Titolo rivista/libro: Journal of medicinal food (Online)

Titolo articolo/sezione: Agave fructans: their effect on mineral absorption and bone mineral content

Autore/i: Garcia-Vieyra MI, Del Real A, Lopez MG

ISSN: 1557-7600

DOI: 10.1089/jmf.2013.0137

Anno: 2014

Volume: 17

Fascicolo: 11

Editore:

Pag. iniziale: 1247

Pag. finale: 1255

JOURNAL OF MEDICINAL FOOD
J Med Food 17 (11) 2014, 1247–1255

DOI: 10.1089/jmf.2013.0137

Agave Fructans: Their Effect on Mineral Absorption and Bone Mineral Content

María Isabel García-Vieyra,[1] Alicia Del Real,[2] and Mercedes G. López[1]

[1]*Departamento de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN, Unidad de Biotecnología e Ingeniería Genética de Plantas, Irapuato, Guanajuato, México.*
[2]*Departamento de Ingeniería Molecular de Materiales, Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, México.*

ABSTRACT In this study we investigate the effect that Agave fructans as new prebiotics have on mineral absorption improvement. Forty-eight 12-week-old C57BL/6J mice were used in this study. Forty mice were ovariectomized and eight were sham-operated controls. Mice were fed standard diets or diets supplemented with 10% Agave fructans or 10% inulin fructans. Calcium and magnesium were evaluated as well as their excretion in feces. Osteocalcin levels were also measured; femur structure was studied by scanning electron microscopy. Other parameters, such as food intake, body weight, glucose, and short-chain fatty acid content, were recorded. Calcium in plasma and bone increased in Agave fructan groups (from 53.1 to 56 and 85 mg/L and from 0.402 to 0.474 and 0.478 g/g, respectively) and osteocalcin increased in all fructan groups (> 50%). Scanning electron microscopy showed that fructans were able to mitigate bone loss. In conclusion, we demonstrated that supplementation with Agave fructans prevents bone loss and improves bone formation.

KEY WORDS: • *calcium* • *functional foods* • *osteocalcin* • *osteoporosis* • *prebiotics*

INTRODUCTION

OSTEOPOROSIS IS ONE OF THE leading health problems in the world. Osteoporosis is a metabolic disease of bones in which the bones become porous and weak, consequently reduces bone mineral density; this condition can be a feature of bone remodeling in some diseases that are associated with age-related joint degeneration. The pathophysiological development of osteoporosis is affected by many factors, including nutrition, which can influence skeletal development, growth, and bone maintenance during adulthood.[1,2] In addition to minimizing bone resorption during old age, maximizing peak bone mass during adolescence through appropriate nutrition and exercise may be key to postponing and even preventing osteoporotic bone fractures.[3]

Functional components are added to foods to provide beneficial health effects. Addition of these components into food system has increased during the last decade. Fructans are one type of functional component that affect physiological and biochemical processes in humans and result in improved health and risk reduction of many diseases.[4] Fructans are composed of fructose polymers containing glycosyl linkages, β(2-1) and/or β(2-6), and possibly one moiety of terminal glucose. They can be linear or branched and are found predominately in chicory roots, Jerusalem artichokes, onions, and Agaves.[4,5]

Experimental studies have shown that fructans are bifidogenic agents that stimulate the immune system, decrease the level of pathogenic bacteria in the intestines, relieve constipation, and decrease the risk of osteoporosis by increasing mineral absorption, especially calcium (Ca).[6] Fermentation of fructans in the cecum-colon produces short-chain fatty acids (SCFAs) that increase cation solubility by decreasing the pH,[7] which might facilitate dissociation of bivalent cation-phytate complexes.[8,9] Many reports also show that fructans enhance not only intestinal calcium absorption but bone calcium content as well. Effects on glucose and hormone metabolism have also been described when fructans are used as supplements.[10,11]

Inulin is the most well known of the fructans; however, another type of fructan is Agave fructans, which are complex and possess a highly branched structure. These fructans are typically referred to as agavins.[12,13] They contain both β(2-1) and β(2-6) glycosyl linkages and have external glucose (graminans) and internal glucose (neofructans) moieties. *In vitro* studies have shown that Agave fructans stimulate the growth of *Bifidobacterium breve* and *Lactobacillus casei* more efficiently than inulin fructans.[14,15]

Manuscript received 7 August 2013. Revision accepted 14 May 2014.

Address correspondence to: Mercedes G. López, PhD, Titular Research, Centro de Investigación y de Estudios Avanzados del IPN, Km. 9.6 Libramiento Norte Carretera Irapuato-León, Irapuato 36821, Guanajuato, México, E-mail: mlopez@ira.cinvestav.mx

The aim of this work was to evaluate the effect of two commercial Agave fructans with different degrees of polymerization on bone mineralization as well as establishing their potential as a new prebiotics, using ovariectomized mouse model of osteoporosis.

MATERIALS AND METHODS

Animals and diets

Experiments were performed in accordance with current legislation regarding animal experiments in Mexico (NOM-062-ZOO-1999). We housed forty-eight 12-week-old female C57BL/6J mice from the experimental unit in Cinvestav Zacatenco-Mexico in a temperature- and humidity-controlled room that was on a 12-h light–dark cycle. There were 40 ovariectomized (OVX) mice and 8 sham-operated controls (SHAM). At 14 weeks of age, mice were divided into five groups and fed for 6 weeks as follows: groups 1 (STD) and 2 (SHAM) were fed a standard diet (group-1 animals were OVX mice), group 3 (RNE) was fed a diet containing 10% inulin (Raftiline) fructans (positive control), and groups 4 (CAF1) and 5 (CAF2) were fed a diet containing 10% commercial Agave fructans. The standard diet was obtained from Lab-Diet (5053 Lab-Diet, Richmond, IN, USA). The food consumed by each mouse was weighed daily. On the sixth week following treatment, the mice were euthanized and femurs were collected.

