbance of ferrithiocyanate complex at 492 nm measured after 1 and 9 minutes of reaction time. The activity was calculated and expressed as units of catalase, as indicated by Cohen *et al.* (23) and it represents the rate of decay of the red color of the ferrithiocyanate complex per minute/g-hemoglobin in the hemolysate.

Glutathione Reductase activity

The enzyme activity was determined in a diluted sample (1:2 in H_2O) of the red cell hemolysate as recommended by Bayoumi and Rosalki (24) and Sauberlich (25). The assay measures the capacity of NADPH to reduce oxidized glutathione. Glutathione reduction is coupled with NADP formation. The rate of oxidation of NADPH to NADP was followed in a spectrophotometer set at 340 nm, after 5 and 10 minutes of reaction time. The reaction mixture was the same as reported in (24) (25) and the enzyme activity was calculated and expressed as units of Glutathione Reductase as indicated by Bayoumi and Rosalki (24) and it is expressed as the decay of NADPH per minute/g-hemoglobin in the hemolysate.

INDICATORS OF IRON NUTRITIONAL STATUS

Blood hemoglobin was determined using the Drabkin spectrophotometric procedure (26) with the Hemoglobin Assay Kit (Mak 115 Sigma). The same technique was used to determine the hemoglobin content of the hemolysate used for erythrocyte Catalase and Glutathione Reductase activity determination.

Hematocrit was determined in total blood, using seable glass micro-hematocrit capillary tubes (Thomas Scientific) which were centrifuged (2500 rpm, 10 min, 4 °C) and the % of hematocrit was determined using a Microhematocrit Tube Reader also from Thomas Scientific.

Liver Iron was determined in 0.1g liver (cut in small pieces). Iron was extracted from the tissue in 1 mL solution containing 50 g trichloracetic acid; 124,2 mL 12 M HCL and 15 mL thioglycolic acid in 500 mL H_2O. Iron was determined using the ferrozine spectrophometric method, described by Bothwell Torrance (27).

LIVER VITAMIN E

Liver Vitamin E was determined in 500 µL of liver homogenates (0.5 g liver in 2 mL H_2O).The method described by Ueda and Igarashi (28) was used. This method measures Vitamin E after saponification and extraction from a liver sample using high pressure liquid chromatography (HPLC). The HPLC conditions were the same as previously described (29). Retinylacetate was used as an internal standard during the complete procedure. This HPLC method cannot tell the tocopherol isomers from the tocotrienol isomers.

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

TBARS were determined in 100 µL of serum or 100 µL liver homogenates (1g liver in 10 mL KCl 1.5%), using the method proposed by Ohkawa *et al.* (30) using 1,1,3,3-tetrahidroxipropane as a standard.

Results

In this study, in the rice bran containing groups, rice bran (Z-15) represented 12.8% of the diet and, according to its composition (16) (21), it contributed with 1.39 g protein and 1.67 g lipids per 100 g diet. This accounted for 7% and 24% of the dietary protein and fat respectively. As shown in Table I, the protein and lipids provided by rice bran were subtracted from the dietary casein or the dietary soybean oil incorporated in the diets that included this bran. This made these diets isocaloric and isonitrogenous with respect to the diets without added rice bran. From the data presented in Table II, it can be calculated that the rats assigned to the diets with rice bran consumed approximately 3.5 g fiber/kg body weight-day. This represents 6.4 times the fiber consumption recommended for a human adult male (8) and it provided only 70% of the fiber intake recommended for experimental rodents (19).

In general, the amount of rice bran incorporated here in the diet attempted to add as much rice bran fiber as possible but keeping its concentration within reasonable limits with respect to the recommendations for humans. At the same time, the replacement of casein and soybean oil for rice protein or rice fat oil was kept to a minimum to avoid negative effects on food consumption and growth.

Table II shows that neither the inclusion of rice bran nor the deletion of VitE in the diet of the studied rats had a significant effect on the amount of food they consumed. This was observed all along the experiment.

