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# Extruded whole grain diets based on brown, soaked and germinated rice. Effects on cecum health, calcium absorption and bone parameters of growing Wistar rats. Part I

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The influence of diets with whole rice processed ingredients on cecum health, calcium absorption and bone parameters was studied using an animal model. Thirty-two male *Wistar* rats were fed with **Control** (C), extruded **Brown** rice (B), extruded **Soaked** whole rice (S) and extruded **Germinated** whole rice (G) diets for 60 days. The cecum weight, cecal content pH, cecal sIgA content, and β-glucosidase and β-glucuronidase activities were determined. Calcium apparent absorption, total bone mineral content and density and right femur parameters (ashes, organic content, calcium and P) were evaluated. The results showed that animals fed with whole grain diets have lower food intake in comparison with the C diet, and decreased cecal content pH (7.06 *vs.* 6.33) and β-glucosidase activity (1.66 *vs.* 0.21 μmol *p*-nitrophenol $g^{-1}$ cc $h^{-1}$). Even though calcium apparent absorption was not different among treatments (~70%), none of the whole grain diets improved calcium related bone parameters over the control fed rats (cellulose as dietary fibre).

## 1. Introduction

Dietary fibre (DF) includes cell wall polysaccharides, lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans.[1] DF has physiological effects on the large bowel microflora diversity. The degradation of DF can lead to the production of fermentation products (*e.g.*, short-chain fatty acids [SCFAs]) and increase in fecal weight by providing substrates for microbial growth. In addition, beneficial gut microflora serves as a brake to colonization by pathogens. Whole grains (WG) are valuable sources of fermentable carbohydrates such as dietary fibre, resistant starch, and oligosaccharides.[2] Epidemiological studies have found that high consumption of WG is associated with a low risk of colorectal cancer.[3–5]

Adequate calcium intake is critical to maintaining the peak bone mass and modifies the rate of bone loss in the latter years. Moreover, convincing evidence has emerged regarding the importance of dietary calcium and bone health for all age groups.[6] The calcium requirement of an individual depends on many factors such as age, sex, pregnancy and ethnicity. Besides the amount of calcium in the diet, its absorption is another critical factor determining its availability for the body. In addition to dietary recommendations, the consumption of functional foods containing ingredients that promote calcium absorption may ensure maximum calcium bioavailability.[7]

It was reported that the phytic acid (PA) content of whole cereal based products is responsible for a negative calcium balance in rats and humans.[8] Soaking, germination and extrusion processes not only have a deep impact on the chemical composition and physical properties of cereal flours, but can also reduce the PA content, which would improve calcium bioaccessibility. In this regard, extrusion cooking causes the mechanical rupture of DF glycosidic bonds, which could increase soluble dietary fibre (SDF).[9]

The aim of this work was to study the physiological effects of diets with different processed whole grain rice flours using an *in vivo* Wistar rat model, focusing on cecal pH and fermentation, dietary calcium absorption and bone mineral balance.

## 2. Materials and methods

### 2.1 Processed whole grain rice flours

Rough and brown long rice Fortuna type (*Oryza sativa* L.) were provided by Los Cerrillos S.A (Santa Fe, Argentina). Three different ingredients based on whole rice flours (extruded

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brown rice, extruded soaked whole rice and extruded germinated whole rice) were produced as follows.

**2.1.1. Extruded brown rice flour.** Brown rice was transformed into grits (particle size ranging from 1190 to 420 μm) using a roller mill (Bühler-Miag, Braunschweig, Germany). The grits were conditioned to 14 g per 100 g of moisture 2 h before the extrusion process using a Brabender planetary mixer P-600 L (Brabender, Duisburg, Germany) at 60 rpm rotation speed. The extrusion process was carried out with a Brabender 20 DN single screw extruder (Brabender, Duisburg, Germany), using the following conditions: 4 : 1 compression ratio screw, 3/20 mm die (diameter/length), 150 rpm screw speed and 160 °C. The feeding rate of the extruder was at full capacity. The extruded samples were milled with a hammer mill (Retsch-Mühle, Braunschweig, Germany) using a sieve of 1.5 mm in order to obtain the extruded brown rice flour (B).

