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Inhibition of the growth of rats by extruded snacks from bean (*Phaseolus vulgaris*) and corn (*Zea mays*)

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Abstract

There is a need to develop new food products with high protein quality and a high caloric value, high acceptability and low costs for low income families. The aim of this investigation was to evaluate in vivo an extruded bean–corn product, supplemented with Ca and Zn, as a potential nutritional snack. Extruded and non-extruded bean-corn flours were fed to rats. Antinutritional factors, rat weight and length, femur weight and heart weight and volume were determined. Microscopy pictures of rat liver were taken. The antinutritional factors present in the studied bean variety did not affect rat growth or internal organ characteristics. Bean-corn diets affected ($p<0.05$) rat weight and produced liver alterations, probably because of interference by bean protein with intestinal or systemic metabolism. Ca and Zn supplementation is not necessary in a bean-corn extruded snack for rats.

Key words: Bean flour, Extrusion, Maize flour, Mineral supplementation, Steatosis

Introduction

Malnutrition is common among neonates and infants in developing countries. There is a need to develop new food products with high protein quality and a high caloric value, high acceptability and low costs for low income families (Kannan et al., 2001; Hussain et al., 2010; Atienzo-Lazos, 2011; Rodriguez-Miranda et al., 2011, 2012).

Legumes play an important role in the diet of many people throughout the world. They are drough resistant and salt-tolkerant (Messina, 1999; Sagarpa, 2004; Akhtar et al., 2010; Rao and Shahid, 2011). Beans are rich in lysine but deficient in sulfur amino acids. On the other hand, corn contains high amounts of sulfur amino acids, but is poor in lysine. A combination of legumes and cereals can result in a product with high protein quality, even comparable to casein (Gujska and Khan, 1990; Stoecker et al., 2006; Dhingra and Jood, 2007; Hussain et al., 2010; Giwa and Ikujenlola-Abiodun, 2010; Atienzo-Lazos, 2011; Rodriguez-Miranda, 2012).

Beans and corn, as well as other plants, have antinutritional factors that affect growth, nitrogen balance, intestinal sugar and amino acid absorption, and the immune system, and reduce the bioavailability of cations (Mamiro et al., 2001; Manary et al., 2002; Devine 2002; Marzo et al., 2002; Nestares et al., 2003; Boccio and Monteiro, 2004; Dhingra and Jood, 2007; Martinez et al., 2012). Phytic and oxalic acid, which are present in cereals and legumes, can bind minerals such as Ca, Fe and Zn, reducing the availability of these minerals in cereal foods (Ockenden, 1997; Zhou and Erdman 1995; Adams et al., 2002; Lönnerdal et al., 2011). Phosphate can cause significant zinc loss and influence its bioavailability. Dehulling can reduce phytic acid content by 60 – 90% in cereals, since phytic acid is located principally in the germ and aluerone layer (Hatzack et al., 2000; Linares et al., 2007; Lönnerdal et al., 2011). Although phytate is the principal chelating agent, water-soluble components are responsible for 39% of the binding power of whole bran (Linares et al., 2007). Other

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authors also found that the highest amount of phytate-P in cereals is found in the bran fraction (Alonso et al., 2000). Another antinutritional factor in beans are lectins. The presence of 0.5% lectins in the rat diet can be enough to introduce impairment of intestinal absorption and produce death (Martinez et al., 2012). The presence of both trypsin inhibitors and lectins in the diet increases fecal nitrogen excretion (Morrison et al., 2005; Tan et al., 2006; Macedo et al., 2007; Wati et al., 2009). Since lectins are located in the cytoplasm of the cotyledon, dehulling has no effect on its content. Detoxification of lectins can be obtained by traditional cooking, but some industrial processes, such as dry heat, do not lead to complete detoxification (Tan et al., 2006). Extrusion of bean-corn flour is more effective than soaking for inactivating antinutritional factors such as lectins (Dhurandhar and Chang, 1990; Zhou and Erdman, 1995; Ockenden et al., 1997; Hatzack et al., 2000; Mamiro et al., 2001; Adams et al., 2002; Martinez et al., 2012). To counter the loss of minerals caused by the phytic acid present in cereals and legumes, flours may be complemented with minerals such as Ca, Fe and Zn from natural sources (Hussein et al., 2010; Ramkumar et al., 2012). There is little information regarding the nutritional effect of extruded bean-corn flours. The purpose of the present study was to evaluate *in vivo* an extruded bean–corn product, supplemented with Ca and Zn.

