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Effects of extruded whole maize, polydextrose and cellulose as sources of fibre on calcium bioavailability and metabolic parameters of growing Wistar rats

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The aim of this study was to evaluate the influence of diets with different types of fibres on Ca bioavailability and metabolic parameters in growing Wistar rats. Twenty four male Wistar rats were fed with 3 different diets: control (C), polydextrose (PD), and extruded whole maize (M) during a 60 day period. Apparent Ca absorption percentage (%Ca Abs), total skeleton bone mineral content (t BMC), total bone mineral density (t BMD), femur (F), spine (S) and tibia (T) BMD, cecum weight, and pH were evaluated. Malondialdehyde (MDA) and lipid (TG and cholesterol) contents in serum and liver were also evaluated. The results showed that rats fed with M and PD had the same cecum weight, but higher than that of C (1.53 ± 0.02 *vs.* 0.94 ± 0.01). There was moderate acidification of the cecal content in rats fed with M compared to C (pH 5.93 *vs.* 6.98) and the fecal weight was 1.06 ± 0.02, 3.07 ± 0.03 and 4.81 ± 0.05 for PD, M and C, respectively. There were significant differences in %Ca Abs between PD and C (87.57 ± 1.20 *vs.* 71.10 ± 1.11). The PD group had the highest values of F-BMD, S-BMD, and T-BMD, but there were no differences between M and C groups. Regarding lipids, there was a significant lowering effect in the M liver triglycerides content. Moreover, liver MDA levels significantly decreased with M and PD diets. The consumption of PD and grain fibres can exert some beneficial gastrointestinal effects such as lowering of the pH, hepatic TG and MDA content related to fibre colon fermentation.

Introduction

High consumption of dietary fibre has been associated with reduced risk of cardiovascular disease, diabetes, hypertension, obesity and gastrointestinal disorders.[1] Grain fibre and polydextrose (PD) are examples of different types of fibres that are highly variable in both their technical and physiological functionalities. They also belong to different categories of the new dietary fibre definitions of the European Union and Codex.[2] Grain fibre is chemically heterogeneous, has insoluble and soluble fractions, and the dietary fibre polymers are organized in a complex hierarchical structure in the cell wall. In addition, grain fibre contains many associated compounds with various biological activities.[3] A diet containing whole-grain cereals (WG) can protect against metabolic disorders, this effect being mainly attributed to the fibre and micronutrients in the outer layer of the grain and in the germ fraction. Also, the protective effects of cereal fibre depend on its solubility and fermentability. Fermentable fibre (soluble arabinoxylans and β-glucans) can decrease blood cholesterol and reduce the post-prandial glycaemic response. Non-fermentable fibre (cellulose and insoluble arabinoxylans) increases the speed of transit time and the feces volume, decreasing the contact between carcinogens and colon epithelial cells. Fibre fermentation may also produce significant quantities of butyrate, which protects epithelial cells against carcinogenesis.[4,5]

In contrast, PD is an industrial product whose beneficial physiological functions must be demonstrated in order to be classified as dietary fibre. It is a soluble non-viscous man-made polymer, widely used as an additive for more than 15 years. It is not digested in the upper gastrointestinal tract, but is partially fermented by the gut microbiota.[3] The beneficial effects of PD on gastrointestinal function have been largely studied. However, few studies have been carried out regarding the PD effect on Ca absorption and bone retention. Moreover, several of them were done in normal adult or gastrectomized rats,[6] but not in growing rats, which is a suitable animal model to evaluate Ca absorption and retention.