**2.1.2. Extruded soaked whole rice flour.** Rough rice was previously washed in a NaClO solution (120 mg $L^{-1}$ available chlorine) for 15 min. After that, the grains were washed with tap water and soaked in a 5.5 g $L^{-1}$ lactic acid solution at 45 °C for 24 h according to Albarracín, González & Drago.[10] Then, the grains were washed with tap water, dried to around 13 g per 100 g moisture using an oven at 40 °C, dehulled, and milled to grits (1190–420 μm particle size) with the roller mill. Then, the sample was extruded at 160 °C and 16.5 g per 100 g of moisture and the extrudate was milled with the hammer mill with a 1.5 mm sieve in order to obtain the extruded soaked whole rice flour (S).

**2.1.3. Extruded germinated whole rice flour.** Rough rice was previously washed in a NaClO solution (120 mg $L^{-1}$ available chlorine) for 15 minutes. After that, the grains were washed with tap water and soaked in distilled water at 20 °C for 24 h. Then, the water was drained, and the moistened rice grains were germinated over 24 h by placing them in an oven at 35 °C (98% relatively humidity), according to the conditions previously studied.[11] The germinated rice was dried over 24 h at 40 °C, dehulled and milled to grits (1190–420 μm particle size) using the roller mill. Then, the sample was extruded at 175 °C and 14 g per 100 g moisture and the product was milled with the hammer mill using a 1.5 mm sieve in order to obtain the extruded germinated whole rice flour (G).

### 2.2. Animals and diets

Thirty two ($n$ = 32) male Wistar rats (52.3 ± 4.5 g) were obtained from the Animal Service Laboratory of the Facultad de Bioquímica y Farmacia, UBA (Buenos Aires, Argentina). Throughout the experiment, the animals were housed in individual stainless steel cages in a temperature (21 ± 1 °C) and humidity (60 ± 10%) controlled room with a 12 h light/dark cycle and allowed free access to deionized water and food. They were divided in 4 groups ($n$ = 8 per group) and fed with one of the following diets over a 60-day period:

**Group C**: rats fed with AIN 93.[12]

**Group B**: rats fed with AIN 93 containing 5 g per 100 g diet of fibre from extruded brown rice (see section 2.1.1) replacing cellulose or maltodextrin.

**Table 1** Composition of: control (C), extruded brown (B), soaked (S) and germinated (G) rice based diets

| Food component | C | B | S | G |
|---|---|---|---|---|
| Casein (g per kg diet) | 170.00 | 98.9 | 100.9 | 145.2 |
| AIN-93 mineral mix (g per kg diet) | 35.00 | 21.4 | 20.6 | 26.4 |
| AIN-93 vitamin mix (g per kg diet) | 10.00 | 8.1 | 8.1 | 10.0 |
| L-Cysteine (g per kg diet) | 3.00 | 3.0 | 3.0 | 3.00 |
| Vitamin A (mL) | 1.00 | 0.8 | 0.8 | 1.0 |
| Soybean oil (g per kg diet) | 69.00 | 54.0 | 52.6 | 68.5 |
| Choline bitartrate (mL) | 7.1 | 5.7 | 5.7 | 7.1 |
| Cellulose (g per kg diet) | 50.00 | — | — | — |
| Whole rice ingredient (B, S or G) (g per kg diet) | — | 808.1 | 808.3 | 699.3 |
| Dextrin (g per kg diet) | 654.9 | — | — | 39.5 |
| Total energy (kcal per kg diet) | 3766 | 3766 | 3766 | 3766 |

**Group S**: rats fed with AIN 93 containing 5 g per 100 g diet of fibre from extruded soaked rice (see section 2.1.2) replacing cellulose or maltodextrin.

**Group G**: rats fed with AIN 93 containing 5 g per 100 g diet of fibre from extruded germinated rice (see section 2.1.3) replacing cellulose or maltodextrin.

The composition of diets is presented in Table 1.

The analysis of the diets confirmed that they were isocaloric and supplied a similar amount of macronutrients, calcium (Ca) (0.5 g per 100 g) and phosphorus (P) (0.3 g per 100 g).

This study was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and was approved by the Committee of Health Guide for the Care and Use of Laboratory Animals of the Facultad de Bioquímica y Farmacia, UBA (Buenos Aires, Argentina).