The commercial Agave fructans were obtained from two different companies. Raftiline fructan was obtained from Orafti (Tienen, Belgium). Raftiline is a linear fructan with an average degree of polymerization (DP) of 25 and CAF1 and CAF2 are branched fructans obtained from *Agave tequilana* with an average DP of 22 and 13, respectively.

The standard diet 5053 contained the following (g/100 g dry bases): protein 23.5 (consisting of soybean and fish proteins); total carbohydrates obtained from maize, wheat, and oats 64.5 (including starch 32, sucrose 3.0, and cellulose 6.0); lipid 12; mineral mixture 6.0; and vitamin mixture 1.3. The fructan diets were pelleted by LabDiet adding 10% of fructans.

Physical measurements

Food intake was evaluated each morning. Body weight (weight gain) and excretion of feces were evaluated once a week.

Blood samples

Blood samples were taken once per week from the tail. The samples were collected in heparin tubes. After centrifugation, the plasma was stored at −80°C until further use.

Blood glucose was measured using a Glucometer Prestige kit (Home Diagnostics, Inc., Ft. Lauderdale, FL, USA). Plasma osteocalcin levels were determined using a mouse Osteocalcin ELISA kit from Immuno-Biological Laboratories, Inc. (Minneapolis, MN, USA).

Short-chain fatty acids

Upon death, the cecal content was collected on ice and frozen at −80°C. The SCFA content was analyzed using gas chromatography and flame ionization detection (GC-FID). Briefly, the cecal and feces samples were weighed and the solutions were acidified with H_2SO_4. An internal standard solution (2-methyl valeric acid) was then added.[16] The SCFAs were extracted by shaking sample solutions with diethyl ether and then centrifuging at 479*g* with a rotor of 2.71 cm. The ether phase (2 μL) was injected directly into an FFAP capillary column of the GC-FID.

Calcium and magnesium measurements

Calcium and magnesium levels were determined using an atomic absorption spectrophotometer. The diet, feces, and femurs were first scraped and then oven-dried at 60°C to a constant weight. The dry samples were weighed and a mixture of nitric-perchloric acid at a ratio of 3:1 was added to digest the samples. Next, the samples were transferred and diluted with 10 mL of distilled water. Reagent blanks were prepared with the same digestion procedures. Blood was diluted with distilled water and subjected directly to atomization.

Calcium and magnesium absorption was calculated using a previously described formula[22] with modifications.

$$\text{Absorption}(\%) = (\text{intake} - \text{fecal excretion})/(\text{intake}) \times 100.$$

Scanning electron microscopy

At the end of the experimental period, the right femur was processed for scanning electron microscopy. The femurs were incubated with 1 mg/mL of proteinase K at 37°C overnight for 12 h. The bones were washed in distilled water two times to remove any debris from the samples. The water was discarded and the samples were incubated in acetone for 12 h. The samples were then washed twice with fresh distilled water, incubated in ethyl ether for 12 h, and washed again, and the solution was discarded to eliminate residue. The femurs were dried at 60°C to a constant weight. The bones were mounted on stubs and coated with gold/palladium using an ion sputter. The samples were then analyzed by SEM and energy dispersive X-ray spectroscopy (EDS).

Microstructure analysis

Trabecular bone area as a fraction of total trabecular tissue area, porosity diameter, and compact bone thickness were measured within the same microscopic field and in the same section of the bone (femoral heads). For each treatment, several measurements were taken from four different mice and the averages were determined.

Statistical methods

Statistical differences between groups were evaluated by one-way ANOVA with Tukey's *post hoc* analysis using STATGRAPHICS Plus 5.1. Differences with a $P < .05$ were considered statistically significant.

Table 1. Food Intake, Body Weight, Feces Excretion, and Glucose Levels of Mice Fed a Standard Diet (STD and SHAM) or a Diet Supplemented with Fructans RNE (Inulin Fructans), or CAF1 and CAF2 (Commercial Agave Fructans)

	STD	*SHAM*	*RNE*	*CAF1*	*CAF2*
Intake, mg/day	2.62 ± 0.34[a]	2.88 ± 0.06[a]	2.52 ± 0.31[a]	2.54 ± 0.22[a]	2.5 ± 0.24[a]
Initial weight, g	21.35 ± 0.87[a]	20.51 ± 0.82[a]	21.41 ± 0.8[a]	21.57 ± 0.56[a]	20.95 ± 0.58[a]
Final weight, g	26.01 ± 0.14[a]	23.89 ± 0.52[b]	24.09 ± 0.28[b]	23.12 ± 0.25[b]	23.48 ± 0.35[b]
Weight gain, g	3.66 ± 0.37[a]	3.38 ± 0.30[a]	2.67 ± 0.22[b]	1.56 ± 0.31[c]	2.53 ± 0.23[b]
Initial fecal excretion, g/day	6.7 ± 0.29[a]	7.65 ± 0.36[a]	6.34 ± 0.16[a]	5.21 ± 0.21[a]	6.65 ± 0.12[a]
Final fecal excretion, g/day	7.34 ± 0.23[b]	8.34 ± 0.12[a]	8.84 ± 0.11[a]	9.31 ± 0.12[a]	10.35 ± 0.15[a]
Initial glucose levels, mM	7.06 ± 0.12[a]	6.09 ± 0.9[b]	6.99 ± 0.10[a]	6.87 ± 0.9[a]	6.93 ± 0.8[a]
Final glucose levels, mM	7.26 ± 0.23[a]	6.49 ± 0.9[a]	6.14 ± 0.08[b]	6.03 ± 0.11[b]	6.07 ± 0.13[b]

Values represent mean ± SD, $n = 10$ for STD, RNE, CAF1, and CAF2; $n = 8$ for the SHAM. Means sharing the same letter do not differ significantly ($P \leq .05$). STD, control group; SHAM, Sham operated; RNE, inulin fructan; CAF1 and CAF2, commercial agave fructans 1 and 2.

