In vivo effect of two different dietary fiber blends on the milk calcium bioavailability

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Abstract Milk is a valuable source of dietary calcium and it becomes important to establish whether incorporation of dietary fiber (DF), a health promoting food constituent, would lead to any undesirable impact on the bioavailability of milk calcium or not. The DF fortified spray dried partly skimmed milk powder with prestandardized fiber Blend-I (psyllium husk, oat fiber, MCC, inulin) and fiber Blend-II (psyllium husk, oat bran, wheat fiber and inulin) was subjected to rat-feeding studies to examine the possible effects on the bioavailability of milk calcium. The differences for calcium absorption and retention among diets containing DF Blend-I, DF Blend-II and cellulose (control) were found to be non-significant. It was evident that the milk calcium bioavailability of the diets containing two fiber formulations tested (at the levels studied) was at par with that of control standard diet containing only cellulose as DF. Therefore, it is reasonable to incorporate these DF blends into dairy products, and thereby add value.

Keywords Calcium bioavailability · Dietary fiber · Psyllium husk · Oat bran · Inulin · Wheat fiber

Introduction

Lack of dietary fiber (DF) in diet is correlated to the occurrence of various disorders such as ischemic heart disease, appendicitis, diverticular disease (pockets in the bowel), hemorrhoids, gall bladder disease, varicose veins (abnormally dilated veins), deep vein thrombosis, hiatus hernia, tumors of the large bowel and colorectal cancer (Mann and Cummings 2009; Anderson et al. 2009). Fiber supplements such as psyllium-seed fiber and methylcellulose or food components such as wheat bran are sometimes used in the treatment of chronic constipation (American Dietetic Association 2008). Several epidemiological studies have indicated a strong link between a high-fiber diet, especially a generous intake of whole grains, and the prevention of coronary heart disease (Anderson et al. 2009). Moreover, high-fiber diets provide benefits to diabetic patients by lowering blood glucose concentration, reducing postprandial insulin levels (Lummela et al. 2009) and antidiabetic drug requirements.

While DF is recognized to play an important role in human health, its presence in diet has under certain conditions (such as presence of high phytate content) been found to have undesirable effects on the nutritional constituents of the food. Such effects include lowered bioavailability of certain minerals, vitamins and protein (Slavin and Jacobs 2010; Zhang et al. 2013). However, the concentration of DF, the presence of non-fiber components such as phytates and other phytochemicals, environmental conditions such as the pH of the gastro-intestinal tract etc. are key determinants of whether DF would lead to any undesirable impact on the nutritional properties of the product. However, with respect to purified DF, the case is not well defined as they have weak binding characteristics and less reliable and less effective adsorption and produces

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outcomes that are less conclusive (Harland and Oberleas 2001).

Milk is appropriately recognized as a nearly complete food. Besides being a source of first-class protein, milk contains important dietary elements such as calcium, potassium, sodium, magnesium and vitamins (Muehlhoff et al. 2013). Calcium is required for the strengthening of bones, nervous system and in controlling hypertension. It has also been reported that calcium, whey protein, sphingomyelin, β -carotene, conjugated linoleic acid (CLA), butyric acid and ether lipids found in milk are anti-carcinogenic in nature (Muehlhoff et al. 2013; Westphal and Böhm 2015). Calcium bioavailability from milk and milk products is always considered superior in developing bone mass (Guéguen and Pointillart 2000). Dairy products, with high digestibility of protein, fat and lactose, and little or no DF content, form an essentially low-residue diet i.e., if consumed without appreciable quantities of foods rich in fiber, it could lead to a less bulky stool. However, DF has now widely been recognized as a health-promoting food constituent (Anderson et al. 2009) and there is an increasing trend of fortifying milk and other food products with DF (Arora et al. 2015; Sharma et al. 2016). Efforts were made by the authors in earlier studies to develop value added yoghurt and *kheer* (a rice-based milk dessert) fortified with different blends of DF (Arora and Patel 2015, 2017). Milk is a valuable source of dietary calcium and it becomes important to establish whether incorporation of DF would lead to any undesirable impact on the bioavailability of milk calcium or not. Thus, the present project was undertaken with the specific objective to assess the effect of partly skimmed milk powder fortified with two different DF blends (I and II) on the milk calcium bioavailability in rats as experimental animals.