### 2.3. Sampling procedures

The body weight (BW) was recorded once a week throughout the study. Food intakes were recorded every three days throughout the experiment. Total and daily intakes were calculated. The efficiency of diets was calculated as the relationship between the weight gained by the animal and the food consumed, *i.e.* weight gain/food intake.

At the end of the experiment (60 days), the rats were anesthetized with an intraperitoneal injection of 0.1 mg per 100 g BW of ketamine hydrochloride + 0.1 mg per 100 g BW of acepromazine maleate. After the rats were killed, ceca were removed and weighed (Mettler Toledo, USA). Cecal contents were weighed and their pH was measured (IQ Scientific ISFET Handheld pH/mV Meter, Cole Parmer, USA). Then, a 1/4 cecal content dilution with 0.01 mol $L^{-1}$ PBS buffer (pH 7.3) was performed, shaken and centrifuged at 3000$g$ for 20 min. In these samples, cecal enzymes were analyzed. An aliquot of the supernatant was combined with 4 μL of the protease inhibitor (Protease Inhibitor Cocktail P8340, Sigma-Aldrich, USA) in order to analyze the secretory IgA (sIgA) content. All cecal diluted samples were stored in a freezer at −80 °C for posterior analysis.

Right femurs were removed according to Albarracín *et al.*[13]

### 2.4. Analytical procedure

**2.4.1. Total skeleton bone mineral content (BMC) and bone mineral density (BMD).** Before the end of the experiment the total skeleton bone mineral content (BMC) and bone mineral density (BMD) were determined *in vivo* under light anesthesia (0.1 mg per 100 g body weight of ketamine hydrochloride + 0.1 mg per 100 g BW of acepromazine maleate) with a total body scanner by dual-energy X-ray absorptiometry (DXA) provided with a specifically designed software for small animals (DPX Alpha, Small Animal Software, Lunar Radiation Corp. Madison WI) as previously described by Albarracín *et al.*[13]

**2.4.2. Cecal secretory IgA content.** Secretory IgA (sIgA) of the cecal content was determined by ELISA (enzyme-linked immunosorbent assay) (BD Biosciences, California, USA). The absorbance was recorded at 450 nm with a microplate reader (Biochrom Asys UVM340 Microplate Reader, Cambridge, UK). The results were expressed as μg sIgA per g cecal content (cc).

**2.4.3. β-Glucosidase and β-glucuronidase activities in cecum.** The enzyme activities were determined as described by Gudiel-Urbano & Goñi[14] with modifications. The rates of release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucoside (N-7006, Sigma-Aldrich) as a substrate for β-D-glucosidase and *p*-nitrophenyl-β-D-glucuronide (N-7009, Sigma-Aldrich) as a substrate for β-D-glucuronidase were determined. The reaction mixture containing 35 μL of cecal content, 23 μL of phosphate buffer 0.1 M (pH 7) and 12 μL of a 5 mM substrate solution was incubated at 37 °C for 10 minutes (in the dark). The reaction was stopped after the addition of 180 μL of 0.25 M $Na_2CO_3$. The concentration of released *p*-nitrophenol was determined from the optical absorbance at 400 nm with a microplate reader (Biochrom Asys UVM340 Microplate Reader, Cambridge, UK). Enzyme activity was determined by using a calibration curve with *p*-nitrophenol as a standard and the result was expressed in μmol *p*-nitrophenol $g^{-1}$ cc $h^{-1}$.

**2.4.4. Mineral analysis.** Feces were dried under infrared light and pounded. Diets and feces were processed by wet ashing with nitric acid using Parr bombs.[15]

The calcium concentration in diets, feces and bones was determined using an atomic absorption spectrophotometer.[16] Lanthanum chloride (6500 mg $L^{-1}$ in the final solution) was added to avoid interferences. The P concentration was measured according to the Gomori method.[17] NIST (National Institute of Standards and Technology) reference material RM 8435 (whole milk powder) was also subjected to identical treatment to verify the accuracy of the analytical procedures and treated with each bath of samples to ensure accuracy and reproducibility of mineral analysis.

In the case of femurs, the amounts of calcium and P were expressed as the total content and the femur Ca/P ratio was calculated.

