

# FEMUR CHARACTERISTICS AND FECAL NITROGEN IN HEALTHY AND CANCER-AFFECTED WISTAR RATS SUPPLEMENTED WITH INULIN AND AGAVE FRUCTANS

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## ABSTRACT

The aim of this study was to evaluate the effect of 10 % supplementation with commercial chicory fructans (I), commercial *Agave tequilana* fructans (ATC), experimental *A. tequilana* fructans (ATE), experimental *A. salmiana* fructans (AS), rice starch (RS), and a control diet (C) on Wistar rats, both healthy and carcinogen-induced to colorectal cancer. It was hypothesized that agave and chicory fructans have similar effects. The supplements evaluated in both experiments maintained the dry weight and length of rat femurs in both groups without producing significant changes. Only healthy rats supplemented with chicory and both *A. tequilana* fructans showed higher fecal nitrogen levels. The femur ash content in rats that were carcinogen-induced to develop colorectal cancer was significantly lower ( $p \leq 0.05$ ) than the content in healthy rats. Minerals in the femurs of both groups of rats were similar. The hypothesis of this work was rejected.

**Keywords:** prebiotics, colorectal cancer, femur mineral content, fecal nitrogen.

## INTRODUCTION

Prebiotic consumption has been related to increased intestinal absorption and bioavailability of some macro minerals such as calcium, magnesium, and phosphorus, as well as micro minerals such as iron and zinc (Kruger *et al.*, 2003; Scholz-Ahrens *et al.*, 2007; Weaver *et al.*, 2011). The potential mechanisms of prebiotics' influence on calcium absorption in the large intestine are related to the increase in calcium solubility as a result of a pH reduction induced by the increase in bacterial synthesis of short-chain fatty acids (SCFA), an increase in the dynamic trans-epithelial interchange of cellular H<sup>+</sup> and Ca<sup>+2</sup> triggered by the SCFA, or an increase in the absorption area growth and levels of calbindin-D in the large intestine due to cellular proliferation promoted by metabolites produced by the resident microbiota, such as butyrate or polyamines (Whisner and Castillo, 2018).

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Prebiotics can increase the intestinal absorption of other minerals such as magnesium and zinc, given that several of their effects are not specific to calcium, such as the microstructure change of intestinal tissue and mineral availability (Raschka and Daniel, 2005). Phytic acid, naturally present and abundant in insoluble fiber, is considered to be responsible for the absorption and retention of some minerals (calcium, magnesium, and zinc) due to its chelation property. In contrast, soluble fiber consumption, such as pectin, gum, resistant starch, lactulose, fructooligosaccharides (FOS), and inulin, has been demonstrated to increase mineral absorption and bioavailability (López *et al.*, 2000). This is due to intake and fermentation increasing SCFA concentrations, decreasing pH, hypertrophying cecal tissue, and promoting phytic acid dissociation and its negative effects on mineral homeostasis (López *et al.*, 2000).

Soluble fiber improves mineral solubility, permeability, and absorption in the gut (Raschka and Daniel, 2005; Scholz-Ahrens and Schrezenmeir, 2007), as well as mineral bioavailability for bones (Scholz-Ahrens *et al.*, 2007). Moreover, diets with high soluble fiber reduce protein utilization while increasing fecal nitrogen excretion (Falcón *et al.*, 2011). Fecal nitrogen originates from dietary protein that escapes digestion, endogenous protein from pancreatic and intestinal secretions, detached epithelial cells, blood urea after diffusing through the intestinal tract, and increased bacterial mass (Beynen *et al.*, 2002; Delzenne *et al.*, 2011).

The aim of this study was to compare the effects of commercial chicory, *A. tequilana* fructans, and standardized stem fructans from *A. salmiana* and *A. tequilana* on femur mineral absorption and nitrogen content in the feces of healthy and carcinogen-induced colorectal cancer Wistar rats. It was hypothesized that agave and chicory fructans have similar effects in this regard.

## MATERIALS AND METHODS

### Tested polysaccharides

Chicory fructans (*Cichorium intybus* L.), known as inulin (Orafti® Synergy 1, Tienen, Belgium), *A. tequilana* fructans F.A.C. Weber (Inufib™, Jalisco, Mexico), and rice starch (Tres Estrellas®, Toluca, Mexico) were the commercial polysaccharides used. In addition, *A. salmiana* and *A. tequilana* fructans extracted from the stems of six individuals in optimum physiological maturity (Aguirre-Rivera *et al.*, 2001) from the Charcas (San Luis Potosí) and Arandas (Jalisco) regions were included as noncommercial polysaccharides, both extracted according to the process developed in our laboratory (Godínez-Hernández *et al.*, 2016).