Materials and methods

Partly skimmed milk powder (SMP)

Partly skimmed milk powder (spray dried) used as the sole source of protein and a part source of calcium for animal feeding was prepared in the Experimental Dairy, National Dairy Research Institute (NDRI), Karnal. For this purpose, buffalo milk was standardized using skim milk to 2.5% and 8.3% solids-not-fat (SNF) and was preheated to 90 °C for 1 min. It was concentrated to 34–35% total solids (TS) in a double-effect falling-film evaporator (SSP Ltd., Faridabad) followed by cooling (to < 10 °C) and holding (at < 5 °C) for overnight. Butylated hydroxyl anisole (BHA) was then added (at 0.01% of concentrate dry matter) after heating the concentrate to 73–75 °C followed by homogenization ($140 \pm 5 \text{ kg/cm}^2$) and spray drying in the nozzle type

spray drier (SSP Ltd., Faridabad) (Feed rate, 10 kg/cm²; Nozzle diameter, 0.5 mm; Inlet air temperature, 197–202 °C; Outlet drier air temperature, 87–93 °C; Product temperature, 60–64 °C). The powder was packed in air-tight polyethylene bags and stored at –1.0 °C till further use.

The prepared milk powder was analysed for its proximate composition. The proximate analysis for moisture, fat (Mojonnier method), protein (Micro Kjeldahl method) and ash (in a muffle furnace set at 550–600 °C) was performed using the procedures outlined in FSSAI (2016). The proximate composition of the product is shown in Table 1. An atomic absorption spectrophotometer (AAS) (Philips, Model: PU9100X, England) fitted with a calcium cathode lamp was used to determine the concentration of calcium in skim milk powder (SMP), rat diets and the composite samples of the urine, and the feces of each rat. The readings were carried out at 422.7 nm wavelength.

Dietary fiber preparations

Commercial DF viz. Vitacel wheat fiber (WF-200), Vitacel oat fiber (HF-600) and Vitacel microcrystalline cellulose (MCC-105) were procured from M/s J. Rettenmair and Sohne GmbH, Germany. Oat bran was obtained from Bagrry's Pvt. Ltd., India and psyllium (*Plantago ovata*) husk was sourced from 'Sat Isabgol', Palani Group, India. Inulin 'Raftiline ST' was sourced from ORAFTI, (Belgium). Cyclotech 1093 sample mill (*Foss Techator*, Sweden) fitted with a 0.5 mm sieve was used to grind the psyllium husk into a fine powder. The DF content of the powdered fiber preparations was estimated by the AOAC (2000) method 991.43 employing TDF-100A kit supplied by Sigma (USA). The moisture, soluble and insoluble DF contents of the fiber preparations are shown in Table 2.

Components of diet(s) for animal feeding studies

The different components of the diet, namely AIN-76 mineral mixture (Cat No. 905455), Vitamin diet fortification mixture (Cat No. 904654), Corn Starch, Corn oil, Cellulose powder Microcrystalline for animal feeding studies were procured from MP Biomedicals, USA and Lactose was procured from CDH, New Delhi. The various components of AIN-76 mineral mixture used in animal diet are shown in Table 3.

Experimental animals

Twenty-nine days old weanling Wistar rats (average body weight $43 \pm 6.5 \text{ g}$) were used following the protocol described by Kansal and Chaudhary (1982). The rats were housed in individual stainless-steel metabolic cages under

Table 1 Proximate composition of partly skimmed milk powder and three diets

Constituent	Partly skimmed milk powder	Diet I (containing Blend I)	Diet II (containing Blend II)	Diet III (control with cellulose)
Moisture (%)	2.09 ± 0.09	5.77 ± 0.02	4.21 ± 0.19	6.40 ± 0.14
Fat (%)	22.29 ± 0.99	10.35 ± 0.08	10.33 ± 0.09	10.43 ± 0.14
Protein (%)	28.79 ± 0.18	10.12 ± 0.02	10.2 ± 0.14	10.39 ± 0.11
Ash (%)	6.33 ± 0.06	2.27 ± 0.01	2.33 ± 0.02	1.53 ± 0.02
Carbohydrates (%) (by difference)	40.50 ± 1.0	71.49 ± 0.07	72.93 ± 0.12	71.246 ± 0.13
Calcium (mg/100 g)	115.13 ± 7.85	443.7 ± 8.12	395.2 ± 6.62	507.6 ± 10.71