**2.4.5. Calcium apparent absorption.** Food intake was determined and feces were collected and weighted during the last three days of the experiment and were used to calculate calcium apparent absorption (%Ca App Abs) as follows (eqn (1)):

$$\%\text{Ca app abs} = [(\text{Ca I–fecal Ca})/\text{Ca I}] \times 100 \quad (1)$$

where Ca I is the daily calcium intake, and fecal Ca is Ca fecal excretion.

### 2.5. Statistical analysis

Data were presented as the arithmetic means ± SEM for each treatment group ($n$ = 8). Differences were tested by one-way analysis of variance (ANOVA) and the statistical differences among samples were determined using the LSD test (least significant difference). Significance was accepted at $p < 0.05$.

## 3. Results and discussion

### 3.1. Food intake and efficiency

Total food intake, body weight gain (BWG) and efficiency are presented in Table 2. The results of total and daily food intake indicated significant differences among the diets. The lowest values correspond to the animals fed with S and B diets. The consumption of both the diets resulted in lower BWG and consequently, lower values of efficiency. Even though animals fed with the G diet presented a lower total intake than the animals fed with the C diet (approximately 12% less), BWG and efficiency did not differ from C.

Studies reported by Coudray *et al.*[18] showed that animals fed with a whole-wheat diet had lower growth than those fed with a control diet, and both animal groups presented higher growth compared to a refined wheat diet. Moreover, the intake was also lower for the groups consuming wheat compared with

Table 2 Total food intake, daily intake, body weight gain (BWG) and efficiency

| Diets | Total intake (g per 60 days) | Daily intake (g per day) | BWG (g per 60 days) | Efficiency (g BW per g diet) |
|---|---|---|---|---|
| C | 1043.87 ± 36.6$^{a}$ | 17.04 ± 0.71$^{a}$ | 225.99 ± 10.65$^{a}$ | 0.27 ± 0.01$^{a}$ |
| B | 785.25 ± 38.4$^{c}$ | 13.28 ± 0.62$^{c}$ | 188.04 ± 10.70$^{b}$ | 0.24 ± 0.01$^{b}$ |
| S | 872.94 ± 30.2$^{bc}$ | 14.53 ± 0.51$^{bc}$ | 215.17 ± 10.98$^{b}$ | 0.24 ± 0.01$^{b}$ |
| G | 913.64 ± 20.4$^{b}$ | 15.55 ± 0.35$^{ab}$ | 267.05 ± 7.81$^{a}$ | 0.29 ± 0.01$^{a}$ |
| ***P*** | ***0.0001*** | ***0.0005*** | ***<0.0001*** | ***0.0001*** |

Data are expressed as mean ± SEM ($n$ = 8 per group). Values with different letters are significantly different ($p < 0.05$). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

the control diet. However, they did not find clinical signs of deficiency in the rats fed with refined wheat grains.

Paturi *et al.*[19] found that the consumption of diets with 7% of inulin or potato fibre (cell wall) decreased food intake by 10% when these were given to Sprague-Dawley rats as compared to a control diet (cellulose). Inulin is a readily fermented, short-chain carbohydrate; and potato fibre, a mix of fermentable (pectin and hemicellulose) and non-fermentable (cellulose) polysaccharides. The researchers indicated that supplementation with dietary fibre could have decreased food intake by promoting satiety. The authors raise a possibility that a colonic brake involving feedback from the large intestine fermentation delayed gastric emptying. This concept called "ileal brake" was defined as a mechanism to control the transit of food through the gastrointestinal tract, in order to optimize digestion and absorption of nutrients.[20]

There are numerous studies in humans that relate low body weight to the consumption of whole grain foods because of the promotion of satiety and spontaneous decrease of food intake.[21–24]

### 3.2. Cecum

Table 3 shows the measured parameters related to the cecum of the animals. The results indicated that there was no change in the weight of the cecum, except in the rats fed with the B diet which had the lowest one. When this value was related to BW no difference was found.