Material and Methods

Bean and corn flour

The bean cultivar Pinto villa (*Phaseolus vulgaris*) and the corn (*Zea mays*) cultivar H-131 were used (INIFAP-Durango, Mexico). Beans and corn were conditioned at 16% humidity for six hours. The flour (<200µm diameter) was obtained with a laboratory mill (Brook Control Gear, West Yorkshire, England). Composite flour containing a mix of 30% bean and 70% corn flour was prepared, since higher bean flour concentrations reduces expansion index and quality of the extrudates.

Extrusion process

The bean-corn flour was extruded with a Brabender Extruder 19/25 D (Germany) with a compression ratio of 1:6, 25 rpm at 150°C and 20% humidity. The duration of the extrusion process was constant for all experiments.

Chemical composition

Fat, protein starch, ash and fiber were determined by AOCS (1983) methods. Results are presented as the mean of two replicates.

Antinutritionals

Trypsin inhibitors were determined as described by Alonso et al. (2000). Phytic acid was determined by the method described previously (AOCS, 1983). Lectins were determined by the method described by Haug and Lantzsch (1983). Results are presented as the mean of three replicates.

In vivo assays

Sprague-Dawly female rats (n = 54), 37 days old, with a mean weight of 100g at the beginning of the study, were used. They were kept in plastic metabolic cages and were divided into nine groups. Each group was formed by six individuals. The groups were fed and named as follows: (1) commercial rat feed, Teklad 2018S Rodent Diet, Harlan, USA (control), (2) bean-corn flour without addition of Ca and Zn (Bean-corn-flour), (3) bean-corn flour supplemented with Ca and Zn (Bean-corn-flour Ca-Zn), (4) bean-corn flour supplemented with Ca (Bean-corn-flour Ca), (5) bean-corn flour supplemented with Zn (Bean-corn-flour Zn), (6) extruded bean-corn flour without supplementation of Ca and Zn (Bean-corn-extrudates), (7) extruded bean-corn flour supplemented with Ca and Zn (Bean-corn-extrudates Ca-Zn), (8) extruded bean-corn flour supplemented with Ca (Bean-corn-extrudates Ca) and (9) extruded bean-corn flour supplemented with Zn (Bean-corn-extrudates Zn). During the 22 days of the experiment, the rats of the control group were fed only with the basal diet described by Tejerina et al. (1977). The diets of the eight other groups were supplemented with the recommended concentrations of minerals and with 27 UI of vitamin E as DL - α- tocopherol (Tejerina et al., 1977). The rats were kept at 22°C and 50% H. Water was given every day.

Body weight and weight gain/day were recorded twice a week and are presented in g as a mean of six replicates per day (Rodriguez et al., 1998). After 22 days the rats were sacrificed. Femur lengths (mm) were measured as described by Rodriguez et al. (1998), and is presented as the mean of six replicates.

Hearts were removed as described by Ceislar et al. (1998). Heart weight is presented in g, while heart volume is presented in ml as the mean of six replicates.

The amount of Ca and Zn present in the diets and femur was determined by atomic absorption (Alonso et al., 2000).

Histological studies

The liver was removed as described by Ceislar et al. (1998). Liver weight is presented in g as the mean of six replicates. Histological sections were made with Hematoxilin - Eosin at the Faculty of Medicine at the Juarez University, Durango, Durango.

Statistical analysis

Data were analyzed by ANOVA using SIGMASTATT (SPSS. Inc.) at $p < 0.05$.