RESULTS

Food intake, body weight, and glucose levels

Over the 6-week experimental period, food intake and initial body weight were the same in all groups (Table 1). However, by the end of the sixth week, the final body weight increased in all five groups of mice. The increase in weight, however, was larger in the STD group (21.35 ± 0.87 to 25.01 ± 0.14 g). Alternatively, weight gain in the STD and SHAM groups was higher (3.66 ± 0.37 and 3.38 ± 0.30 g, respectively) (Table 1). The results in Table 1 show that glucose levels decreased in all mice independently of the diet but there was a significant decrease in mice fed with fructans over the course of the experiment.

Fecal excretion

In general, groups that were fed diets supplemented with fructans showed a tendency of increased fecal excretion compared with the STD group (CAF1 = 9.31 ± 0.12 g, CFA2 = 10.35 ± 0.15 g, RNE = 8.84 ± 0.11 g, STD = 7.34 ± 0.23 g, and SHAM = 8.34 ± 0.12 g) (Table 1). However the increment was not significant.

Short-chain fatty acids

With respect to SCFA concentration in the cecum, the groups that were fed diets supplemented with fructans (CAF1, CAF2, and RNE) showed increased SCFA content compared with the standard diet groups (STD and SHAM). However, the CAF1 group had the highest concentration of SCFAs (Table 2) and acetate and butyrate in the cecum (34.72 ± 1.01 mmol/kg and 18.67 ± 2.13 mmol/kg, respectively). CAF2 had the highest concentration of propionate (25.64 ± 1.03 mmol/kg). The concentration of SCFAs in the colon showed the same trend observed for the cecum where CAF1 had a greater rise in total concentration of SCFAs (Table 2) and in the individual concentrations of acetate and butyrate (44.03 ± 1.91 mmol/kg and 16.21 ± 2.00 mmol/kg, respectively).

Calcium and magnesium analysis

Calcium content in plasma (mg/L) was similar in all mice groups at the beginning of the experiment (Fig. 1). Nevertheless, the STD group showed decrement on calcium content in the third and sixth weeks (63.5 and 53.1, respectively) from a starting content of 67.8 mg/L. The groups that were fed fructan-supplemented diets and the SHAM group presented a slight, but not statistically significant, decrease in calcium content. No difference was observed in magnesium content for the entire duration of treatment (Fig. 1).

The most relevant results of this study pertain to the effect of the prebiotics used on mineral absorption and retention in bone (Table 3). The CAF1 mice showed the highest increase in apparent absorption (38%) and retention (20%) of the

Table 2. Short-Chain Fatty Acid Concentration in the Cecum and Colon (mmol/kg)

	STD	*SHAM*	*RNE*	*CAF1*	*CAF2*
Cecum					
Acetate	27.92 ± 1.23[b]	28.26 ± 0.98[b]	34.10 ± 3.05[a]	34.72 ± 1.01[a]	32.64 ± 1.72[a]
Propionate	17.82 ± 0.40[b]	18.64 ± 1.28[ab]	23.50 ± 1.04[a]	21.75 ± 1.18[a]	25.64 ± 1.03[a]
Butyrate	12.54 ± 1.19[b]	11.32 ± 1.08[b]	17.28 ± 0.64[a]	18.67 ± 2.13[a]	16.15 ± 0.76[a]
Total SCFA	58.28	58.22	74.88	75.14	74.43
Colon					
Acetate	33.39 ± 1.51[b]	35.21 ± 1.27[ab]	41.34 ± 1.73[a]	44.03 ± 1.91[a]	41.25 ± 1.43[a]
Propionate	12.18 ± 0.59[b]	13.62 ± 1.89[b]	16.85 ± 2.55[a]	18.60 ± 1.71[a]	20.97 ± 2.91[a]
Butyrate	10.63 ± 0.30[b]	11.39 ± 1.91[ab]	13.06 ± 1.76[a]	16.21 ± 2.00[a]	15.42 ± 2.41[a]
Total SCFA	56.28	60.22	71.25	78.84	77.64

Values represent mean ± SD, $n = 10$ for STD, RNE, CAF1, and CAF2; $n = 8$ for the SHAM.
Means sharing the same letter do not differ significantly ($P \leq .05$).