Diets based on WG rice exerted a change in the pH of the cecum (<0.0001). This shift towards lower pH can be attributed to the fermentation of WG fibre, and SCFA production. The C diet contains cellulose that is resistant to fermentation, so the pH of the cecum content was higher than the other WG based diets.[19] Previous studies[13] agree with this, since it was found that consumption of extruded WG maize also decreased the pH in the cecum because of the fermentation of fibre in comparison with the C diet with cellulose. Several researchers demonstrated a decrease of the pH in human's cecum by consumption of fibre from different WG.[25–28] It was known that a decrease in pH promotes intestinal health by altering the absorption of potentially toxic metabolites and preventing the proliferation of pathogenic bacteria.[29]

On the other side, Weaver *et al.*[30] studied the fermentation of different types of fibre and indicated that both, SDF (inulin, polydextrose, synergy, soluble corn fibre, *etc.*) and insoluble dietary fibre (IDF) (resistant starch) were fermentable. The difference was that SDF produced greater amounts of SCFAs such as acetate and butyrate than IDF ones and both the fibres produced even greater amounts of SCFAs than the cellulose component of the C diet.

Table 3 also shows the sIgA content of the cecum. There were no differences among the animals consuming different diets. There have not been any studies until now about the effect of consumption of whole rice on sIgA. Type A immunoglobulins are a specialized form of antibodies found particularly in mucosal sites, although they are also present in serum and elsewhere. Luminal sIgA is able to bind and block microbial antigens, thereby interfering with the mechanisms of mucosal penetration and/or helping the immune system to fight off invading microbes once they are within reach. It also induces bacterial agglutination. Growing evidence indicates that sIgA uses a high-affinity binding system to neutralize microbial toxins and pathogens, and a low-affinity binding system to prevent commensal bacteria from reaching the mucosal surface.[31]

Regarding enzyme activities in the cecal content (Table 3), significant differences in β-glucosidase activity were observed. The consumption of all WG diets decreased this activity and particularly, the B diet presented the lowest value.

The activity of β-glucosidase tends to involve the attack of the numerous plant glycosides encountered in the human diet, leading to the release of toxic aglycones, which are, in most cases, carcinogenic.[32] A reduced glucosidase activity in the cecum lumen indicates a possible protection afforded by the WG rice diet to the body. Some dietary fibers appear to affect the composition and activity of the lumen microbiota. Therefore, the identification of bacterial enzymes with toxicological importance would provide a great deal of useful information regarding the influence of diet with regard to the modulation of the lumen microbiota.[33]

It is possible that the incorporation of whole rice fibre in the diet can reduce bacterial activation of dietary pro-carcinogens, thus preventing exposure of colon cells to luminal mutagens.

Feces excretions (g) in dry basis during the whole experiment period were recorded (Fig. 1). It was observed that rats fed with the C diet excreted more feces than those fed with

**Table 3** Cecum weight, cecum weight 100 g per BW, cecal content pH, sIgA content, β-glucosidase and β-glucuronidase activities

| Diets | Cecum weight (g) | Cecum weight/BW (g per 100 g BW) | Cecal content pH | sIgA (μg $g^{-1}$ cc) | β-Glucosidase (μmol *p*-nitrophenol $g^{-1}$ cc $h^{-1}$) | β-Glucuronidase (μmol *p*-nitrophenol $g^{-1}$ cc $h^{-1}$) |
|---|---|---|---|---|---|---|
| C | 1.09 ± 0.07$^a$ | 0.37 ± 0.02 | 7.06 ± 0.06$^a$ | 12.65 ± 5.38 | 1.66 ± 0.14$^a$ | 4.16 ± 0.45 |
| B | 0.81 ± 0.09$^b$ | 0.33 ± 0.02 | 6.40 ± 0.06$^b$ | 5.39 ± 0.99 | 0.21 ± 0.05$^c$ | 4.68 ± 0.56 |
| S | 1.11 ± 0.06$^a$ | 0.40 ± 0.04 | 6.38 ± 0.04$^b$ | 8.59 ± 2.70 | 1.09 ± 0.14$^b$ | 3.63 ± 0.43 |
| G | 1.18 ± 0.05$^a$ | 0.40 ± 0.03 | 6.33 ± 0.07$^b$ | 4.55 ± 1.42 | 1.07 ± 0.18$^b$ | 4.96 ± 0.74 |
| ***p*** | ***0.0041*** | *0.2508* | ***<0.0001*** | *0.1953* | ***<0.0001*** | *0.3662* |

Data are expressed as mean ± SEM ($n$ = 8 per group). Values with different letters are significantly different ($p$ < 0.05). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