Means of triplicate determinations with ± SD values

Table 2 Moisture, soluble and insoluble DF contents of the constituent fiber preparations used in Blend-I and/or Blend-II

Constituent fiber preparation	Moisture content (% by wt)	Soluble fiber content (% on dry basis)	Insoluble fiber content (% on dry basis)	Total DF content (% on dry basis)
Psyllium husk	10.82	63.52	31.28	94.80
Oat fiber (HF-600)	8.98	3.00	93.00	96.00
MCC	5.72	0.00	97.00	97.00
Inulin	2.42	94.00	0.00	94.00
Oat bran	7.44	9.00	6.00	15.00
Wheat fiber (WF-200)	6.52	2.50	94.50	97.00

Means of triplicate determinations

Table 3 Components of AIN-76 mineral mixture (MP Biomedicals, USA) used in animal diet

S. no.	Mineral	Amount (g/kg mineral mixture)
1.	Calcium phosphate, dibasic	500.00
2.	Sodium chloride	74.00
3.	Potassium citrate, monohydrate	220.00
4.	Potassium sulfate	52.00
5.	Magnesium oxide	24.00
6.	Manganous carbonate	3.50
7.	Ferric citrate	6.00
8.	Zinc carbonate	1.60
9.	Cupric carbonate	0.30
10.	Potassium iodate	0.01
11.	Sodium selenite	0.01
12.	Chromium potassium sulfate	0.55
13.	Sucrose, finely powdered	118.00

controlled light, temperature and humidity conditions. The rats were divided into 3 groups of 5 each according to weight so that the animals in each group were within ± 5 g of mean weight. To conduct the present study, proper approvals were taken from the Animal Ethics Committee, National Dairy Research Institute, Karnal and all the proper procedures were followed strictly as directed.

Preparation of diets

Both the test and control diets were prepared so as to make them isocaloric and 15 g of each diet could provide nearly 40 mg calcium per day to each rat so as to provide them the minimum required/threshold quantity of calcium for normal growth and survival (Hunt et al. 2008), otherwise the

absorption and/or retention of calcium might also get affected. The individual ingredients (Table 4) were accurately weighed and mixed together (except corn oil). The dry mix of the diets was sieved (sieve mesh size no. 22, aperture size: 0.965 mm; made up of stainless steel). It was then dry blended in an electrical blender (batch-type horizontal semi cylindrical mixer of a 10 kg capacity with a double-spiral agitator, make: APV Engineering, Calcutta) initially for 5 min, then further for 10 min while slowly adding the required quantity of corn oil for uniform mixing. The proximate composition of each diet is given in Table 1.

Dietary fiber content of DF-fortified milk powder

For the purpose of animal feeding experiments, a dry formulation of the fiber-fortified milk was obtained by blending of the dry fiber preparations with partly skimmed milk powder. Two such formulations were obtained for the two fiber blends (I and II) and were named as Group I (diet consisting of DF Blend-I) and Group II (diet consisting of DF Blend-II) while a third group (Group III) diet contained only cellulose. The fiber-fortification of partly skimmed milk powder was done in the same ratio as in the milk used for developing fiber-fortified yoghurt and fiber-fortified *kheer* (Arora and Patel 2015, 2017).

From the Table 4 it can be seen that the total source of DF in diet I was 15.96% and in diet II was 16.01%. However, in the control group (III) there was only one source of DF viz. cellulose (5.0%, all insoluble fiber). On correcting for the moisture present in the individual DF (Table 2) it was found that the total DF in diet I was 13.8% on dry matter basis (dmb) (soluble fiber being 10.6% dmb

and insoluble fiber 3.2% dmb), while the total DF in diet II was 11.7% dmb (soluble fiber being 10.6% dmb and insoluble fiber 1.1% dmb).

Feeding of experimental animals

Feeding of the test and control diets was carried out for the first 3 days preliminary adjustment period followed by 7 days' balance period. Fifteen grams of the diet was given to each rat such that each rat could receive nearly 40 mg calcium per day. The diet was served after the addition of a known quantity of just enough distilled water (5.6–6.6 ml) to impart it a dough-like consistency. Distilled water was provided ad libitum to rats (distilled water is free from calcium therefore rats were allowed to drink water as much as they needed).