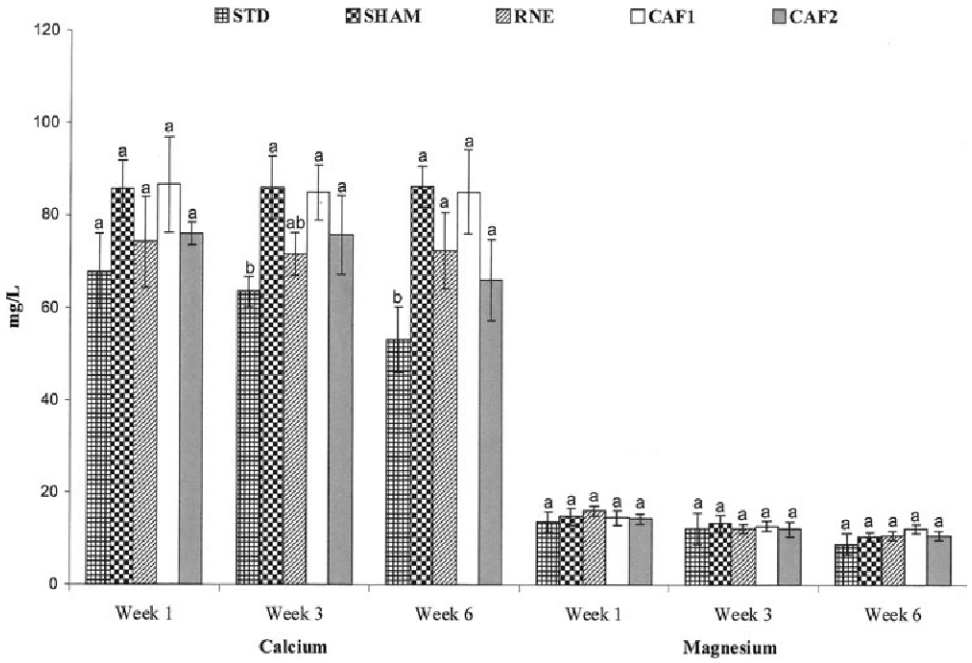

FIG. 1. Calcium and magnesium content in the plasma of mice fed a standard diet (STD and SHAM) or a diet supplemented with inulin fructans (RNE) or Agave fructans (CAF1 and CAF2). Bars represent mean ± SEM. Mean values with different letters were significantly different ($P \leq .05$).

mineral in bone. However, the other groups also showed an incremental increase in absorption and retention (CAF2: 36% and 18%; RNE: 34% and 18%, respectively). On other hand, calcium and magnesium contents in the plasma increased in both mice groups that were fed diets supplemented with Agave fructans. Moreover, the levels of calcium in the plasma show slight changes over the course of the experiment and, in the sixth and final weeks, the groups that were fed diets supplemented with fructans were able to overcome the imbalance in calcium homeostasis caused by ovariectomy. Magnesium levels, however, were maintained for the duration of the experiment.

Figure 2 shows results for calcium and magnesium bone content analyses. Fructan intake caused a statistically significant increase in Ca concentration compared with the STD group. Differences were observed in Mg concentrations in the femurs between the experimental groups with the CAF2 group showing the highest Mg levels and the STD group showing the lowest (CAF2 = 4.41 ± 0.63 mg, CAF1 = 3.91 ± 0.19 mg, RNE = 3.66 ± 0.49 mg, SHAM = 4.29 ± 0.46 mg, and STD = 3.01 ± 0.14 mg).

We also determined calcium content in the vertebral column (Fig. 3). The two groups that were fed diets supplemented with Agave fructans (CAF1 and CAF2) showed high vertebral calcium content (0.464 ± 0.007 g and 0.442 ± 0.015 g, respectively) that was similar to the SHAM group (0.455 ± 0.013 g). The magnesium content of the three fructan groups increased significantly compared with the STD group. These results confirm more accurately the preventive effect of Agave fructans on bone loss induced by ovariectomy.

Osteocalcin levels

Interestingly, osteocalcin concentrations (ng/mL) in the plasma were significantly increased in the third and sixth weeks in all mice fed with fructans compared with the STD group (Fig. 4) (51.72 ± 6.0, week 1; 54.6 ± 6.9, week 3; and 45.11 ± 3.7, week 6, vs. 48.27 ± 5.9, week 1; 31.60 ± 3.2, week 3; and 27.25 ± 3.8, week 6, respectively).

We observed a significant increment (up to 50%) on osteocalcin in all groups that were fed diets supplemented with fructans compared with the standard group. Osteocalcin is a

TABLE 3. CALCIUM AND MAGNESIUM ABSORPTION

	STD	*SHAM*	*RNE*	*CAF1*	*CAF2*
Calcium					
Intake (mg)	11.50 ± 0.070	12.64 ± 0.93	12.57 ± 0.38	12.24 ± 0.79	12.23 ± 0.72
Excretion (mg)	8.40 ± 0.52	8.87 ± 0.39	8.23 ± 0.47	7.54 ± 0.35	7.77 ± 0.41
% Absorption	26.93[b]	29.87[b]	34.55[a]	38.42[a]	36.46[a]
Magnesium					
Intake (mg)	2.23 ± 0.04	2.45 ± 0.05	2.12 ± 0.03	2.13 ± 0.03	2.13 ± 0.05
Excretion (mg)	1.37 ± 0.05	1.48 ± 0.02	1.26 ± 0.04	1.29 ± 0.02	1.27 ± 0.03
% Absorption	38.66[ab]	39.46[a]	40.29[a]	39.63[a]	40.42[a]

Values represent mean ± SD, $n = 10$ for STD, RNE, CAF1, and CAF2; $n = 8$ for the SHAM.
Means sharing the same letter do not differ significantly ($P \leq .05$).

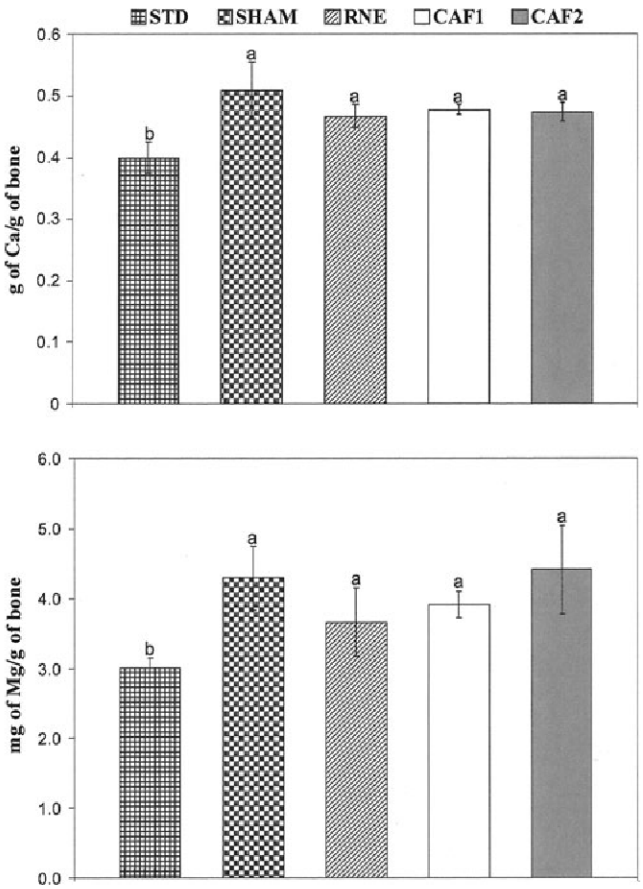

FIG. 2. Effect of the diet on calcium and magnesium content of the femur of mice fed a standard diet (STD and SHAM) or a diet supplemented with inulin fructans (RNE) or Agave fructans (CAF1 and CAF2). Bars represent mean ± SEM. Mean values with different letters were significantly different ($P \leq .05$).