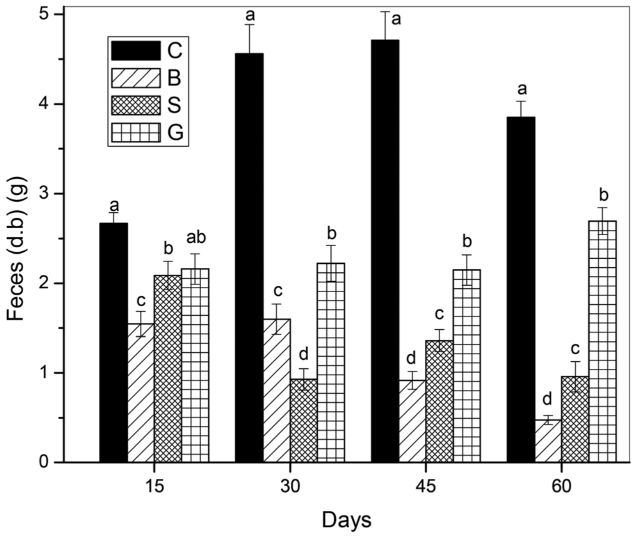


Fig. 1 Feces excretion (dry basis) at 15, 30, 45 and 60 days. Values with different letters are significantly different ($p < 0.05$). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

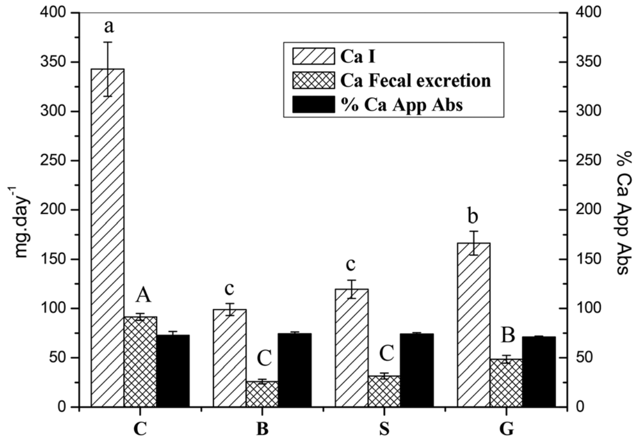


Fig. 2 Ca daily intake (Ca I), Ca daily fecal excretion (Ca fecal excretion) and Ca apparent absorption (%Ca app abs). Values with different letters are significantly different ($p < 0.05$). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

WG rice diets at the end of the experiment (3.85 *vs.* 2.69; 0.96; 0.47 for C, G, S and B respectively) ($p < 0.0001$). The lower excretion of these animals would be related to the higher content of fermentable fibre of these diets, which was degraded by colonic bacteria and thus resulted in a smaller mass, compared to the C diet with cellulose. Also, these animals had a lower intake of food. B, G and S rice ingredients used for diets presented 1.15, 0.60 and 0.55 g per 100 g of SDF, respectively. Therefore, it is justified that the animals fed with the B diet excrete lower mass of feces. Paturi *et al.*[19] found that the animals fed with a cellulose diet (control diet) had higher fecal excretion than those fed with diets including inulin or potato fibre diets, because cellulose produces a faster transit through the GI tract. Also, Ranhotra, Gelroth, Glaser & Rag[34] studied the consumption of diets based on a WG meal from different types of grains (wheat, oats, rye and barley) and found the same trend, the rats fed with a cellulose diet had higher fecal excretion than those consuming diets with higher levels of soluble fibre (oats and barley).

### 3.3. Calcium apparent absorption

Fig. 2 shows calcium daily intake, excretion and apparent calcium absorption of animals fed with different diets. The intake and excretion were significantly lower ($p < 0.0001$) in animals fed with WG based diets in comparison with the C diet. Otherwise, the percentage of calcium apparent absorption was not different ($p$: 0.2113).

The higher intake of calcium was related to the higher food consumption of some groups (C and G diets). The fact that calcium apparent absorption was the same among the groups indicates that the lower cecal pH obtained for the animals fed with WG rice diets would contribute to but did not promote calcium absorption beyond C diet. Taking into account that the C diet has no source of PA, and the PA content was 511.62, 479.8 and 477.7 mg per 100 g for extruded B, G and S ingredients, respectively, the final level of PA in the different ingredients did not affect calcium absorption since all groups had the same absorption rate compared to a diet without PA (C diet).