### Animals and diets

A total of 36 male, two-month-old Wistar rats with an average body weight of 200–250 g were used for each experiment. The animals were provided by the Centro de Biociencias of the Universidad Autónoma de San Luis Potosí. The experimental unit was regarded as each individual rat, which were housed in separate polypropylene

cages in a climate-controlled environment with a temperature between 20 and 25 °C, relative humidity between 30 and 60 %, and an inverted circadian cycle of 12 h of light and 12 h of darkness. Animals were kept in accordance with the local ethical guidelines for cautions and proper maintenance (NOM 062-ZOO-1999). Protocols were approved by the institutional ethics committee (CONBIOÉTICA-24-CEI-003-20160830).

The animals were given a one-week adaptation period with a *per capita* portion of 25 g of standard rodent feed (Chow® 5008, Brentwood, MO, USA) and *ad libitum* water. Moreover, six treatments were assigned at random (n = 6): standard commercial feed (C) or 90 % same feed plus 10 % of the following polysaccharides: commercial inulin (I), commercial fructans from *A. tequilana* (ATC), experimental fructans from *A. tequilana* (ATE), experimental fructans from *A. salmiana* (AS), and commercial rice starch (RS). Commercial fructans contain a higher proportion of FOS, whereas AS fructans have a lower polymerization degree (Regalado-Rentería *et al.*, 2020).

During the 20-week experimental trial, each feed portion for all treatments, with or without supplementation, was administered every day at the start of the dark period. Standard composition of the feed was 12 % moisture, 23 % protein, 6.5 % fat, 4 % fiber, 8 % ash, and 46.5 % nitrogen free extract: with an energy content of 84.12 Kcal per 25 g. At the end of the experiments, all animals were sacrificed with pentobarbital sodium (0.063 g mL<sup>-1</sup>) (SEDALPHARMA®, Pet's Pharma, Mexico) at an intraperitoneal dose of 40 mg kg<sup>-1</sup> live weight.

#### **Inducement of incipient colorectal carcinogenesis**

After eight weeks of treatment, the colon cancer preneoplastic stage was induced by administering two intraperitoneal doses of 15 mg of the carcinogenic drug azoxymethane (AOM) (Sigma-Aldrich®, San Louis Missouri, USA, A2853) by kg of body weight, dissolved in sterile solution saline (0.85 %) (Dávila-Céspedes *et al.*, 2014), one at the end of the eighth week and the other at the end of the ninth week of the experimental period.

#### **Mineral content and femur characterization**

At the end of the experimental period, both femurs per animal were dried and immersed in a 0.85 % saline solution. The muscular tissue was removed and the femurs were dried (at 40 °C for 36 h) (Shel-Lab FX-14, USA) until constant weight (Sartorius BPAA1S, Germany) and their length was measured with a digital vernier (Digital Cadena® Model A020, China).

Ash content was estimated using the left femur (550 °C after 6 h) (LINDBERG SB, 51844, Mexico). The macro and micro mineral content of the pulverized right femur was estimated after the organic matter was removed with concentrated H<sub>2</sub>NO<sub>3</sub>. Calcium, magnesium, phosphorus, and potassium content were determined using an optical emission spectrometer (Thermo Scientific iCAP 7000 Series, USA) and atomic emission spectrometry with plasm coupled inductively (ICP-OES); sodium, iron, zinc,

and sulfur were determined using mass plasma spectrometry coupled inductively (ICP-MS) with a quadrupole system in a spectrophotometer of optical emission coupled to a mass spectrometer.

#### Nitrogen content in feces

At the end of the experiment, sample feces were collected and dried to constant weight 15 (40 °C for 36 h) (Shel-Lab FX-14, USA); then, 100 mg of dry feces were used to estimate the 16 nitrogen content, according to AOAC 993.13 methodology using a FLASH 2000 17 N/Protein analyzer (Thermo Scientific™, Series No. 2015FO106).