The spilled food was collected and dried at $102 \pm 2^\circ\text{C}$ overnight. Food consumed was calculated by the difference between dry solids present in the feed served and dry solids in the residual, unconsumed feed. During the balance feeding period, faeces and urine, from individual rats were collected daily into separate aluminium containers.

Standard curve for calcium estimation

Calcium carbonate (Qualigens, AR grade) was dried at 105°C for 2 h. To get the standard solution of 1000 ppm of calcium, 2.496 g of the dried calcium carbonate was dissolved in 25 ml of 3 N HCl, and diluted to 250 ml with distilled water (Ranganna 2001). To this solution, 5 ml of 7.5% of the EDTA (disodium salt) solution was added (Bassett et al. 1978) and the volume made up to 1.0 l using 0.3 N HCl (1000 ppm concentration). From this 1000 ppm

Table 4 Components of the various diets for different rat groups

Component	Group I	Group II	Group III (control)
Partly skimmed milk powder (%)	34.73	34.73	34.73
Vitamin mix (%)	2.00	2.00	2.00
AIN-76 Mineral mix (%)	1.30	1.30	1.30
Corn starch (%)	44.50	44.43	55.44
Corn oil (%)	2.26	2.26	2.26
Psyllium husk (%)	0.79	0.87	–
Microcrystalline cellulose (%)	2.45	–	–
Oat fiber (%)	1.05	–	–
Wheat fiber (%)	–	0.58	–
Oat bran (%)	–	2.91	–
Inulin (%)	11.67	11.65	–
Cellulose (%)	–	–	5.00
Total (%)	100.75	100.73	100.73
Total source of DF (%)	15.96	16.01	5.00

Group I: Diet consists of Blend-I of DF; Group II: Diet consists of Blend-II of DF; Group III: Diet consists only cellulose as DF

stock solution, 1, 2, 4, 8, 16, 32 and 64 ml was taken into separate one-litre volumetric flasks and the volume made up to 1.0 l with 0.3 N HCl to develop standard solutions of 1, 2, 4, 8, 16, 32 and 64 ppm calcium concentrations, respectively. The solutions were estimated for calcium by employing atomic absorption spectrophotometer at 422.7 nm. The absorbance values were plotted against the corresponding concentrations and the linear regression model fitted to data.

Determination of calcium in feces and urine samples

The procedure described by Kansal and Chaudhary (1982) was used. The pooled fecal material from each rat collected during the 7-day balance period was dried overnight at $102 \pm 2^\circ\text{C}$. The dried feces (W) were weighed and ground into a powder with a pestle and mortar and kept in air-tight plastic containers in a refrigerator until required for analysis. An accurately weighed quantity, w (0.6 g) of each sample was charred in a silica dish, cooled and ashed overnight in a muffle furnace at $525 \pm 5^\circ\text{C}$, followed by cooling in a dessicator, weighing and adding to it 10 ml of 6 N HCl (Ranganna 2001). This solution was heated to dryness using an electrical heating coil (Bajaj Electricals). Fifteen milliliters of 3 N HCl were carefully added to it avoiding splashing. The solution was heated just enough to bring to boil and then cooled and filtered through Whatman No. 41 ashless filter-paper into a 100 ml volumetric flask (prerinsed with distilled water and 3 N HCl). Further 10 ml of 3 N HCl was added to the same silica dish and heated until the solution just began to boil, followed by cooling and filtering through the same filter-paper into the volumetric flask. The dish was washed thrice with distilled water, and the washings were collected into the same volumetric flask after passing through the filter paper. To the washings collected along with the filtrate, 0.5 ml of 7.5% of the EDTA (disodium salt) solution was added. The flask content was cooled and its volume made up to 100 ml with distilled water. The absorbance of samples was measured with an atomic absorption spectrophotometer (at a wavelength of 422.7 nm) and the concentration of calcium (ppm) was estimated from the standard curve. A blank determination was carried out following the same procedure without sample. The calcium concentration (in ppm) of the sample was converted to mg calcium present in the total fecal material using the following formula:

$$\text{Calcium in mg/100 g feces} = \frac{(a - b) \times v \times 100}{w \times 1000}$$

where a, concentration of calcium in the sample solution, $\mu\text{g/ml}$; b, concentration of the calcium in the sample blank, $\mu\text{g/ml}$; v, final volume made up of the ash solution or digest

of the sample, ml; w, weight of the fecal sample taken for analysis, g.