Numerous studies indicate that polysaccharides and oligosaccharides may improve the bone status through their influence on mineral absorption and retention. A diet with FOS (long and short chain fructo-oligosaccharides) improved calcium absorption[35] and inulin-FOS increased calcium retention.[36] Furthermore, polydextrose can be fermented in the colon and produces SCFAs which reduced pH values, contributing to an increase in the absorption and retention of calcium.[13,37,38] However, in this study it was not possible to observe differences in the calcium apparent absorption in the rats fed with WG rice diets.

### 3.4. Mineral content and density of bones

Table 4 shows the results of densitometries obtained at the end of the experiment. The total bone mineral content (t BMC) and the total bone mineral content related to body weight (t BMC t60) were lower in all animals fed with extruded WG rice compared to the C group. Animals which consumed B and S diets had lower t BMC than those fed with the G diet. The differences with the C diet remained when the results were expressed in terms of BW (t BMC t60). It was noted that t BMD t60 was lower in animals fed with S and G diets, compared with the C and B diets, which have similar values. By contrast, mineral density in femur, tibia and spine (F-BMD, T-BMD and S-BMD, respectively) was lower for all rats fed with WG diets compared with those fed with the C diet. These results suggest that the consumption of these diets based on extruded whole rice resulted in a deterioration of the mineral content and density in relation to the C diet. Albarracín *et al.*[13] observed that the intake of extruded whole corn improved t BMC and t BMC t60, but not BMD of animals. In this study, the animals fed with the C diet and extruded whole corn diet had the same

 

Table 4 Total skeleton bone mineral content (t BMC) (expressed as mg and mg per g BW) and bone mineral density of total body (t BMD t60), femur (F-BMD t60), spine (S-BMD t60), and proximal tibia (T-BMD t60) at 60 days

| Diets | t BMC (mg) | t BMC t60 (mg per g BW) | t BMD t60 (mg $cm^{-2}$) | F-BMD t60 (mg $cm^{-2}$) | S-BMD t60 (mg $cm^{-2}$) | T-BMD t60 (mg $cm^{-2}$) |
|---|---|---|---|---|---|---|
| C | 4051 ± 288$^a$ | 11.8 ± 0.6$^a$ | 259.75 ± 2.5$^a$ | 259.25 ± 11.1$^a$ | 242.50 ± 6.5$^a$ | 219.5 ± 8.1$^a$ |
| B | 997 ± 95$^c$ | 4.62 ± 0.2$^c$ | 252.25 ± 2.6$^a$ | 188.38 ± 3.9$^c$ | 187.50 ± 3.3$^c$ | 176.0 ± 4.6$^{bc}$ |
| S | 1282 ± 113$^c$ | 5.79 ± 0.2$^b$ | 241.12 ± 2.1$^b$ | 198.38 ± 4.3$^c$ | 191.88 ± 1.3$^c$ | 173.6 ± 3.1$^c$ |
| G | 1963 ± 182$^b$ | 6.11 ± 0.4$^b$ | 243.50 ± 1.6$^b$ | 213.63 ± 5.0$^b$ | 200.71 ± 2.7$^b$ | 187.7 ± 3.2$^b$ |
| ***p*** | ***<0.0001*** | ***<0.0001*** | ***0.0001*** | ***<0.0001*** | ***<0.0001*** | ***<0.0001*** |

Data are expressed as mean ± SEM ($n$ = 8 per group). Values with different letters are significantly different ($p$ < 0.05). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

Table 5 Right femur parameters at 60 days: ashes, ashes/OC ratio, femur calcium and P content and Ca/P ratio

| Diets | Ashes (mg 100 per g) | Ashes/OC (mg per mg) | Femur calcium (mg per 100 g) | Femur P (mg per 100 g) | Femur Ca/P |
|---|---|---|---|---|---|
| C | 55.03 ± 0.55$^a$ | 1.22 ± 0.03$^a$ | 21.50 ± 1.6$^b$ | 12.30 ± 0.1$^a$ | 1.78 ± 0.2$^b$ |
| B | 47.56 ± 0.2$^c$ | 0.91 ± 0.01$^c$ | 27.05 ± 1.4$^a$ | 9.99 ± 0.05$^d$ | 2.60 ± 0.1$^a$ |
| S | 46.80 ± 0.8$^c$ | 0.88 ± 0.01$^c$ | 28.94 ± 1.50$^a$ | 10.49 ± 0.2$^c$ | 2.57 ± 0.2$^a$ |
| G | 49.53 ± 0.3$^b$ | 0.98 ± 0.01$^b$ | 22.80 ± 0.50$^b$ | 11.08 ± 0.1$^b$ | 2.06 ± 0.01$^b$ |
| ***p*** | ***<0.0001*** | ***<0.0001*** | ***0.0020*** | ***<0.0001*** | ***0.0006*** |