#### Experimental design and statistical analysis

A completely randomized design was used in both groups of rats, healthy and carcinogen-induced colorectal cancer. One-way analysis of variance (ANOVA) and the Tukey test ( $p \leq 0.05$ ) were used. SAS 9.2 (SAS Institute Inc., 2019) was used for statistical analysis. The Student's *t*-test was used to compare all variables between the two experiments: healthy rats and carcinogen-induced colorectal cancer rats; the error probability was set at 5 % ( $p \leq 0.05$ ).

### RESULTS AND DISCUSSION

#### Femur mineral content and characterization

There were no differences ( $p > 0.05$ ) in dry weight and femur length in both experiments, in contrast to the results found by Lobo *et al.* (2006) and Rivera-Huerta *et al.* (2017), who observed an increase in femur mass, density, and volume in response to FOS supplementation, attributing this to fructan consumption decreasing bone turnover through suppression of osteoclast resorption.

There were no differences ( $p > 0.05$ ) among treatments for K, Zn, and Fe in healthy rats (Table 1) or colorectal cancer rats (Table 2). In contrast, there were some differences ( $p \leq 0.05$ ) in healthy rats and colorectal cancer rats for Ca, P, Na, and Mg (Tables 1 and 2).

**Table 1.** Mineral content (mg g<sup>-1</sup> dry matter) in femurs of healthy Wistar rats.

Treatment	Ca	P	Na	S	Mg	K	Zn	Fe
C	644.83± 5.65a	294.34± 4.76b	29.09±0.89b	17.60±0.22a	11.47±0.60a	1.54±0.14a	0.95±0.17a	0.21±0.11a
I	611.17±29.94ab	322.71±31.00ab	34.72±2.31a	17.88±0.36a	10.55±0.65ab	1.86±0.31a	0.91±0.10a	0.20±0.03a
CAT	599.94±23.76ab	343.97±24.10ab	25.56±0.78b	17.89±0.07a	9.71±0.81ab	1.55±0.07a	1.13±0.04a	0.25±0.06a
EAT	607.01±20.14ab	335.17±20.18ab	27.74±1.39b	17.94±0.65a	9.41±0.82b	1.67±0.28a	0.77±0.03a	0.28±0.07a
AS	600.85±5.65ab	343.77±5.21ab	25.32±0.88b	17.56±0.36a	9.60±1.02ab	1.52±0.32a	1.07±0.25a	0.31±0.09a
RS	586.37±12.60b	356.14±2.14a	27.04±2.14b	17.95±0.26a	9.77±0.07ab	1.65±0.21a	0.83±0.13a	0.25±0.04a
<i>p</i> -value	0.0484	0.0393	<0.0001	0.8391	0.0311	0.4359	0.3316	0.2807

C: control; I: commercial inulin; CAT: commercial fructans from *A. tequilana*; EAT: experimental fructans from *A. tequilana*; AS: fructans from *A. salmiana*; RS: rice starch. a,b: Means with different letters in the same column are statistically different ( $p \leq 0.05$ ).

**Table 2.** Mineral content (mg g<sup>-1</sup> dry matter) in femurs of carcinogen-induced colorectal cancer Wistar rats.

Treatment	Ca	P	Na	S	Mg	K	Zn	Fe
C	648.42± 5.79a	290.62±4.56b	29.14±0.66ab	17.41±0.46a	11.52±0.04a	1.64±0.25a	0.95 ±0.02a	0.30±0.12a
I	610.75±24.09ab	332.21±39.17ab	26.66±0.76b	17.61±0.75a	10.65±0.98a	1.13± 0.13a	0.82±0.03a	0.17±0.01a
CAT	607.86±10.89ab	337.40±11.02a	25.59±0.58b	17.53±0.15a	9.02 ±0.36b	1.30±0.19a	1.00±0.22a	0.28±0.01a
EAT	616.81±12.29ab	324.68±9.53ab	27.66±1.76ab	17.74±0.66a	10.59±0.49a	1.45±0.22a	0.83±0.17a	0.21±0.06a
AS	589.46± 7.39b	354.15±6.72a	26.18±1.31b	17.59±0.51a	10.09±0.45ab	1.18±0.24a	1.11±0.17a	0.22±0.05a
RS	627.69± 2.88ab	309.22±3.21ab	30.79±0.52a	18.15±0.17a	11.24±0.18a	1.58±0.24a	1.11±0.41a	0.24±0.05a
<i>p-value</i>	0.0167	0.0074	0.0049	0.7307	0.0021	0.1376	0.4292	0.2645

C: control; I: commercial inulin; CAT: commercial fructans from *A. tequilana*; EAT: experimental fructans from *A. tequilana*; AS: fructans from *A. salmiana*; RS: rice starch. a,b: Means with different letters in the same column are statistically different ( $p \leq 0.05$ ).