In agreement with the results of food intake, Table II also shows that the rats assigned to the four study groups showed similar weight gains. This is also presented in Figure 1, which shows the changes in body weight seen in the rats during the experiment. From this figure, it is apparent that the increase in body weight with time, of all rats, followed a similar pattern and that there was significant overlap among the four studied groups, particularly until day 10. Table II also shows that in agreement with the food intake and the growth data just discussed, feed efficiency (growth x 100/food consumed) was similar in the four groups of rats included in this experiment.

Table III shows the mean fecal weight measured during the last 4 days of the experiment. From the table, it is apparent that the two groups consuming the diets with added rice bran excreted substantially more wet or dry fecal matter than those assigned to the diets without rice bran (approximately 2.8 times higher in both cases). Also, the fecal moisture was 3.1 times higher in the feces of the rats consuming rice bran, whereas the ratio of fecal weight/food consumed was also higher (2.54 times) in the rats consuming the rice bran.

Table IV shows that blood hematocrit, hemoglobin and liver iron were similar in the four groups of rats,

Table II. *Food intake and growth of young male rats consuming diets that provided sufficient or deficient Vitamin E with or without rice bran during fifteen days.*

Experimental groups				
Vitamin E (VitE)	+	+	-	-
Rice Bran (RB)	-	+	-	+
Consumption	*(g/15 days)*			
Food	200.5±31.33^{a}	211.2±15.15^{a}	194.5±27.72^{a}	206.5±15.28^{a}
Rice Bran (RB)	0^{a}	27.0±1.79^{b}	0^{a}	26.4±1.81^{b}
Dietary Fiber[1]	0^{a}	10.6±0.70^{b}	0^{a}	10.3±0.71^{b}
VitE (mg/15days)[2]	30.1±4.35^{c}	37.3±2.46^{d}	0^{a}	5.5±0.38^{b}
Rat growth				
Initial weight (g)	118.8±10.51^{a}	118.6±9.91^{a}	118.3±10.33^{a}	119.0±11.74^{a}
Final weight (g)	193.3±30.12^{a}	199.1±22.35^{a}	193.2±25.01^{a}	199.5±19.92^{a}
Growth (g/15 days)	75.0±23.81^{a}	80.4±15.3^{a}	78.2±11.83^{a}	81.1±10.34^{a}
Feed Efficiency (%)	36.8±8.21^{a}	37.8±5.43^{a}	39.6±1.92^{a}	39.2±2.81^{a}
N	7	7	7	7

Entries are means ± SD of 7 rats. Means with different superscript letters $^{(a\text{-}d)}$ are statistically different at 5%. 1. Calculated from the dietary fiber content of rice bran Z-15 which was 39.2% (16). 2. Calculated from the VitE provided by the (+VitE) diet (15 mg/100 g diet or 7.5 UI/100 g diet) (19) and or the total tocol content of the same rice bran (0.0209%) (14)(21).

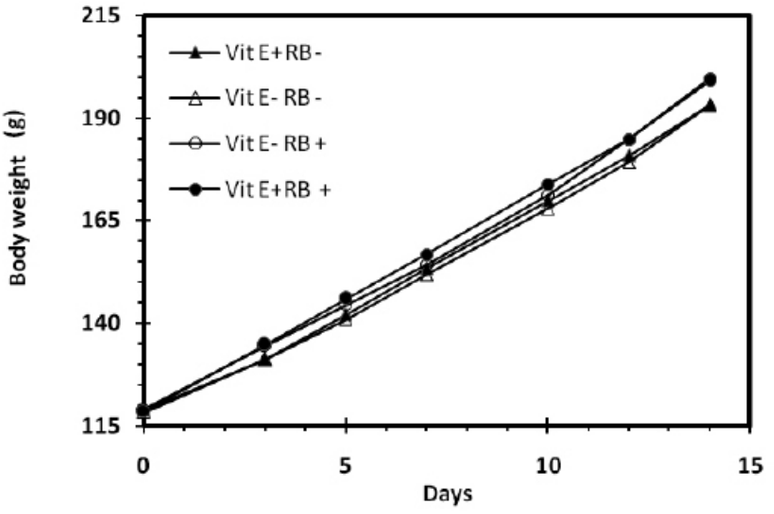

Figure 1. *Body weight change of rats (7 per group) fed Vitamin E sufficient (VitE+) or Vitamin E deficient (VitE-) diets with (RB+) or without (RB-) rice bran Z-15 during 15 days.*