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Calcium (Ca) nutrition adequacy is necessary for bone accretion during growth to achieve an optimum peak bone mass.[7] Increasing Ca intake would be the most effective strategy for avoiding Ca deficit. Ca absorption averages only 30% in adults and most of the absorption occurs in the small intestine. However, the solubilization of Ca by lowering the pH, through microbial fermentation in the large intestine, has been proposed as one of the mechanisms responsible for the increase in Ca absorption observed following ingestion of highly fermentable indigestible materials.[6] Non-digestible oligosaccharides can modify the colonic microbiota by increasing the proliferation and activity of beneficial flora,[8] which induces changes in the enzymatic activity and produces compounds that enhance paracellular and transcellular absorption of Ca.[9] Several types of fructans including inulin, oligofructose, or a mixture of short and long chain products[10] have been investigated in relation to their effect on Ca absorption and bone health.[11,12]

In the case of WG, phytic acid inhibits absorption of Ca and other minerals. However, there are different technological processes, such as extrusion, that can reduce the content of phytic acid and improve Ca bioavailability.

On these bases, we compared the effect of extruded whole maize, cellulose and PD as sources of fibre on Ca absorption, Ca retention and changes in the acidity of the cecal content in growing rats with adequate dietary calcium intake. Also, malondialdehyde (MDA) as an indicator of oxidative stress, and triglycerides and cholesterol contents from liver and serum were evaluated as lipid related metabolic parameters.

Materials and methods

Materials

Extruded maize. Commercial whole maize (*Zea mays*) grits (supplied by Molino Matilde, Santa Fe), with a particle size between 1190 and 420 μm, were used in the experiments. The grits were conditioned to the extrusion moisture level (13.9 g per 100 g) 2 hours before extrusion, using a Brabender planetary mixer P-600 L (Germany) at a rotation speed of 60 rpm. The extrusion process was carried out with a Brabender 20 DN single screw extruder, using the following conditions: 4 : 1 compression ratio screw, 3/20 mm (diameter/length) die, 175 rpm screw speed and 160 °C temperature. The feeding rate of the extruder was at full capacity. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the metering and die sections were both kept at the same temperature by using the heat control device of the extruder. The extruded samples were milled with a roller mill (Retsch Mühle 5657 Haan, West-Germany) in three successive stages, 6 mm, 3 mm and 1 mm.

Rats and diets

Twenty four male Wistar rats (43.0 ± 4.5 g) were obtained from the Animal Service Laboratory, Facultad de Farmacia y Bioquímica, UBA (Argentina). Throughout the experiment, animals were allowed free access to deionized water and food, and 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. They were fed with one of the 3 following diets ($n = 8$ per group) during a 60 day period (Table 1):

- Control group (C): rats fed with a semi-synthetic diet prepared according to the American Institute of Nutrition Diet (AIN 93)[13] having 5 g per 100 g cellulose.
- Polydextrose group (PD): rats fed with the AIN 93 containing 5 g per 100 g diet of polydextrose (Litesse®, Danisco) replacing cellulose.
- Maize group (M): rats fed with the AIN 93 containing 5 g per 100 g diet of fibre from extruded maize replacing cellulose.

The analyses 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 approved by the Committee of Health Guide for the Care and Use of Laboratory Animals of the Facultad de Farmacia y Bioquímica, UBA.

Sampling procedures

Body weight (BW) was recorded once a week throughout the study. Food intakes were recorded every three days throughout the experiment and total intake, daily intake and daily calcium intake (Ca I) were calculated.

At the end of the experiment, 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. An abdominal incision was made; blood was withdrawn from the abdominal aorta and centrifuged at 3000–3500 rpm for 20 minutes at 4 °C. The obtained serum samples were stored at −80 °C and examined within the following 3 days for TG, proteins and MDA analysis.