Therefore,

$$\begin{aligned} \text{Calcium (mg) in W g material (i.e. in the total feces obtained)} \\ = \frac{(a - b) \times v \times 100 \times W}{w \times 1000} \end{aligned}$$

The urine from individual rats was collected daily into separate glass bottles by carefully rinsing with toluene, the funnel and the attached urine-collecting plastic container of the metabolic cage. The collection was made for the balance period of 7 days. The pooled urine from each rat was carefully transferred to a silica dish in installments and evaporated on an electrical heating coil (Bajaj Electricals). To ensure quantitative transfer of the material each glass bottle was rinsed twice with toluene (10 ml) and deionised distilled water (10 ml). It was then evaporated to dryness. The dried material was then ashed overnight in a muffle furnace at $525 \pm 5^\circ\text{C}$. The ash quantity was determined and its calcium content estimated in the same way as for ash of the fecal samples above. A blank was also run simultaneously and calcium quantity worked out as

$$\text{Calcium (mg) in urine} = \frac{(a - b)}{10}$$

where a, concentration of the calcium in the sample solution, $\mu\text{g/ml}$; b, concentration of the calcium in the sample blank, $\mu\text{g/ml}$.

The absorption and retention of calcium by experimental animals were calculated as follows:

$$\text{Calcium absorption (mg)} = \text{Calcium intake (mg)}$$

$$- \text{Calcium loss in feces (mg)}$$

$$\text{Calcium retention (mg)} = \text{Calcium absorption (mg)}$$

$$- \text{Calcium loss in urine (mg)}$$

Calcium intake was calculated from the measured amount of calcium present in a diet and the amount of that diet consumed by a rat.

The data obtained was statistically analyzed for variance through the analysis tool in ANOVA using Excel-2000 (Microsoft).

Results and discussion

The calcium balance in rats fed on the diets containing two different fiber blends, Blend I was 15.96% of total DF and in Blend II was 16.01% of total DF in comparison to control (containing cellulose) is shown in Table 5. The calcium absorption and retention were found to be 94.4% and 94.2% for the Group-I fed on the diet containing DF Blend-I and 96.3% and 96.0% for the Group-II fed on the

Table 5 Calcium balance (mean $\pm$ standard error) in rats fed on the diets containing fiber Blend-I, Blend-II and control

Group	Intake (mg)	Ca absorption		Ca retention	
		(mg)	(%)	(mg)	(%)
I (Blend-I)	297.4 $\pm$ 22.9	282.1 $\pm$ 25.7	94.4 $\pm$ 1.9	281.5 $\pm$ 25.7	94.2 $\pm$ 1.9
II (Blend-II)	247.2 $\pm$ 22.9	238.7 $\pm$ 24.9	96.3 $\pm$ 1.32	238.0 $\pm$ 25.0	96.0 $\pm$ 1.4
III (control)	291.4 $\pm$ 26.5	262.7 $\pm$ 36.3	88.8 $\pm$ 4.9	262.2 $\pm$ 36.3	88.7 $\pm$ 4.9
F-ratio		Within treatments (animals)	1.57 ^{NS}	Within treatment	1.56 ^{NS}
		Between treatments (diets)	2.71 ^{NS}	Between treatments	2.82 ^{NS}

NS non-significant ($p > 0.05$)

Group I: Diet consists of Blend-I of DF; Group II: Diet consists of Blend-II of DF; Group III: Diet consists only cellulose as DF

diet containing DF Blend-II as against 88.8% and 88.7% for the control (Group-III), respectively. It can be further seen from the Table 5 that these values were statistically not significantly different (ANOVA, $p > 0.05$) with regard to the diets or fiber types for both the absorption and the retention of calcium in the growing rats. The absorption % was similar in all the three groups and there was no significant difference in the absorption of calcium within the group as well as between the groups.

Toma and Curtis (1986) reported after reviewing 13 human and 13 animal studies that the addition of 15–20 g of fiber from a variety of sources like wheat bran, neutral detergent fiber (NDF), soybean fiber and cellulose did not significantly affect the bioavailability of iron and calcium thus regarding 15–20 g of fiber/day as the safe level for the consumer at large.

The slightly higher values for the fiber-added diets than the control found herein could be attributed to the presence of inulin in the fiber blends. Similar observations of enhanced calcium absorption due to inulin and other fructooligosaccharides were reported by Takahara et al. (2000). They also quantified the enhanced calcium and magnesium absorption with inulin and fructooligosaccharides by increased femoral bone volume and mineral concentration in rats.