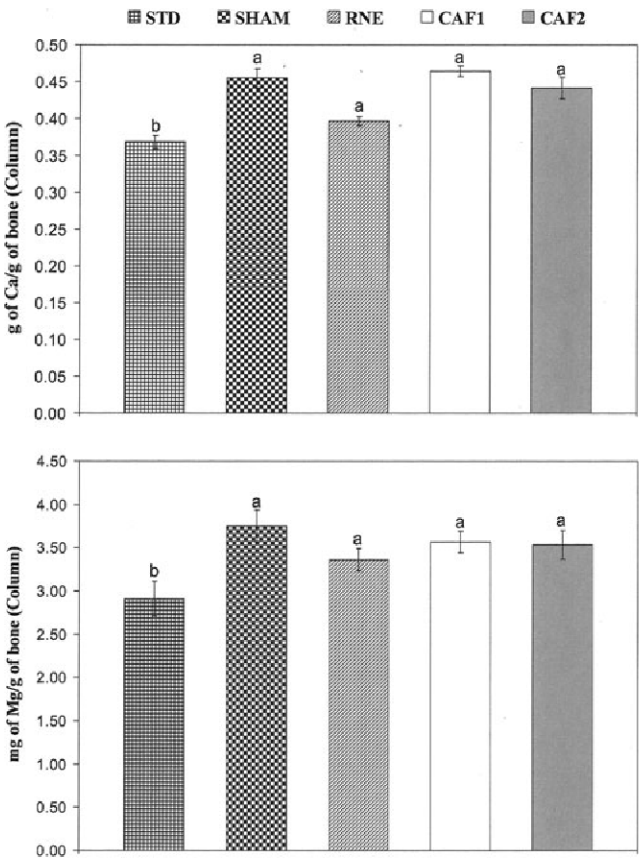

FIG. 3. Differences in calcium and magnesium content in the vertebral column of mice fed a standard diet (STD and SHAM) or a diet supplemented with inulin fructans (RNE) or Agave fructans (CAF1 and CAF2). Bars represent mean ± SEM. Mean values with different letters were significantly different ($P \leq .05$).

marker of osteoblast activity and therefore a marker of bone formation. An increase in osteocalcin concentration indicates high osteoblastic activity and increased bone formation.

Calcium absorption

A high concentration of Ca was observed in the feces of the control group compared with the fructan groups (Table 3); however, the differences were not significant. Likewise, there was no difference in magnesium content between groups; consequently, the CAF1 group presented a higher mean apparent Ca absorption compared with the STD group for the duration of the experiment (42% increase). The mean fecal concentration of Mg was not significant (CAF1 = 1.29 ± 0.02 mg, CAF2 = 1.27 ± 0.03 mg, RNE = 1.26 ± 0.04 mg, and STD = 1.37 ± 0.05 mg). Apparent intestinal absorption did not show significant differences either (Table 3).

Scanning electron microscopy analysis

SEM images (Fig. 5) of the trabecular bone showed important differences in the groups that were fed diets supplemented with Agave fructans compared with the control. The STD group's trabeculae were thinner, the connectivity of the trabecular structure was poor, and some trabeculae lost continuity. In contrast, the fructan groups showed less loss of trabecular volume. The EDS analysis showed important differences in the mineral composition of the bone between the STD and fructan groups (Table 4). The mineral composition was drastically diminished in the STD group compared with the Agave-fructan-treated group, which is confirmed by the atomic absorption spectrometry results. The results also allowed us to distinguish differences between the groups that were fed diets supplemented with the different sources of fructans.

Microstructural analyses showed that the groups taking CAF1 and CAF2 fructans displayed low porosity (8.66 and 8.56 μm, respectively), whereas the STD group displayed higher porosity (10.75 μm). The same results were observed with regard to trabeculae diameter. Specifically, the standard group's trabeculae had smaller diameters compared with the Agave-fructan-treated groups (Fig. 6).

Thickness of the compact bone was also affected by the ovariectomy. The STD group presented thinner compact bone (80.69 μm); however, in the CAF2 and CAF1 groups, this decrease in thickness was prevented by the consumption