Table III. *Fecal mass of male growing rats consuming diets that provided sufficient or deficient Vitamin E with or without rice bran during fifteen days.*

	Experimental groups			
Vitamin E (VitE)	+	+	-	-
Rice Bran (RB)	-	+	-	+
Fecal matter	*(g/4 days)*[1]			
Fecal weight (wet)	0.58±0.07[a]	1.63±0.21[b]	0.61±0.18[a]	1.68±0.34[b]
Fecal weigh (dry)	0.29±0.05[a]	0.83±0.10[b]	0.30±0.08[a]	0.82±0.13[b]
Fecal moisture	0.21±0.11[a]	0.71±0.22[b]	0.24±0.14[a]	0.68±0.34[b]
	(mg/g)			
Fecal weight (wet)/ Food intake	10.85±1.09[a]	26.94±5.78[b]	11.76±2.81[a]	30.51±6.39[b]
N	7	7	7	7

Entries are means ± SD of 7 rats. Means with different superscript letters (a-b) are statistically different at 5%. 1. Feces were collected during the last 4 days of the experiment.

indicating that rice bran did not affect the iron status of these animals. In contrast, liver VitE was different between all dietary groups. Liver VitE of rats fed on the –VitE/RB- diet was 10 times lower than the animals fed on the +VitE/RB- diet. However, liver VitE of rats fed on the -VitE/RB+ was only 2.6 times lower than the +VitE/RB- group. Moreover, dietary rice bran further increased by 1.43 times the liver VitEin rats consuming the VitE-sufficient diet (+VitE/RB-).

Table IV indicates that the TBARS measured both in blood serum and in liver of the rats did not differ between the four dietary groups. In contrast, the table shows that the erythrocyte capacity of detoxifying H_2O_2 via catalase or of utilizing NADPH to reduce glutathione via glutathione reductase were 35% and 39% higher respectively in the group fed on the diet deficient in VitE without rice bran (-VitE/RB-). This was prevented by dietary VitE and/or rice bran.

Discussion and Conclusions

Even though the health benefits of dietary fiber have long been appreciated (31) and more recently, (1) the value of dietary whole cereal grains has been established, consumption of these food ingredients in the western world is still very low (8-11). Nutrition policy makers in different parts of the world (9) have

Table IV. *Iron status, plasma and liver TBARS, erythrocyte catalase and glutathione reductase activities and liver Vitamin E of young male rats consuming diets that provided sufficient or deficient Vitamin E with or without rice bran, during fifteen days.*

	Experimental groups			
Vitamin E (VitE)	+	+	-	-
Rice Bran (RB)	-	+	-	+
Blood				
Hematocrit (%)	33.14±6.70[a]	33.14±3.01[a]	29.47±3.13[a]	32.71±3.25[a]
Hemoglobin (g/dL)	12.54±0.97[a]	13.17±0.81[a]	12.39±1.21[a]	11.20±1.94[a]
Plasma				
TBARS (nmoles/mL)	15.48±3.03[a]	13.39±1.67[a]	14.13±2.03[a]	15.23±1.00[a]
Erythrocytes				
Catalase (U/g Hb)	8.94±1.40[a]	8.27±1.87[a]	11.90±1.1b[b]	9.15±1.70[a]
Glutathione reductase (U/g Hb)	3.09±0.44[a]	2.99±0.53[a]	4.85±1.06[b]	3.07±0.52[a]
Liver				
Weight (g)	9.9±2.8[a]	9.3±1.5[a]	9.5±1.8[a]	10.1±1.6[a]
Iron (µg/g)	63.89±22.09[a]	67.00±18.75[a]	64.04±9.35[a]	71.21±13.33[a]
VitE (µg/g)	25.07±7.26[c]	35.89±11.27[d]	2.51±0.79[a]	9.57±2.35[b]
TBARS (nmoles/g)	163.20±7.26[a]	151.20±19.96[a]	169.70±27.51[a]	160.81±30.74[a]
N	7	7	7	7