The right femur was excised for biochemical analysis. The right tibia was resected and fixed by immersion in buffered formalin for 48 h, decalcified in 10% ethylene-diamine tetraacetic acid (EDTA) (pH 7) for 25 days and embedded in paraffin. An 8 to 10 μm thick longitudinally oriented section of subchondral bone was obtained at the level of the middle

Table 1 Composition of control (C), maize (M) and polydextrose (PD) diets

Ingredient (g per kg diet)	C	M	PD
Maize starch	397.5	177.3	397.5
Casein	200.0	154.1	200.0
Maize dextrine	132.0	59.0	132.0
Sucrose	100.0	44.7	100.0
Soybean oil	70.0	62.1	70.0
Mineral mix (AIN-93M-MX)	35.0	28.6	35.0
Vitamin mix (AIN-93-VX)	10.0	10.0	10.0
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
tert-Butylhydroquinone	0.01	0.01	0.01
Cellulose	50.0	—	—
Maize	—	458.7	—
Polydextrose	—	—	50.0

third, including primary and secondary spongiosa. It was stained with haematoxylin–eosin, and microphotographed (AXIOSKOP, Carl Zeiss) to determine the bone volume (BV%) on the central area of the metaphyseal bone displayed on the digitalized image.[14]

The liver was excised, weighed and stored at −80 °C for the measurement of triglycerides, cholesterol and MDA.

The cecum from each rat was excised, weighed and split open, and the pH of the cecal content was recorded.

Analytical methods

Bone measurements. At the end of the experiment ($t = 60$) 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 specifically designed software for small animals (DPX Alpha, Small Animal Software, Lunar Radiation Corp., Madison, WI) as previously described.[15] In brief, all rats were scanned using an identical scan procedure. Precision was assessed by measuring one rat five times with repositioning between scans on the same and on different days. The coefficient of variation (CV) was 0.9% for total skeleton BMD and 3.0% for BMC. The analysis of the different subareas was carried out on the image of the animal on the screen using a ROI for each segment. The CV for BMD was 2.2% for the femur. To minimize the inter-observer variations all analyses were carried out by the same technician.

Femurs were cleaned of any adhering soft tissue, dried at 100 °C for 72 hours, and fat was extracted by immersion for 15 days in a chloroform–methanol (3 : 1) mixture, which was removed and replaced every three days. Finally, bones were dried for 48 h at 100 °C. The fat-free and dried bones were weighed and ashes were obtained at 700 °C until white and crystalline. Thereafter, they were dissolved in HCl and diluted for Ca and P analysis. The amounts of Ca and P were calculated as the total content and percentage content of dried fat-free tissue and the femur Ca/P ratio was also calculated.

Feces. Feces were dried under infrared light and pounded. Diets and feces were wet-ashed with nitric acid using Parr bombs.[16]

Mineral analysis. Ca concentration in diets, feces and bones was determined using an atomic absorption spectrophotometer.[17] Lanthanum chloride (6500 mg L^{-1} in the final solution) was added to avoid interferences. P concentration was measured according to the Gomori method.[18] NIST 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 batch of samples to ensure the accuracy and reproducibility of the mineral analysis.

Apparent calcium absorption. Food intake and feces recorded during the last three days of the experiment were used to calculate apparent Ca absorption (%Ca Abs) as follows (eqn (1)):

$$\%\text{Ca Abs} = [(\text{Ca I} - \text{fecal Ca})/\text{Ca I}] \times 100 \quad (1)$$

Cholesterol. Weighed dried livers (250 mg) were extracted with Folch reagent [chloroform–methanol (2 : 1)].[18] An aliquot of 100 μl was evaporated and reconstituted in distilled water for determining cholesterol by an enzymatic method using a commercial kit purchased from Wiener Lab® (Colestat Enzimático, Wiener Lab, Rosario, Argentina).

Triglycerides. Serum triglycerides were determined by an enzymatic method using a commercial kit purchased from Wiener Lab (TG Color GPO/PAP AA, Wiener Lab, Rosario, Argentina). Liver triglycerides were extracted by the Folch method[19] and then measured using the same kit used for serum triglycerides.

Proteins. Protein content was determined according to the method of Lowry *et al.* (1951),[20] using bovine serum albumin as the standard.