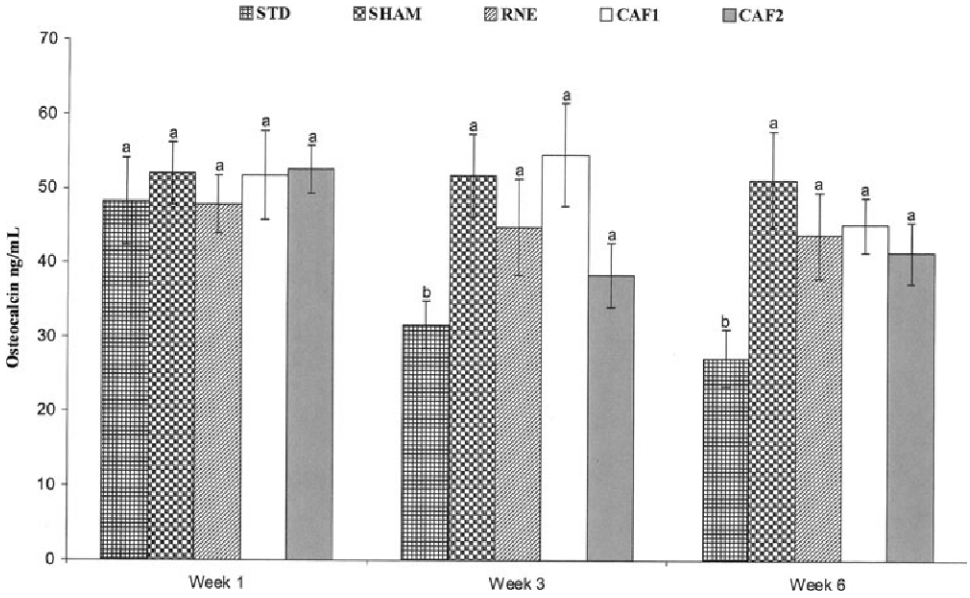

FIG. 4. Osteocalcin levels in the plasma of mice fed with Agave fructans. Bars represent mean ± SEM. Mean values with different letters were significantly different ($P \leq .05$).

of Agave fructans (104.32 μm and 116.71 μm, respectively) (Fig. 6). The compact bone thickness values of CAF2 and CAF1 were very similar to the SHAM control (121.09 μm).

DISCUSSION

Vegetable products rich in nondigestible dietary carbohydrates may affect the bioavailability of minerals.[17] Nevertheless, in this study, when fructans were ingested there was no decrease in Ca and Mg absorption. In the present study, we evaluated the effect of two different commercial fructans from Agave plants on Ca and Mg intestinal mineral absorption and mineral retention. Our results indicate that ovariectomized mice that were fed diets containing 10% fructans, regardless of type (linear or branched), show decreased weight gain in comparison to the STD and SHAM mice. A 15% decrement in glucose levels in comparison to the standard group occurred in mice that consumed diets supplemented with Agave fructans CAF1 and CAF2; likewise, a 13% decrement was observed in the mice that were fed fructans from RNE.

Our results are consistent with other studies[11,17] that showed that glycemia positively correlates with body weight gain. However, the mechanisms by which these

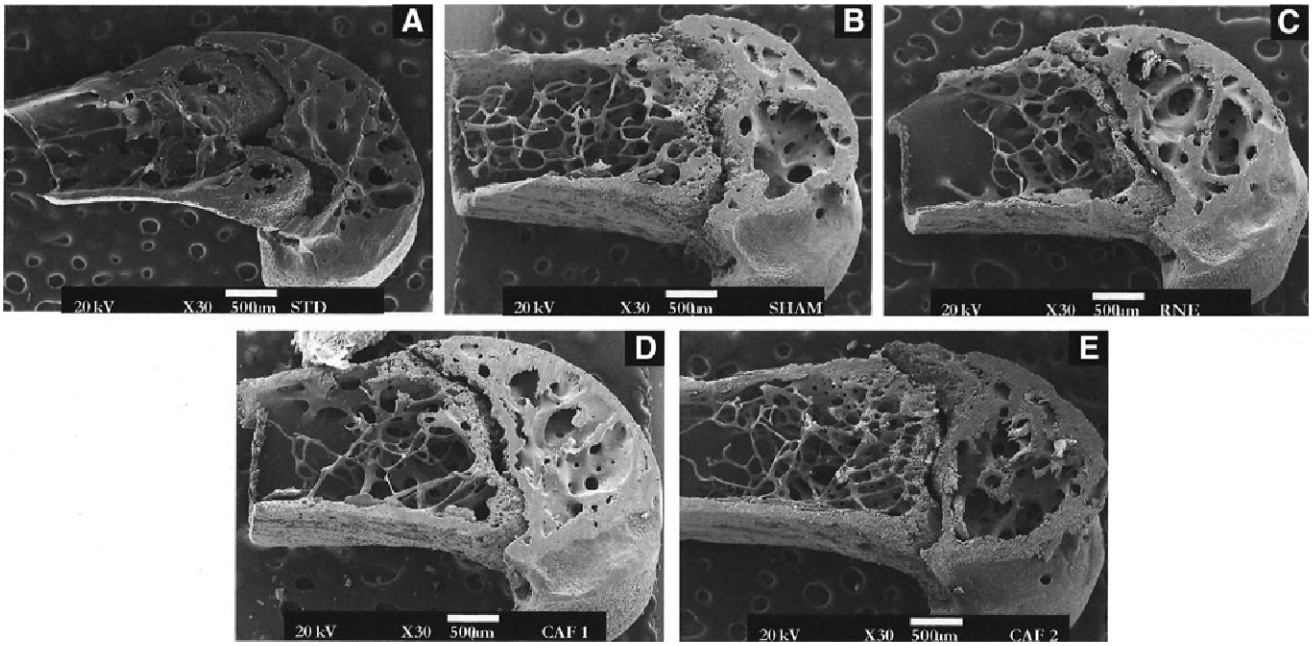

FIG. 5. Scanning electron micrographs of femoral heads of mice. **(A)** STD group, **(B)** SHAM group, **(C)** RNE Group, **(D)** CAF1 group, and **(E)** CAF2 group.

Table 4. Energy Dispersive Spectroscopy Semiquantitative Analysis of Femurs

	Ca^{++}	*P's concentration (g/100 g)*	*P (%)*	*P's concentration (g/100 g)*
STD	13.38	27.15 ± 1.57^{d}	7.38	15.79 ± 1.21^{b}
SHAM	27.11	53.09 ± 2.69^{a}	11.59	31.58 ± 1.01^{a}
RNE	19.98	39.16 ± 1.79^{c}	10.25	30.10 ± 1.33^{ab}
CAF1	24.89	48.79 ± 1.05^{b}	12.27	32.39 ± 1.11^{a}
CAF2	23.91	47.69 ± 1.89^{b}	11.51	31.64 ± 1.91^{a}

Values represent mean ± SD; $n = 4$. Means sharing the same letter do not differ significantly ($P \leq .05$).