Entries are means ± SD of 7 rats. Means with different superscript letters (a-d) are statistically different at 5%.

established recommendations for intakes that are not met by the vast majority of the population. In the US, the recommended intake of fiber for adults is in the range of 25 to 38 g/day (11). This is met by less than 5% of most age and gender subgroups. Most Americans need to nearly double their fiber consumption to meet their daily recommendation (11). The fact that the most commonly consumed foods are low in dietary fiber (30) justifies the addition of fiber to these foods as a practical solution to solve this dietary fiber gap condition (11). Accordingly, the effects of incorporating rice bran, a high fiber dietary ingredient with antioxidant properties, in the diets of rats are presented here.

It is accepted that fiber and satiety are related. However, this relationship depends on many factors (8) and therefore, it is difficult to generalize. The results of this experiment showed that the diets designed here were well accepted and resulted in similar growth rates, food intake and feed efficiency ratios, both in the groups with or without rice bran. This is consistent with previous reports (12) (13) regarding the fact that rice bran, being a source of good quality nutrients, did not affect negatively the nutritional status of the rats studied. Similar results have been reported in rats fed on wheat bran (32). The results of these study are also consistent with short term human studies showing that diets providing different amounts of fiber from beans (3 g and 12 g) have similar effects on satiety and caloric consumption (33). In contrast, long term human studies (9 weeks) show that in obese individuals, corn dextrin fiber caused a reduction both in hunger feeling and caloric intake and that these reductions were proportional both to experimental time and fiber concentration (33). Lastly, our results show that a VitE deficiency lasting 15 days was insufficient to affect rat growth.

One of the most noticeable effects of consuming fiber is an increase in stool weight and laxation (8). This effect laid the foundations for the dietary fiber theory (5) and the beneficial effects of fiber on gastrointestinal health. In this study, rice bran incorporated at 12.8 % in the diet provided 5.02 g fiber per 100 g diet and its consumption increased wet and dry fecal mass by approximately 3 times. The increment in fecal weight associated with fiber consumption may result from the following factors: a) water held by the fiber; b) components of the fiber remaining unfermented in the intestine and excreted in the feces; and c) a fermentable fraction of fiber, which, working as prebiotics, increases bacterial mass and fecal weight (8). In this study the feces of the rats consuming rice bran had both more water and dry matter indicating that the increment in fecal mass associated with the consumption of rice bran resulted from the first two factors. Since the intestinal microflora was not assessed in these rats, it is not possible to say whether this factor contributed to the increase in fecal weight seen in the rats that consumed rice bran. However, this is likely to have occurred since, without the fermentation of the rice bran fiber by gut bacteria, it would have been impossible for rats consuming rice bran to have the same growth and feed efficiency that the rats that did not consume rice bran.

Rice bran fiber contains mainly cellulose and hemicellulose with less than 1% of soluble β glucans (34). Single stomach animals like rats lack the enzymes necessary to digest these carbohydrates, but the intestinal microflora (intestinal microbiota) can metabolize and utilize them for their own multiplication and growth in the gut. In addition, the bacteria liberate products such as short chain fatty acids that can be used as a source of energy by the host, converting the indigestible fiber into digestible energy (31).The intestinal microbiota has many other functions as well in host physiology and metabolism, particularly by modulating the immune response both in the intestine and elsewhere in the organism (35). Thus, shifts in gut microbiota composition and density alter immunity and inflammation even in organs distal from the intestine (36). Recent studies have suggested an association between gut microbiota and obesity, metabolic syndrome, diabetes as well as allergies and autoimmune diseases (36-38). The indigestible carbohydrates in fiber have a trophic effect among saccharolytic bacteria (lactobacilli and bifidobacteria), present in gut microbiota. This effect of fiber is considered desirable since it is associated with an immune system more tolerant toward innocuous antigens producing a less inflammatory environment in the gut and elsewhere in the organism (6) (7) (31).