MDA. Determination of the MDA content in serum was measured using the thiobarbituric acid (TBA) method[21] with some modifications. Briefly, 0.25 mL of the sample was added to 1 mL of stock solution containing 150 g L^{-1} TCA, 3.75 g L^{-1} TBA and 0.25 mol L^{-1} HCl and heated in a water bath at 100 °C for 30 minutes. After cooling, the mixture was centrifuged and the absorbance measured at 535 nm. The MDA level in the serum was expressed as nmol g^{-1} protein.

The MDA content in the liver was measured using the TBA method,[22] with some modifications. Briefly, 0.9 mL of liver homogenate (1/10) of the sample was combined with 0.09 mL of 4 g per 100 mL BHT in ethanol and 0.9 mL of 20 g per 100 mL TCA. The mixture was centrifuged at 5000 × *g* for 10 minutes. Then, 0.9 mL of the supernatant was combined with 0.9 mL of 7 g L^{-1} TBA. The mixture was heated in a water bath at 100 °C for 1 h, and the absorbance was measured at 535 nm. The MDA concentration of the sample was calculated using an extinction coefficient of 1.56 mM^{-1} cm^{-1}.

Data 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 (least significant difference) test. The significance was established at $p < 0.05$.

Results and discussion

Effects of different diets on total and daily intake, body weight gain and efficiency

Table 2 shows the total intake, daily intake, body weight gain and efficiency. Differences between M and PD with respect to C were observed for total intake and daily intake. Only animals fed with PD had significantly lower BWG than the C (diet having cellulose as the source of fibre). However, efficiencies were the same for all diets. The diets were designed to provide similar lipid, protein, and carbohydrate contents, but in the case of M, the proportion of available carbohydrates varied according to the fibre content of the diet.

Table 2 Total intake, daily intake, body weight gain (BWG) and efficiency[a]

Diets	Total intake (g per 60 days)	Daily intake (g per day)	BWG (g per 60 days)	Efficiency (g BW per g diet)
C	1134.44 ± 24.92[a]	19.21 ± 0.42[a]	284.44 ± 12.13[a]	0.27 ± 0.01[a]
M	982.58 ± 28.15[b]	16.58 ± 0.50[b]	251.91 ± 15.55[ab]	0.26 ± 0.01[a]
PD	981.28 ± 26.10[b]	16.49 ± 0.43[b]	241.55 ± 9.38[b]	0.26 ± 0.01[a]
p value	0.0008	0.0006	0.0531	0.6631

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Different letters mean significant differences between samples analyzed by the LSD test ($p < 0.05$).

Effects of different diets on cecum and fecal weight and the pH of cecal content

Table 3 shows the cecum weight, cecum weight g per 100 g BW, fecal weight and the pH of cecal content. The rats fed with M and PD showed a significant increase of cecum weight compared to C ($p < 0.0001$). This was also observed when cecum weight g per 100 g BW was considered. Regarding fecal weight, significant differences among samples were observed. The rats fed with C had a higher fecal weight and those fed with PD, a lower one.

The total weight of the cecum was directly related to the soluble fibre content of the diets, while fecal weight was inversely related. Dietary fibre affects bowel function by increasing the fecal volume and weight (bulking effect), improving stool consistency, decreasing transit time, and increasing stool frequency, all of which ease defecation and prevent upset in the stomach and gut.[23] The bulking effect is mainly due to non-fermentable fibre, but fermentable fibre can increase the bacterial mass and stool frequency. Thus, rats fed with C diet having cellulose (non-fermentable fibre) had a lower weight of the cecum and a higher fecal weight, while rats fed with a PD diet having total fermentable fibre showed the opposite behavior, and the M group having both fermentable and non-fermentable fibre showed intermediate behavior.

The cecal enlargement was accompanied by significant acidification of the cecal content in the case of M (Table 3), probably due to grain fibre fermentation throughout the large intestine.[3] Also, several human studies have concluded that the fecal pH was reduced following grain fibre consumption.[24–27]

Effects of different diets on daily Ca intake, fecal Ca and apparent Ca absorption

Table 4 shows the daily Ca intake (Ca I), daily fecal Ca excretion, and percentage of apparent calcium absorption (%Ca Abs).