In both healthy and colorectal cancer rats, the highest Ca concentrations were found in the control treatment. Thus, the increased intestinal absorption and bioavailability of Ca and P assumed for the fructans (Kruger *et al.*, 2003; Raschka and Daniel, 2005) was not reflected in a higher concentration in the femur, as reported by García-Vieyra *et al.* (2014). However, it is likely that this occurred for the P concentration in rats that were carcinogen-induced to colorectal cancer for the AS treatment (Table 2), corresponding to *A. salmiana* fructans. For all treatments with fructans, the Ca-P ratio changed from 1.6:1 to 1.9:1, which is less than the normal 2:1 ratio (Rivera-Huerta *et al.*, 2017).

In general, supplementation with fructans has little effect on femur mineral concentration. It should be noted that inulin (I) induced the highest Na content in healthy rats, but one of the lowest values in colorectal cancer rats; in addition, in the experiment with colorectal cancer rats, *A. salmiana* (AS) fructans induced the lowest Ca content and the highest P content, in a 1.6:1 ratio.

#### Nitrogen content in feces

The experiment with healthy rats revealed a significant ( $p \leq 0.05$ ) increase in nitrogen content in feces for three out of the four fructan treatments (Table 3). In contrast, in the experiment with unhealthy rats, all treatments had similar nitrogen fecal content. In general, high levels of nutritional fiber increase N excretion in feces. Moreover, diets high in soluble fiber reduce protein utilization while increasing fecal nitrogen excretion (Falcón *et al.*, 2011). Feces nitrogen is derived from dietary protein that escapes digestion and endogenous proteins such as pancreatic and intestinal secretions, detached epithelial cells, and blood urea after diffusion through the intestinal gut (Beynen *et al.*, 2002).

Fructans, in addition to interfering with protein digestion because they are indigestible, are a basic substrate for a variety of intestinal acidophilous bacteria (Rendón-Huerta *et al.*, 2011); therefore, supplementation with these prebiotics significantly increases

**Table 3.** Effect of supplementation on fecal nitrogen content ( $\text{mg g}^{-1}$  dry matter).

Treatment	Healthy	Colorectal cancer
C	$36.0 \pm 3.3\text{b}$	$38.3 \pm 2.2\text{a}$
I	$43.8 \pm 1.8\text{a}$	$44.4 \pm 1.7\text{a}$
CAT	$43.6 \pm 2.6\text{a}$	$41.8 \pm 5.5\text{a}$
EAT	$44.3 \pm 3.3\text{a}$	$37.7 \pm 4.1\text{a}$
AS	$40.5 \pm 4.6\text{ab}$	$37.7 \pm 5.7\text{a}$
RS	$34.9 \pm 2.5\text{b}$	$38.7 \pm 3.9\text{a}$
<i>p-value</i>	0.0014	0.3756

C: control; I: commercial inulin; CAT: commercial fructans from *A. tequilana*; EAT: experimental fructans from *A. tequilana*; AS: fructans from *A. salmiana*; RS: rice starch. a,b: Means with different letters in the same column are statistically different ( $p \leq 0.05$ ).

the quantity of these bacteria in the cecum, gut, and feces, which in turn incorporate a significant portion of residual N, lower the pH, and thus reduce ammoniacal absorption in the colon (Cani and Delzenne, 2011; Delzenne *et al.*, 2011). Reduced intestinal ammonia absorption reduces urea generation in the liver, causing N excretion to shift from urine to feces. As a result, fructans favor the extra renal route of N excretion while increasing N elimination via the feces (Younes *et al.*, 1999).

#### Contrast between experiments

Aspects probably involved in the prebiotics-mediated stimulation of mineral absorption, solubility, and bone mineralization are due to the decrease in pH in the intestinal lumen caused by the production of short-chain fatty acids, aside from indirectly stimulating passive calcium absorption by increasing its solubility via lowering the pH with the exchange of cellular proton ( $\text{H}^+$ ) for luminal cation ( $\text{Ca}^{++}$ ) (Scholz-Ahrens *et al.*, 2007).