Ca^{++}, calcium; P, phosphorus.

carbohydrates promote alternate systemic effects are not understood.

The underlying mechanism of increased mineral absorption by SCFAs is not completely understood.[18,19] In an *in vitro* experiment with Ussing chambers, addition of SCFAs and lowering of mucosal pH caused significant decreases in tissue conductance; these changes in tissue conductance correlated well with calcium fluxes and resembled luminal composition *in vivo*.[19–21] Previous *in vivo* studies have repeatedly shown that the intake of different inulin fructans can variably increase mineral intestinal absorption in humans and other animals.[17,21–23] In agreement with these reports, we found that the ingestion of all tested fructans increased total SCFA concentration in the cecum and colon and that the increment was more pronounced in mice that consumed CFA1 fructans (23% cecum and 25% colon).

The calcium plasma results agree with calcium content of the femur reaffirming the beneficial effect of diets supplemented with fructans. Our data correlate well with previous works; independent of the type of fructans administrated to mice (linear or branched), diets with fructans prevent calcium imbalance.[1,3,8] However, CAF1 shows the best results, more likely due to its structure, which is longer than RNE and CAF2 and highly branched than CAF2. This structure could facilitate access to hydrolytic enzymes in the mice large intestine.

The sites and mechanisms of Ca intestinal absorption are different from those of Mg intestinal absorption.[24,25] The intestinal absorption of Mg by passive diffusion from the distal part of the small intestine and in the first part of the colon is very important.[26]

It is well known that ovariectomy induces bone loss and a decrease in trabecular structure. In the present study, we found that the ingestion of Agave fructans markedly reduced bone loss caused by ovariectomy and conserves the trabecular structure as shown in Figure 5. On the other hand, an increase of dietary calcium alone impeded the absorption of other important minerals, including magnesium,[27] and may impede absorption of other trace elements that are important for bone matrix formation.[28,29] Our SEM micrographs show that trabecular bone volume was drastically reduced in the STD group, but in the Agave fructan groups, this reduction

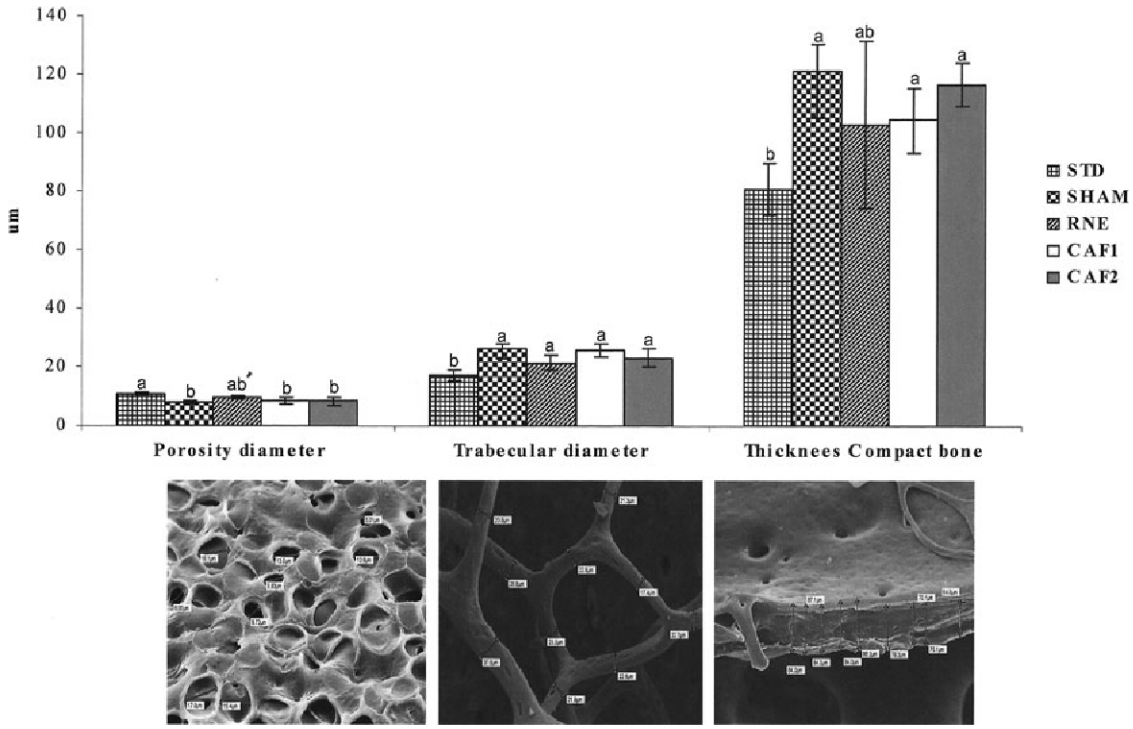

FIG. 6. Analysis of changes in bone microstructure. STD, standard group; SHAM, sham operated group; RNE, Raftiline group; CAF1 and CAF2, commercial Agave fructans 1 and 2 groups, $n = 4$. Mean values with different letters were significantly different ($P \leq .05$).

did not occur. The CAF1 group shows better preservation of the bone structure. Table 4 lists the concentrations of calcium and phosphorus determined by SEM/EDS femur analysis.

As previously shown by SEM analysis, the loss of both compact and trabecular bone structure becomes more evident with microstructural analysis of the STD group. We believe that fructans prevent bone imbalances resulting from ovariectomy as demonstrated by the observed increase in the bone formation marker osteocalcin.

The slight differences observed in Ca and Mg absorption may be related to three important structural features: (1) linearity, (2) degree of polymerization, and (3) the presence of β(2-6). Agave fructans are branched, neoserie fructans, and have both β(2-1) and β(2-6) linkages, while inulin fructans (Raftiline) consist of a linear chain of fructose molecules linked only by β(2-1).