Table 3 Cecum weight, cecum weight g per 100 g BW, fecal weight and cecal content pH[a]

Diets	Cecum weight (g)	Cecum weight (g per 100 g BW)	Fecal weight (g)	Cecal content pH
C	3.05 ± 0.09[b]	0.94 ± 0.01[b]	4.81 ± 0.05[a]	6.98 ± 0.06[a]
M	4.49 ± 0.25[a]	1.53 ± 0.02[a]	3.07 ± 0.03[b]	5.93 ± 0.09[b]
PD	4.51 ± 0.19[a]	1.51 ± 0.01[a]	1.06 ± 0.02[c]	6.85 ± 0.06[a]
p value	<0.0001	<0.0001	<0.0001	<0.0001

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Different letters mean significant differences between samples analyzed by the LSD test ($p < 0.05$).

Table 4 Daily Ca intake (Ca I), daily fecal Ca excretion, and percentage of apparent calcium absorption (%Ca Abs)[a]

Diets	Ca I (mg per day)	Fecal Ca excretion (mg per day)	%Ca Abs (%)
C	93.84 ± 2.84[a]	106.21 ± 4.47[a]	71.10 ± 1.11[b]
M	76.39 ± 2.26[b]	63.91 ± 9.57[b]	75.10 ± 2.15[b]
PD	82.46 ± 2.14[b]	44.28 ± 8.97[b]	87.57 ± 1.20[a]
p value	0.0002	0.0001	<0.0001

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Different letters mean significant differences between samples analyzed by the LSD test ($p < 0.05$).

Both Ca I and fecal Ca were lower in the case of rats fed with M and PD compared to C. However, %Ca Abs was similar for rats fed with M and C and higher in the case of PD.

The decrease of pH promotes the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*[28] and improves absorption of some minerals.[29] However, only the diet with higher fermentable fibre content (PD) increased Ca absorption.

Effects of different diets on the mineral content and density of bones

Table 5 shows the total skeleton bone mineral content (t BMC and t BMC *t*60) and bone mineral density of total body (t BMD *t*60), femur (F-BMD *t*60), spine (S-BMD *t*60), and proximal tibia (T-BMD *t*60) at the end of the experiment ($t = 60$).

The PD group had higher t BMC than C ($p = 0.0093$), but when t BMC was expressed as mg g^{-1} BW, PD showed the highest value ($p < 0.0001$) and the M group was significantly higher than C. When the bone mineral content was expressed by areas (bone mineral density), t BMD *t*60 from M was lower than the other groups ($p = 0.0055$), but F-BMD, S-BMD, and T-BMD were not different compared with C, whereas PD had the highest values of F-BMD, S-BMD, and T-BMD.

Regarding BV% of the metaphyseal tibia and in agreement with T-BMD, the PD group presented higher values than M and C, although without significant differences with C (27.9 ± 1.5, 24.6 ± 2.0, and 22.9 ± 1.6 for PD, C, and M groups, respectively).

Table 6 shows the right femur parameters at the end of the experiment: ash content, organic content (OC), the ash/organic content ratio, Ca and P content and Ca/P ratio. Although for PD the value of F-BMD *t*60 was higher than for the other groups, there were no significant differences among samples in the right femur parameters.