In this study, some aspects of iron status were evaluated, essentially for two reasons: the first relates to the concern that fiber and fiber-associated compounds like polyphenols and phytic acid reduce iron absorption (8); and the second is that erythrocytes are very susceptible to VitE-deficiency and this deficiency produces hemolytic anemia in susceptible humans as it is in the case of premature infants (39). However, in this experiment, neither rice bran nor VitE-deficiency significantly altered rat iron status. Rice bran Z-15 is high in fiber, phytic acid and polyphenols (16), but it is also high in iron (15). The presence of phytic and polyphenols in rice bran might have been expected to diminish liver iron. That this was not the case suggests that elevated levels of iron in Z-15 rice bran were sufficient to compensate for any negative effects of phytic acid and polyphenols. Also, it is worth mentioning that the iron status is controlled by the hepcidine-ferroportine axis (40). In this system, the liver detects changes in systemic iron and responds by increasing (high iron or inflammation) or decreasing (low iron or anoxia) hepcidine production. This liver hormone acting on the only cell exporting transport system known in the organism, ferroportine, reduces or increases iron absorption and cell iron transport. This axis probably explains the lack of a negative effect of rice bran on iron homeostasis seen in this study and also the notion (8) that as long as the diet provides sufficient iron, fiber has no negative effects on iron status.

Hemolytic anemias are characterized by low hematocrit and hemoglobin but with iron deposits that are normal or elevated (40). In this study, the rats assigned to the group deficient in VitE without rice bran had the lowest hematocrit (29.5%) of the whole experiment but this value was not statistically different from those measured in the other groups. This indicates that 15 days on a VitE deficient diet was insufficient to produce a clear case of hemolytic anemia. Since these rats also grew like VitE-sufficient rats, the only evidence that these rats were deficient was that they had a liver VitE content that was 10 times lower than control rats.

It is worth noting that the severe reduction in liver vitamin E resulting from the consumption of the VitE-deficient diet during 15 days was partially prevented by rice bran. Accordingly, the rats fed on the VitE-deficient diet with rice bran had approximately 4 times more liver VitE than those fed on the deficient diet without rice bran. The contribution of rice bran to the liver VitE concentration was also seen in the rats fed on the VitE-sufficient diet with rice bran since these rats had liver VitE concentration 43% higher than the rats fed on the same diet without rice bran. The liver concentration of VitE is important in relation to the antioxidant defense mechanisms in the whole animal, since the liver participates in VitE distribution to the different tissues by the action of α-Tocopherol Transfer Protein (α-TTP) (41) and as shown here, it responded very rapidly to dietary VitE deficiency. The data showed that the rats fed on the diet devoid of VitE had 2.51 μg VitE per g liver and this VitE did not come from the diet consumed during the previous 15 days. Therefore, the liver VitE, originating from the diet in the rats fed on the diet (+VitE/RB-) was 25.07-2.51 μg, and in the case of the rats (-VitE/RB+), it was 9.57-2.51 μg or 22.56 and 7.06 μg VitE/g respectively. Since 7.06 is 31% of 22.56, this indicates that, in this study, rice bran was 31% as effective as dietary VitE in increasing the concentration of VitE in the liver. Since according to the composition of the diet (19), the rats fed on the diet (+VitE/RB-) consumed 15 IU α-tocopherol (Table II), then rice bran Z-15, fed at the concentration used in this experiment, was equivalent to 31% of this amount or 4.65 IU of α-tocopherol. This liver VitE reserve associated with rice bran consumption probably resulted from a reduction in VitE requirements and also from theVitE content of rice bran. Rice bran may reduce VitE requirements because it contains a variety of antioxidants such as polyphenols, oryzanol and a complex variety of tocols (15) (16), which, by replacing Vitamin E in its antioxidant function in the intestine and/or systemically, could reduce the need to use the vitamin. This suggests that rice bran may have a sparing effect on VitE. Also, the fiber content of rice bran may provide a less inflammatory environment reducing oxidative stress and the VitE requirement. Regarding the possible contribution of the VitE present in rice bran to the liver concentration of this vitamin in the rats assigned to the groups consuming rice bran, it is important to indicate that total tocol content of this rice bran was 20.9 mg/100g but only 23% were tocoph-

erols, 77% were tocotrienols, whereas its α-tocopherol concentration accounted for only 12.6% (14) (21). In this regard, it is worth noting that even though tocotrienols are isomers of Vitamin E with well recognized health beneficial properties (42), their availability (43) as well as their tissue retention (17) is small compared to α-tocopherol (all-rac-α-tocopherol) which is the only form of VitE capable of satisfying the daily requirements of this vitamin (41). The contribution of rice bran to liver VitE may therefore be small.