Data are expressed as mean ± SEM ($n$ = 8 per group). Values with different letters are significantly different ($p$ < 0.05). Control diet (C); extruded brown (B); soaked (S) or germinated (G) rice based diets.

efficiency of the diet (0.26 ± 0.01 g BW per g diet), although there was a higher intake of food (1134.44 ± 24.92 *vs.* 982.58 ± 28.15 g per 60 days) and calcium (365.12 ± 19.9 *vs.* 281.25 ± 11.7 mg Ca per day).

The lower intake of WG rice diets resulted in a low intake of calcium, which negatively affected bone health regardless of the calcium absorption rate. The G diet was the best in comparison with B and S, because the mineral content and density of bones were near to those found in the animals fed with the C diet, although the calcium intake was lower.

Table 5 shows the results of the right femur composition from animals at the end of the experiment ($t$ = 60 days). The femur is a representative bone tissue because it is subject to fair remodeling by the ongoing exercise stimulus and even is a representative parameter of bone strength, associated with the risk of possible fractures.[18] The femur ash content was lower in animals fed with WG rice diets in relation to C, and the animals fed with the G diet had the highest value among them. The ash/OC ratio of animals consuming WG diets was lower than that of C in all cases. This would indicate that there is lower mineral deposition in the collagen matrix of bone than in the C group.

In the case of B and S groups, the femur calcium content was higher than that of the C and G groups. Otherwise, the femur P content was lower, so these animals had a higher Ca/P ratio in the femur. Calcium and phosphorus should be bioaccessible and in appropriate amounts in the diet for adequate bone mineralization. The Ca/P ratio of bone in healthy adults is approximately 2.[39] In this study, this ratio was greater in rats fed with B and S diets and reached the 2 value in the case of G diet.

# 4. Conclusions

We studied the effect of WG diets in a model which uses growing rats (physiological status) and not an injury model as usually is utilized. Animals fed with B and S diets presented lower weight gain and consequently lower diet efficiency. No change in the weight of the cecum was observed, but diets based on WG rice produced a change in the cecal pH towards lower values, attributed to the fermentation of WG fibre and the production of SCFAs. Even though the sIgA content had no significant differences among different groups, all WG based diets decreased β-glucosidase activity, indicating a possible protection afforded by WG. Low intake of WG diets resulted in low intake of calcium, which negatively impacted in bone health regardless of its absorption rate. Even though there were no differences in calcium apparent absorption we could conclude that high reduction of PA content, in the case of WG rice ingredients, contributed to maintain the same rate of calcium absorption in comparison with the C diet. From the nutritional point of view, the G diet among rice based ingredients with different treatments, germinated rice was considered the best since bone parameters were closer to the control.

# Conflict of interest

The authors declare no conflict of interest.

# Abbreviations

| | |
|---|---|
| %Ca app abs | Calcium apparent absorption percentage |
| B | Extruded brown rice |

| | |
|---|---|
| BMC | Bone mineral content |
| BMD | Bone mineral density |
| BW | Body weight |
| BWG | Body weight gain |
| C | Control diet |
| Ca I | Ca intake |
| DF | Dietary fibre |
| F-BMD | Femur bone mineral density |
| FOS | Fructooligosaccharides |
| G | Extruded germinated rice |
| GI | Gastrointestinal |
| IDF | Insoluble dietary fibre |
| LSD | Least significant difference |
| OC | Organic content |
| PA | Phytic acid |
| S | Extruded soaked rice |
| S-BMD | Spine bone mineral density |
| SCFAs | Short chain fatty acids |
| SDF | Soluble dietary fibre |
| SEM | Standard error of the mean |
| SIgA | Secretory IgA |
| t BMD | Total bone mineral density |
| T-BMD | Tibia bone mineral density |
| WG | Whole grain |

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