Table 5 Total skeleton bone mineral content (t BMC) (expressed as mg and mg g^{-1} BW) and bone mineral density of total body (t BMD *t*60), femur (F-BMD *t*60), spine (S-BMD *t*60), and proximal tibia (T-BMD t60) (mg cm^{-2}) at the end ($t = 60$) of the experiment[a]

Diets	t BMC (mg)	t BMC *t*60 (mg per g BW)	t BMD *t*60 (mg cm^{-2})	F-BMD *t*60 (mg cm^{-2})	S-BMD *t*60 (mg cm^{-2})	T-BMD *t*60 (mg cm^{-2})
C	3690 ± 230^{b}	11.7 ± 0.6^{c}	261.6 ± 2.4^{a}	258.4 ± 3.4^{b}	231.5 ± 3.8^{b}	220.1 ± 5.4^{b}
M	4210 ± 200^{ab}	15.2 ± 0.5^{b}	253.4 ± 2.3^{b}	256.6 ± 5.0^{b}	230.8 ± 3.9^{b}	214.5 ± 3.5^{b}
PD	4760 ± 230^{a}	17.1 ± 0.7^{a}	264.1 ± 1.6^{a}	276.2 ± 3.0^{a}	248.5 ± 3.6^{a}	239.9 ± 8.4^{a}
p value	0.0093	<0.0001	0.0055	0.0037	0.0044	0.0193

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Different letters mean significant differences between samples analyzed by the LSD test ($p < 0.05$).

Table 6 Right femur parameters at the end of the experiment: ashes, organic content (OC), ash/organic content ratio, Ca and P contents and Ca/P ratio[a]

	Ashes (mg per 100 g)	OC (mg per 100 g)	Ash/OC (mg per mg)	Femur Ca (mg per 100 g)	Femur P (mg per 100 g)	Femur Ca/P
C	59.46 ± 0.24	40.54 ± 0.24	1.47 ± 0.01	23.14 ± 0.76	11.93 ± 0.31	1.95 ± 0.07
M	58.76 ± 0.48	41.24 ± 0.48	1.43 ± 0.03	21.27 ± 0.40	11.70 ± 0.33	1.79 ± 0.05
PD	59.70 ± 0.29	40.30 ± 0.29	1.48 ± 0.02	22.60 ± 0.50	11.89 ± 0.42	1.92 ± 0.08
p value	0.1638	0.1638	0.2168	0.1010	0.8921	0.2101

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Differences were analyzed by the LSD test ($p < 0.05$).

Taking into account the results related to Ca bioavailability, although the PD group had a lower Ca I, it also had lower fecal losses, resulting in higher apparent Ca absorption. This effect was not related to a lower cecal pH, but could be related to a higher cecum weight reached by fermentation, since PD is a fermentable fibre. The lower fecal weight corresponding to the PD group is related to the higher content of fermentable fibre present in this diet. The higher Ca absorption of rats fed with PD was accompanied by higher total skeleton bone mineral content (t BMC). Also, femur, spine and proximal tibia bone mineral densities at the end of the experiment were higher than those of the C group. This means that Ca from a diet with PD as a source of fibre has higher bioavailability than a diet with only cellulose (C). In a previous work performed in ovariectomized rats (a model that simulates menopause), PD had a prebiotic effect because it increased fermentation and Ca absorption and prevented the loss of bone mass compared to the control (cellulose).[30]

The effect of maize fibre is less clear since, despite lower cecal pH, Ca absorption from M was the same as that of the C diet. The lower pH, higher cecum weight and lower fecal weight than C are indicative that some fermentation took place, but this was not sufficient to increase Ca absorption and bioavailability measured as the bone mineral density of specific bones. However, maize extrusion has a positive effect on Ca absorption because M showed similar Ca absorption to C and it is well-known that phytic acid from whole maize could decrease Ca absorption. Moreover, rats fed with M presented higher t BMC (mg per g BW) and t BMD *t*60 than C.

Femur bone is a representative bone tissue because it is subject to fair remodeling by the ongoing exercise stimulus. Femur ashes, Ca and P contents from PD were not different than those from M and C groups. However, t BMC was greater for PD (Table 5) because there are other types of bone mineralized together with the right femur, which contribute to t BMC.