The dry weight, length, ashes, and main minerals in the bones were compared in both experiments on healthy and carcinogen-induced colorectal cancer patients (Table 4). There were differences ( $p \leq 0.05$ ) in ashes percentage with the lowest value for the unhealthy rats, possibly due to the bone demineralization presented by cancer patients due to their pathological and degenerative physiological processes (Rivera-Huerta *et al.*, 2017). There were statistical similarity ( $p > 0.05$ ) for the rest of the variables evaluated.

**Table 4.** Femur parameters and minerals (mg g<sup>-1</sup>) by state of health.

Item	Healthy	Colorectal cancer	<i>p</i> -value
Ashes (%)	67.28 ± 8.91a	57.78 ± 3.23b	0.001
Dry weight (g)	0.88 ± 0.02a	0.92 ± 0.11a	NS
Length (mm)	42.42 ± 2.21a	41.48 ± 2.23a	NS
Ca	609.67 ± 33.92a	612.82 ± 22.31a	NS
P	331.62 ± 25.51a	329.12 ± 24.42a	NS
Na	28.4 ± 3.62a	27.3 ± 1.92a	NS
S	17.8 ± 1.14a	17.6 ± 1.49a	NS
Mg	10.0 ± 0.92a	10.3 ± 0.77a	NS
K	1.64 ± 0.21a	1.35 ± 0.11a	NS
Zn	0.94 ± 0.11a	0.98 ± 0.12a	NS
Fe	0.25 ± 0.03a	0.23 ± 0.04a	NS

a,b: Means with different letters between rows are statistically different ( $p \leq 0.05$ ).

## CONCLUSIONS

The supplements tested in both experiments resulted in no significant changes in femur dry weight or length. Only healthy rats supplemented with chicory and *Agave tequilana* fructans had higher fecal nitrogen.

The femur ash content in rats affected by colorectal cancer was notably lower than in healthy rats. The minerals in the femurs of rats in both experiments were similar.

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## REFERENCES

- Aguirre-Rivera JR, Charcas-Salazar HF, Flores-Flores JL. 2001. El maguey mezcalero potosino. Universidad Autónoma de San Luis Potosí, Consejo Potosino de Ciencia y Tecnología: San Luis Potosí, México. 87 p.
- Beynen AC, Baas JC, Hoekemeijer PE, Kappert HJ, Bakker MH, Koopman JP, Lemmens AG. 2002. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. *Journal of Animal Physiology and Animal Nutrition* 86 (9–10): 298–305. <https://doi.org/10.1046/j.1439-0396.2002.00386.x>