Results and discussion

Chemical composition

The fat, protein, starch, ash and fiber content of corn, beans and bean-corn flour are summarized in Table 1. The fat and starch content in corn was higher ($p < 0.05$) than in bean, while protein and fiber were higher in beans ($p < 0.05$) than in corn. The starch, protein, fat and ash contents of bean were comparable to results obtained by other authors (Gujska and Khan, 1991; Atienzo-Lazos, 2011; Rodriguez-Miranda et al., 2011, 2012). The protein and fiber content were higher ($p < 0.05$) in the bean-corn flour mixture than in corn. Our results are consistent with other studies, which showed a higher amount of protein and fiber in beans than in corn (Sagarpa, 2004; Rao and Shahid, 2011). Mixing bean and corn flour can significantly ($p<0.05$) increase dietary protein and fiber as shown in this work and other findings, whereas composite flours can improve the nutritional value of food (Dhingra and Jood, 2007; Giwa and Ikujenlola-Abiodun, 2010; Hussain et al., 2010).

Table 1. Fat, protein, starch, ash and fiber content of milled bean, corn and of the bean-corn flour mixture (70/30).

Sample	Chemical characteristics [%]				
	Fat	Protein (N x 6.25)	Starch	Ash	Fiber
Control diet (Harlan)	6.0±0.08[a]	18.8±0.15	41.2±0.22	5.9±0.03	3.8±0.05
Corn	4.8±0.09	12.0±0.06	65.5±0.32	3.1±0.06	4.3±0.09
Bean	1.8±0.13	18.6±0.18	51.9±0.50	3.7±0.09	11.0±0.20
Bean-corn flour	3.9±0.09	13.3±0.11	59.8±0.38	2.3±0.05	8.1±0.09

a standard deviation of the mean.

The bean-corn flour had 37.8% less ($p < 0.05$) ash content than beans, due to dehulling of beans before extrusion.

The bean–corn flour used for rat diets and for extrusion contained 35, 29 and 61% less fat, protein and ash content than the control diet (Table 1).

Lectin activity and Trypsin inhibitors

Lectin activity is summarized in Table 2. Relatively high lectin activity was detected in the bean-corn flour mixture before extrusion. Other authors have also found high levels of lectin activity in legumes (Macedo et al., 2007; Wati et al., 2009). Extruded bean-corn flour showed no lectin activity, as found by other authors (Ockenden et al., 1997; Atienzo-Lazos, 2011). Extrusion is more effective at inactivating lectins than dry heat (Rodriguez-Miranda, 2012). Lectin activity was destroyed by the extrusion conditions used in this study. Cooking at 100°C also inactivates lectin (Dhurandhar and Chang, 1990).

Trypsin inhibitor activity was not present in the control diet and it was completely destroyed by extrusion at 150°C in 30 sec. (Table 2). Dhurandhar and Chang (1990) found a trypsin inhibitor activity after 30 min of cooking beans at 100°C. Other researchers also found that traditional cooking shows a rest trypsin inhibitor activity. Generally, extrusion inactivation of trypsin inhibitors was more effective than in other studies (Dhurandhar and Chang, 1990), which reported 43 to 87% of activity after boiling for 1 hour. Other methods such as dry roasting have been reported to be ineffective in destroying trypsin inhibitor activity. Extrusion is a very effective process for inactivating protein-antinutrients such as lectins and trypsin inhibitors in beans. Bean products containing a high amount of protein-antinutrients do not support the normal growth of rats even when the diet is supplemented with essential amino acids (Martinez et al., 2012).

Table 2. Trypsin inhibitors and lectin activity of control, bean-corn flour mixture and extrudates.

Diets	Trypsin inhibitors [TIU/g]	Lectin activity [HU/mg protein]
Control (Harlan)	0	0
Bean-corn flour mixture	10856.9±1.56[a]	640
extrudates	0	0

a standard deviation of the mean.