Effects of diets on TG, cholesterol and MDA

Table 7 shows TG and MDA contents in serum, and TG, cholesterol and MDA contents from liver. The results indicated that rats fed with the different diets had the same serum triglyceride content. However, hepatic TG content was lower in

Table 7 Triglycerides (TG), malondialdehyde (MDA) and cholesterol contents from serum and liver[a]

	Serum		Liver		
Diet	TG (g L^{-1})	MDA (nmol per g protein)	TG (μmol per g liver)	Cholesterol (mg per g liver)	MDA (nmol per 100 g protein)
C	0.75 ± 0.15	39.20 ± 3.23^{a}	14.95 ± 0.82^{a}	22.88 ± 0.61^{ab}	22.94 ± 0.66^{a}
M	0.74 ± 0.08	47.01 ± 2.66^{a}	11.42 ± 0.74^{b}	23.82 ± 0.64^{a}	12.51 ± 0.58^{b}
PD	0.78 ± 0.14	30.94 ± 2.00^{b}	12.42 ± 1.67^{ab}	21.72 ± 0.57^{b}	14.62 ± 1.08^{b}
p value	0.9779	0.0013	0.0403	0.0685	<0.0001

[a] Data are expressed as mean ± SEM ($n = 8$ per group). Different letters mean significant differences between samples analyzed by the LSD test ($p < 0.05$).

M than in C (23.6%) and hepatic cholesterol was lower for rats fed with PD. Also, Adam *et al.* (2001 and 2003)[31,32] showed a decrease of triglyceride and cholesterol contents from serum and liver in rats fed with a diet based on whole cereals. In the case of studies on humans, Jenkins *et al.* (2002)[33] showed the effect of soluble fibre on lowering serum cholesterol to a small, but significant degree. However, there were many studies that found no such effect on cholesterol content.[34,35] Rats fed with PD showed lower serum MDA content than M and C. Regarding hepatic MDA content, it was lower for PD and M than the C group (around 45% and 36%, respectively). These results could mean that whole maize and PD as sources of fibre decrease oxygen reactive species and other free radicals, PD having a higher antioxidant effect.

In the case of M consumption, the effects on this oxidative stress marker may be due to grain antioxidant compounds like ferulic acid, lignins, phytic acid, zinc, copper, selenium, and manganese present in the grain envelope and vitamin E in the germ.[36,37] Also, fermentation of grain fibre contributes to the release of associated antioxidant phenolic compounds, such as ferulic acid, into the bloodstream.[38]

In the case of the PD diet, there was no evidence of previous studies regarding the reduction of hepatic and serum MDA content.

Conclusions

This study clearly showed that the consumption of PD as a source of fibre increased Ca bioavailability from the diet. In the case of extruded whole maize, there was no inhibitory effect on Ca absorption or bioavailability, probably due to the extrusion process, which hydrolyses phytates from whole grain.

Both PD and grain fibres can exert some beneficial gastrointestinal effects such as lowering the cecal pH, hepatic TG and MDA contents related to colon fermentation. However, due to the large chemical heterogeneity, grain fibre is not a well-defined term, and cereal foods that are processed differently and based on different grains can produce a variety of functionalities.

Since the functions of different fibres vary, it is recommendable to eat a varied diet that provides dietary fibre from different food sources.

Abbreviations

%Ca Abs	Apparent Ca absorption percentage
BHT	Butyl hydroxy toluene
BMC	Bone mineral content
BMD	Bone mineral density
BV	Bone volume
BW	Body weight
BWG	Body weight gain
Ca I	Ca intake
C	Control diet
F-BMD	Femur bone mineral density
LSD	Least significant difference
M	Extruded maize diet
MDA	Malondialdehyde
OC	Organic content
PD	Polydextrose diet
S-BMD	Spine bone mineral density
SEM	Standard error of the mean
TBA	Thiobarbituric acid
T-BMD	Tibia bone mineral density
t BMD	Total bone mineral density
TCA	Trichloroacetic acid
TG	Triglycerides
WG	Whole grain

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