- Cani PD, Delzenne NM. 2011. The gut microbiome as therapeutic target. *Pharmacology and therapeutics* 130 (2): 202–212. <https://doi.org/10.1016/j.pharmthera.2011.01.012>
- Dávila-Céspedes A, Juárez-Flores BI, Pinos-Rodríguez JM, Aguirre-Rivera JR, Oros-Ovalle AC, Loyola-Martínez ED, Andrade-Zaldívar H. 2014. Protective effect of *Agave salmiana* fructans in azoxymethane-induced colon cancer in Wistar rats. *Natural Product Communications* 9 (10): 1503–1506. <https://doi.org/10.1177/1934578X1400901025>
- Delzenne NM, Neyrinck AM, Cani PD. 2011. Modulation of the gut microbiota by nutrients with prebiotic properties: Consequences for host health in the context of obesity and metabolic syndrome. *Microbial Cell Factories* 10 (1): S10. <https://doi.org/10.1186/1475-2859-10-S1-S10>
- Falcón VM del R, Barrón HJM, Romero BAL, Domínguez SMF. 2011. Efecto adverso en la calidad proteica de los alimentos de dietas con alto contenido de fibra dietaria. *Revista Chilena de Nutrición* 38 (3): 369–375. <https://doi.org/10.4067/s0717-75182011000300012>
- García-Vieyra MI, Del Real A, López MG. 2014. Agave fructans: their effect on mineral absorption and bone mineral content. *Journal of Medicine Food* 17 (11): 1247–1255. <https://doi.org/10.1089/jmf.2013.0137>
- Godínez-Hernández CI, Aguirre-Rivera JR, Juárez-Flores BI, Ortiz-Pérez MD, Becerra-Jiménez J. 2016. Extracción y caracterización de fructanos de *Agave salmiana* Otto ex Salm-Dyck. *Revista Chapingo Serie Ciencias Forestales y del Ambiente* 22 (1): 59–72. <https://doi.org/10.5154/r.rchscfa.2015.02.007>
- Kruger MC, Brown KE, Collett G, Layton L, Schollum LM. 2003. The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. *Experimental Biology and Medicine* 228 (6): 683–688. <https://doi.org/10.1177/153537020322800606>
- Lobo AR, Colli C, Filisetti TM. 2006. Fructooligosaccharides improve bone mass and biomechanical properties in rats. *Nutrition Research* 26 (8): 413–420. <https://doi.org/10.1016/j.nutres.2006.06.019>
- López HW, Coudray C, Levrat-Verny MA, Feillet-Coudray C, Demigné C, Rémésy C. 2000. Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. *The Journal of Nutritional Biochemistry* 11 (10): 500–508. [https://doi.org/10.1016/S0955-2863\(00\)00109-1](https://doi.org/10.1016/S0955-2863(00)00109-1)
- Raschka L, Daniel H. 2005. Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. *Bone* 37 (5): 728–735. <https://doi.org/10.1016/j.bone.2005.05.015>
- Regalado-Rentería E, Aguirre-Rivera JR, Godínez-Hernández CI, García-López JC, Oros-Ovalle AC, Martínez-Gutiérrez F, Martínez-Martínez M, Ratering S, Schnell S, Ruíz-Cabrera MÁ, Juárez-Flores BI. 2020. Effects of agave fructans, inulin and starch on metabolic syndrome aspects in healthy Wistar rats. *ACS Omega* 5 (19): 10740–10749. <https://doi.org/10.1021/acsomega.0c00272>
- Rendón-Huerta JA, Juárez-Flores BI, Pinos-Rodríguez JM, Aguirre-Rivera JR, Delgado-Portales RE. 2011. Effects of different kind of fructans on *in vitro* growth of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium lactis*. *African Journal of Microbiology Research* 5 (21): 2706–2710. <https://doi.org/10.5897/ajmr11.455>
- Rivera-Huerta M, Lizárraga-Grimes VL, Castro-Torres IG, Tinoco-Méndez M, Macías-Rosales L, Sánchez-Bartéz F, Tapia-Pérez GG, Romero-Romero L, Gracia-Mora MI. 2017. Functional effects of prebiotic fructans in colon cancer and calcium metabolism in animal models. *BioMed Research International* 2017: 9758982. <https://doi.org/10.1155/2017/9758982>

- SAS Institute Inc. 2019. Statistical Analysis System version 9.4. Windows. SAS Institute, Inc., Cary, NC, USA. 1028 p.
- Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Asil Y, Glüer CC, Schrezenmeir J. 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *The Journal of Nutrition* 137 (3): 838S–846S. <https://doi.org/10.1093/jn/137.3.838s>
- Scholz-Ahrens KE, Schrezenmeir J. 2007. Inulin and oligofructose and mineral metabolism: The evidence from animal trials. *The Journal of Nutrition* 137 (11): 2513S–2523S. <https://doi.org/10.1093/jn/137.11.2513s>
- Weaver CM, Martin BR, Nakatsu CH, Armstrong AP, Clavijo A, McCabe LD., McCabe GP, Duignan S, Schoterman MHC, van den Heuvel EGHM. 2011. Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. *Journal of Agricultural and Food Chemistry* 59 (12): 6501–6510. <https://doi.org/10.1021/jf2009777>
- Whisner CM, Castillo LF. 2018. Prebiotics, bone and mineral metabolism. *Calcified Tissue International* 102 (4): 443–479. <https://doi.org/10.1007/s00223-017-0339-3>
- Younes H, Alphonse JC, Behr SR, Demigné C, Rémésy C. 1999. Role of fermentable carbohydrate supplements with a low-protein diet in the course of chronic renal failure: experimental bases. *American Journal of Kidney Diseases* 33 (4): 633–646. [https://doi.org/10.1016/S0272-6386\(99\)70213-1](https://doi.org/10.1016/S0272-6386(99)70213-1)

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