Body weight of rats

Table 3 shows the data obtained in the growth experiments where the control diet and bean corn flour diets, either supplemented with Ca and/or Zn, raw or extruded, were fed to young rats. Both raw and extruded bean-corn flour caused a weight loss ($p < 0.05$) within 22 days, compared to the weight of rats (100g) at the beginning of the experiment. All rats fed with raw or extruded bean corn flour lost around 2.5 g/day. After 22 days the rats started to die. Other authors have shown that heat-treated bean protein incorporated into a basal diet produced a normal rate of growth in rats. These authors also showed that other legumes, with no heat treatment, led to weight gain, although growth was inferior to that when fed casein protein. There was no significant improvement in the nutritive value of extruded bean-corn flour compared to raw bean-corn flour, even though trypsin inhibitors and lectins were inactivated (Tables 2 and 3). Marked inhibition of growth was observed with diets consisting of 100% bean–corn flour, even after extrusion. The results show that growth inhibition is not necessarily due to the trypsin inhibitors and lectin activity present in the bean flour. Apparently, trypsin inhibitors stimulate pancreatic enzyme secretion, which causes pancreatic hypertrophy (Oates and Morgan. 1982). Other researchers have shown lower weight gains and food conversion efficiency in rats fed with legume seeds, due to lower protein and lipid intake (Erlwanger et al., 1999; Grant et al., 1995a; Grant et al., 1995a, 1995b; Nell et al., 1992; Olivera et al., 2003). Protein plays a major role in nutrition; it has been demonstrated that a diet based only on plant protein is not enough to obtain a comparable body weight to rats fed with milk protein. In a human diet, bean protein should be a complement in the diet but not a substitute for animal protein.

Contrary to findings by other authors (Olivera et al., 2003), the protein inhibitors present in the bean–corn flour did not affect rat growth. The poor growth of rats fed with bean–corn flour and bean–corn extrudates cannot be explained by the presence of trypsin inhibitors and lectins. Lower rat weight and weight loss may be explained by diet composition. The fat, protein and ash content in the bean–corn diets is significantly ($p < 0.05$) lower than in the control diet (Table 1). The quality of protein in the bean–corn flour is also lower than that of the casein protein present in the control diet. Both may explain the lower body weight of rats fed with bean–corn mixtures, compared to rats fed with the control diet (Table 3). Complementation of bean flour with corn flour was not enough to compensate for the lack of casein protein in the diet. Although the starch and fiber content in the bean–corn flours is higher ($p < 0.05$) than in the control diet (Table 1), this does not compensate for the lower protein content. Legume proteins are less digestible than high quality protein such as lactalbumin (Dhingra and Jood, 2007; Martinez et al., 2012; Martinez et al., 2012; Olivera et al., 2003). Legume protein is rarely retained by rats (Nielsen 1991; Rubio, 2000), probably because it may interfere with intestinal or systemic metabolism (Rubio et al., 1995), but not due to the low digestibility of the dietary protein or amino acid deficiency. The bean–corn flour and extruded diets have high fiber concentrations (Table 1). Insoluble fiber can also be responsible for the low energy availability of legume diets (Olivera et al., 2003).

Ca and Zn supplementation had no effect ($p > 0.05$) on rat weight and weight gain compared to the un-supplemented bean-corn flour and extruded diets. In this case the amount of Ca and Zn used to supplement bean–corn flour was not sufficient to show a positive effect on body weight (Table 3). The chelating effect of phytic acid was not saturated with the amount of Ca and Zn applied. The control diet showed a much higher ($p < 0.05$) ash concentration than the bean–corn flour, due the high concentration of Ca present in casein (Belitz and Grosch, 1999) and to the fiber in the control diet. As well as protein, starch has an effect on rat weight (Olivera et al., 2003), depending on the concentration of resistant starch present in the starch samples (Table 3). Legume starch also affects food conversion efficiency and body lipid and protein increase in the body. The energy intake of rats fed with legume starch is less than in those fed cereal starch (Grant et al., 1995; Olivera et al., 2003).

Table 3. Body weight, weight gain and length of rats after 22 days of diet, fed with bean-corn flour mixture and extrudates, with or without Ca and/or Zn supplementation.