Oat fiber was found to enhance the absorption of calcium in milk (Frolich et al. 1988) whereas wheat bran and oat bran were observed to have no impact on calcium metabolism in humans and rats, respectively (Bagheri and Gueguen 1982; Spencer et al. 1987).

The bioavailability of minerals depends upon several factors including the phytate and oxalate concentration of the fiber, complex calcium binding and the level of the fiber in the diet (Harland and Oberleas 2001; Ross et al. 2011; Vavrusova and Skibsted 2014). Whereas certain DF like pectin and alginate can bind minerals and vitamins, and

reduce their absorption in the small intestine, these DF (locust bean gum, pectin and alginate) were absent in both the DF Blends-I and Blend-II. Since the two fiber formulations in the present study contained mostly purified fiber commercially available preparations, they were essentially free from the mineral-binding agents such as phytates and oxalates, the calcium bioavailability of the Group-I and Group-II was found to be at par with that of the control (Group-III).

Burckhardt (2013) had detailed an update on calcium requirement, intake, absorption, deficiency and recommendations. The bioavailability of minerals is a key consideration while developing strategies for preventing mineral deficiencies through fortification (Tait and Teucher 2002). Perales et al. (2006) reported an increase in calcium bioavailability by calcium fortification of milk. They found that the solubility, dialysis, transport, and uptake values for calcium are higher ($p < 0.05$) for calcium fortified milks than for nonfortified milks in an in vitro study. Calcium bioavailability may also vary for different dairy products. Unal et al. (2005) investigated the in vitro calcium bioavailability of different dairy products like different kinds of milk, yogurt, cheese and infant formulas. They found no difference among milk, yogurt and infant formula groups in terms of calcium bioavailability. However, all cheese kinds were considerably different from each other ($p < 0.05$). Data has been collected by Fardet et al. (2018) showing different kinetics of bioavailability of calcium, amino acids and fatty acids according to the physico-chemical parameters of dairy products, including compactness, hardness, elasticity, protein/lipid ratio, P/Ca ratio, effect of ferments, size of fat globules, etc.

Bosscher et al. (2003a) studied calcium, iron, and zinc availabilities from dairy infant formulas supplemented with soluble dietary fibers and modified starches in vitro. They found that calcium availability from the standard formula

was elevated by 30% after inulin supplementation (17.2%), whereas locust bean gum (11.9%) and high esterified pectin (11.7%) reduced availability by approximately 10%. Bosscher et al. (2003b) further showed that addition of locust bean gum to infant formulas increased the viscosity of the intestinal contents. Locust bean gum influenced calcium availability in infant formulas by means of its physical properties to act as thickening agent, rather than its chemical ability to form complexes. Similarly, Luccia and Kunkel (2002) found reduction in relative calcium bioavailability to less than 90% by diets containing 10% fiber from psyllium and 10% fiber from Metamucil and induced significant negative changes in calcium contents of weanling Wistar rats. Such high level of psyllium normally forms a very high viscous gel which might be responsible for such negative effect. Psyllium solution at such high level is also very slimy (both in water and in milk) and is difficult to swallow by humans. Its high value of consistency coefficient was found to be related to low sensory viscosity ratings (Arora et al. 2016). Therefore, in the present study, the high level of psyllium was not incorporated in either of the two DF blends (0.79% and 0.87% psyllium was present in Group-I and Group-II, respectively). Further, the presence of inulin (11.7%) might have cancelled any negative effect of psyllium on calcium absorption and/or retention resulting in an overall no significant effect on calcium bioavailability.

Conclusion

The fiber fortified partly skimmed milk powder with Blend-I (containing 15.96% total DF) and the fiber fortified partly skimmed milk powder with Blend-II (containing 16.01% total DF) were subjected to rat-feeding studies to examine the possible effects of the incorporation of DF on the calcium bioavailability, in terms of calcium absorption and retention. The differences for calcium absorption and retention among diets containing fiber Blend-I, Blend-II and cellulose (control) were found to be non-significant (ANOVA, $p > 0.05$). The findings indicate that the calcium bioavailability was similar from the diets containing either of the two DF blends than the one with standard composition. Hence, these two DF blends could be a part of regular diet (at the levels studied) in order to meet the daily recommended dietary allowance for DF.

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