Due to their hemoglobin and oxygen content, red blood cells are very susceptible to oxidation. In these cells, hemoglobin represents 95% of their total protein. At steady state conditions, 3% of this hemoglobin is oxidized daily to methemoglobin with liberation of superoxide anion. Superoxide is subsequently transformed into H_2O_2 by super-oxide dismutase. Hydrogen peroxide is not the only component causing oxidative stress in red blood cells. However, it may cause oxidative injury (44), such as erythrocyte membrane fragility and hemolytic anemias, particularly in cases of VitE-deficiency (40). In fact, erythrocyte membrane fragility towards H_2O_2 is used as an indicator of VitE status (39). The red cell has two main enzymatic systems for neutralizing H_2O_2: Catalase and Glutathione Peroxidase. Catalase decomposes H_2O_2 into water, whereas Glutathione Peroxidase, a selenium containing enzyme, destroys peroxides in general, both organic and H_2O_2, using reduced glutathione as a co-substrate (44). In this study, the erythrocyte decomposition of hydrogen peroxide was expressed as catalase activity and the erythrocyte capacity of reducing glutathione at the expense of NADPH was expressed as glutathione reductase activity. The importance of having sufficient reduced glutathione and NADPH in red cells is demonstrated by the tendency of developing hemolytic anemia in patients with inherited Glucose-6-Phosphate Dehydrogenase deficiency and limited capacity of producing NADPH for reducing glutathione (40).

In agreement with the previous discussion, this study showed that erythrocytes of the rats consuming the diet deficient in VitE (-VitE/RB-), by having higher catalase and glutathione reductase activities, had an increased capacity to neutralize H_2O_2 and to use NADPH for reducing glutathione respectively. This was not seen in the two groups consuming the diets with VitE or in the group consuming the diet deficient in VitE with rice bran. This indicates that rice bran compensated for the lack of vitamin E, since in the rats in this group (-VitE/RB+) just as in the groups consuming this vitamin, there was no need to increase these enzyme activities. It was probably the increase in the activity of these enzymes, occurring during the production of new red cells in rats that were growing and expanding their blood volume, what protected them from developing the hemolytic anemia of VitE deficiency. The fact that rice bran avoided the need to increase erythrocyte antioxidant enzymes is an indication that the effect of this bran was not only limited to the liver but it also had an antioxidant effect beyond this organ. The capacity of rice bran to spare VitE is important because a large segment (>92%) of Americans fail to meet their daily VitE requirements (45). This is particularly of concern among obese individuals presenting metabolic syndrome who have an increased VitE requirement and who, at the same time, reduce their fat intake for weight loss objectives (45). In this condition, the use of foods that have a high fiber content together with antioxidant capacity, such as rice bran, may be an appropriate choice.

In this study, TBARS in plasma and liver were similar in the rats consuming the diets with or without VitE. This indicates that fifteen days were insufficient for eliciting an overt oxidative stress in these rats, probably due to both a residual VitE still present in the system and also to the activation of other antioxidant mechanisms that compensated for this deficiency.

In conclusion, this study shows that in growing rats, Venezuelan Rice Bran Z-15, was well accepted and resulted in growth and feed efficiencies similar to those seen in rats fed on diets without rice bran. In these rats, rice bran increased fecal mass and it did not interfere with iron metabolism. In addition, this bran had a clear antioxidant capacity since its consumption resulted in higher liver VitE and prevented the stimulation of antioxidant enzymes seen in VitE deficient rats.

The results of this *in vivo* study show that rice bran, a by-product of whole rice processing, is a food ingredient high in fiber and antioxidant capacity which may be useful in human diets for reducing both the fiber and Vitamin E gaps.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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