Diet	Rat weight [g]	weight gain/day [g]
Control (Harlan)	127.5 ± 4.61* $_a$	2.0 ± 0.40 $_a$
Bean-corn flour	78.0 ± 3.83 $_b$	-2.4± 0.13 $_b$
Bean-corn flour Ca-Zn	76.8 ± 4.55 $_b$	-2.4± 0.28$_b$
Bean-corn flour Ca	73.6 ± 3.17 $_b$	-2.9 ± 0.17$_b$
Bean-corn flour Zn	81.5 ± 1.69 $_b$	-2.7 ± 0.25$_b$
Bean-corn extrudates	87.0 ± 2.15 $_b$	-2.2± 0.22$_b$
Bean-corn extrudates Ca-Zn	77.1 ± 2.52 $_b$	-2.3 ± 0.20 $_b$
Bean-corn extrudates Ca	81.9 ± 1.65 $_b$	-2.3 ± 0.25 $_b$
Bean-corn extrudates Zn	78.8 ± 4.04 $_b$	-2.9 ± 0.24 $_b$

*standard deviation of the mean.; a-b Different letters indicate significant differences at $p < 0.05$.

Femur length, heart weight and volume

The femur lengths were comparable for all diets (Table 4). Those rats fed with Ca- and Zn-supplemented diets showed no differences, which indicates that supplementation was not needed to assure good femur growth.

Neither heart weight nor volume was affected by diets based on bean–corn flour or extruded bean-corn flour (Table 4). Other authors have also shown that diets based on legumes do not alter the weight of internal organs such as small intestine, heart and pancreas (Grant et al., 1993, 1995b; Olivera et al., 2003). Legume starch does not affect heart size (Erlwanger et al., 1999; Nell et al., 1992; Olivera et al., 2003). These results demonstrate that trypsin inhibitors and lectins have no effect on heart weight, heart volume or femur length. The results also show that the chelating effect of phytic acid present in cereals and legumes did not affect femur length in rats.