Microstructural analyses and SEM revealed important differences that indicate that Agave fructans may have better overall effects. Additionally, in the majority of the performed experiments, mice in the CAF1 group showed the most beneficial effects. Agave fructans can be used as an alternative source of fructans.

In conclusion, structural differences between the types of fructans used did not alter cecal or colonic fermentation, the production of end products of bacterial metabolism (SCFAs), or the stimulation of Ca and Mg digestive absorption and the accumulation in the bone. To our knowledge, this is the first report on the effects of Agave fructans on mineral absorption.

ETHICAL STANDARDS

No humans were involved in this study. The experiments with animals were performed in accordance with (NOM-062-ZOO-1999) of the Mexican legislation.

ACKNOWLEDGMENT

María Isabel García-Vieyra appreciates the scholarship provided by Consejo Nacional de Ciencia y Tecnología (CONACYT) de México.

AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

1. Heaney PR: Calcium, dairy products and osteoporosis. *J Am Coll Nutr* 2000;19:83–99.
2. Bosscher D, Van Loo J, Franck A: Inulin and oligofructose as functional ingredients to improve bone mineralization. *Int Dairy J* 2006;16:1092–1097.
3. Kruger MC, Brown KE, Collett G, Layton L, Schollum LM: The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. *Exp Biol Med* 2003;228:683–688.
4. Mussatto SI, Mancilha IM: Non-digestible oligosaccharides: a review. *Carbopol* 2006;68:587–597.
5. Vijn I, Smeekens SJ: Fructan: more than a reserve carbohydrate? *Plant Physiol* 1999;120:351–360.
6. Roberfroid MB: Inulin type fructans: Functional food ingredients. *J Nutr* 2007;137:2493–2502.
7. Kolida S, Gibson GR: Prebiotic capacity of inulin type fructans. *J Nutr* 2007;137:2503–2506.
8. Greger L: Non digestible carbohydrate and mineral bioavailability. *J Nutr* 1999;129:14345–14355.
9. Lopez HW, Coudray C, Levrat VM, Feillet CC, Demigné C, Rémésy C: Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of the phytic acid on mineral homeostasis in rats. *J Nutr Biochem* 2000; 11:500–508.
10. Cani PD, Dewever C, Delzenne NM: Inulin type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* 2004; 92:521–526.
11. Delzenne N, Kok N: Effects of fructans-type prebiotics on lipid metabolism. *Am J Clin Nutr* 2001;73:456–458.
12. López MG, Mancilla-Margalli NA, Mendoza-Díaz G: Molecular structures of fructans from *Agave tequilana* Weber var. azul. *J Agric Food Chem* 2003;27:7835–7840.
13. Mancilla-Margalli NA, López MG: Water soluble carbohydrates and fructan structure patterns from Agave and Dasylirion species. *J Agric Food Chem* 2006;54:7832–7839.
14. Urías-Silvas JE, López MG: *Agave* spp. and *Dasylirion* sp. fructans as a potential novel source of prebiotics. *Dyn Biochem Process Biotech Mol Biol* 2009;3:59–64.
15. Santiago-García PA, López MG: Prebiotic effect of Agave fructans and mixtures of different degrees of polymerization from *Agave angustifolia* Haw. *Dyn Biochem Process Biotech Mol Biol* 3:52–57.
16. Pietro-Femia A, Piero-Dolara CL, Gianninil A, Biggeri A, Salvadori M, Clune Y, Collins KJ, Paglierani M, Caderni G: Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* on azoxymethane-induced colon carcinogenesis in rats. *Carcinogen* 2002;23:1953–1960.
17. Younes H, Coudray C, Bellanger J, Demigné C, Rayssiguier Y, Rémésy C: Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *Br J Nutr* 2001;86:479–485.
18. Urías-Silvas JE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM: Physiological effects of dietary fructans extracted from *Agave tequilana* Gto. and Dasylirion spp. *Br J Nutr* 2008; 99:254–261.
19. Levrat MA, Remesy C, Demigne C: High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. *J Nutr* 1991;121:1730–1737.
20. Suzuki T, Hara H, Kasai T, Tomita F: Effects of difructose anhydride III on calcium absorption in small and large intestines of rats. *Biosci Biotechnol Biochem* 1998;62:837–841.
21. Ladislav R, Hannelore D: Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. *Bone* 2005;37:728–735.
22. Coudray C, Feillet-Coudray C, Tressol JC, Gueux E, Thien S, Jaffrelo L, Mazur A, Rayssiguier Y: Stimulatory effect of inulin on intestinal absorption of calcium and magnesium in rats is

modulated by dietary calcium intakes short- and long-term balance studies. *Eur J Nutr* 2005;44:293–302.
23. Scholz-Ahrens KE, Schaafsma G, Van den-Heuvel EG, Schrezenmeir J: Effects of prebiotics on mineral metabolism. *Rev Am J Clin Nutr* 2001;73:459S–464S.
24. Norman AW: Intestinal calcium absorption: a vitamin D-hormone-mediated adaptive response. *Am J Clin Nutr* 1990;51: 290–300.
25. Nellans HN: Intestinal calcium absorption. Interplay of paracellular and cellular pathways. *Miner Electrolyte Metab* 1990;16: 101–108.
26. Hardwick LL, Jones MR, Brautbar N, Lee DB: Site and mechanism of intestinal magnesium absorption. *Miner Electrolyte Metab* 1990;16:174–180.
27. Coudray C, Tressol JC, Gueux E, Rayssiguier Y: Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *Eur J Nutr* 2003;42:91–98.
28. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, Ellis KJ: A combination of prebiotic short- and long-chain inulin type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr* 2005;82:471–476.
29. Tahiri M, Tressol JC, Arnaud J, Bornet F, Bouteloup-Demange C, Feillet-Coudray C, Ducros V, Pepin D, Brouns F: Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J Bone Miner Res* 2001;16:2152–2160.