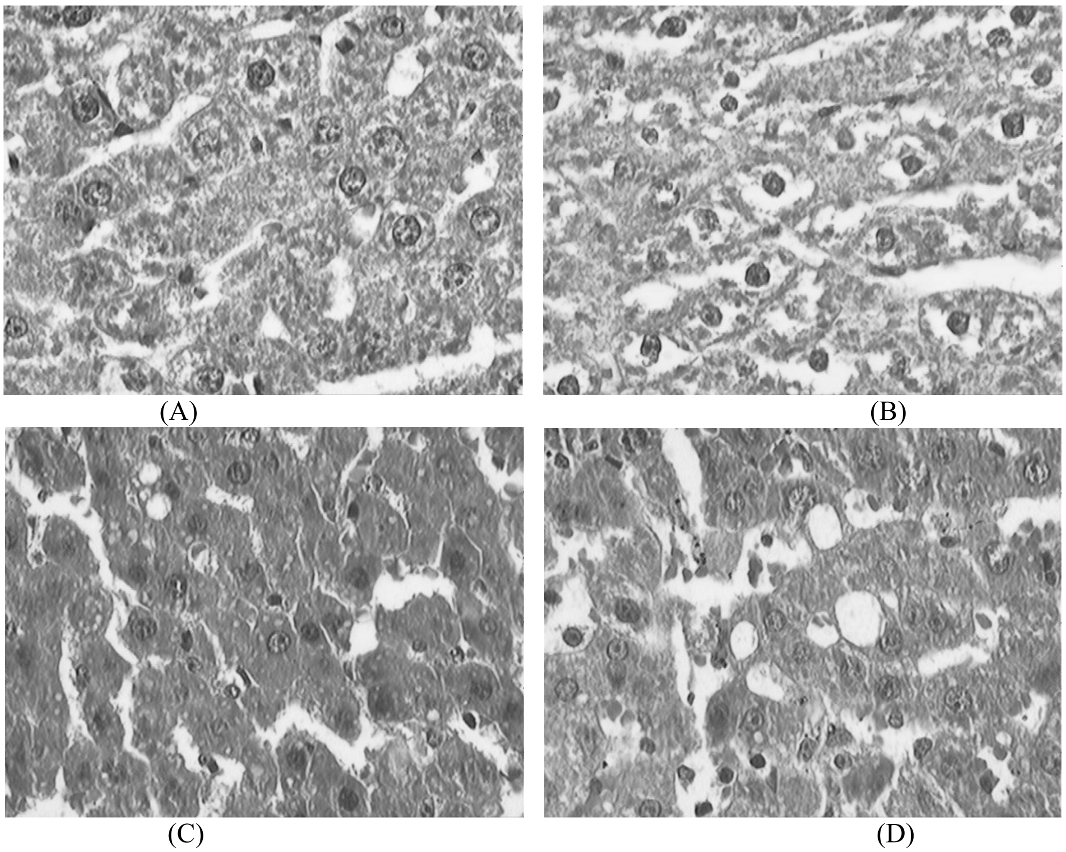

Figure 1. Microscopic pictures of liver with no signs of steatosis in rats fed with the control diet (A), liver with signs of vascular formation in rats fed with bean–corn flour supplemented with Ca (B), liver with signs of microvascular steatosis in rats fed with bean–corn flour supplemented with Zn (C), liver with signs of macrovesicular steatosis in rats fed with extruded bean–corn flour (D)(40X).

Liver

No signs of steatosis were observed in the liver of rats fed with the control diet. The addition of Zn to the extruded bean-corn flour produced a small amount of microvesicular steatosis or none at all (Figure 1). Bean–corn flour supplemented with Ca showed liver balonization. When extruded bean–corn flour was not supplemented with Ca or Zn, intense macrovesicular steatosis (as lipid vacuole accumulation) in the hepatocytes of the liver was observed. Severe gastrointestinal malabsorption is a cause of steatosis. Lipids accumulate when lipoprotein transport is disrupted and/or when fatty acids accumulate. Since we know that legume proteins may cause intestine enlargement and malfunction (Nielsen, 1991; Rubio, 2000; Olivera et al., 2003; Martinez et al., 2012), this may be responsible for the gastrointestinal malabsorption leading to steatosis in the rats fed with bean–corn flour and extrudates. The concentration of trypsin inhibitors and the lectin activity present in the bean–corn flour (Table 2) did not induce changes in the liver of the rats. Bowman-Birk-type inhibitors may have little or no effect on some rat species (Rubio et al., 1995). Extruded diets caused the most severe liver alteration, while Zn supplementation seems to reduce liver alterations induced by bean–corn flour extruded diet.

Table 4. Heart weight, heart volume and femur length of rats fed with bean-corn flour mixture and extrudates, with or without Ca and/or Zn supplementation.

Diet	Heart weight [g]	Heart volume [ml]	Femur length [mm]
Control (Harlan)	0.80 ± 0.100[a]	0.42±0.083	26.0±0.72
Bean corn flour			
no Ca nor Zn	0.70 ± 0.100	0.66±0.083	25.9±0.42
with Ca and Zn	0.70 ± 0.058	0.65±0.076	25.3±0.42
with Ca	0.77 ± 0.033	0.50±0.000	25.5±0.22
with Zn	0.80 ± 0.058	0.50±0.000	25.5±0.22
Extruded bean corn flour			
no Ca nor Zn	0.70 ± 0.058	0.60±0.058	26.8±0.31
with Ca and Zn	0.80 ± 0.000	0.70±0.050	25.5±0.24
with Ca	0.85 ± 0.050	0.50±0.250	26.3±0.20
with Zn	0.77 ± 0.088	0.78±0.117	25.8±0.60

a standard deviation of the mean.

Conclusions

The demonstration that a diet based only on bean-corn flour significantly affects rat weight and produces liver alterations, may have important implications in the field of human nutrition. The chelating effect of phytic acid present in cereals and legumes did not affect ($p < 0.05$) femur length in animals and probably also would not in humans. As shown in this study, trypsin inhibitors and lectins present in composite bean-corn flours had no negative effect on the growth and internal organs of rats. Trypsin inhibitors and lectin can be totally inactivated by extrusion. Care must be taken in translating the above findings to humans, until further investigation is done, since not all of the population is vegetarian, and diets contain more than just beans and corn. For this reason bean–corn flour extruded snacks can be used for human nutrition as a complement in the diet.

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