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Calcium Bioavailability of Textured Vegetable Protein Fortified With Calcium

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ABSTRACT

The purpose of this research was to increase the calcium (Ca) content of textured vegetable protein (TVP) by fortifying with 0.4% calcium carbonate or 0.3% calcium chloride and to compare the calcium bioavailability using rats. The calcium bioavailability was determined in terms of the calcium retention, absorption and intake in the bone and serum levels of phosphorus and alkaline phosphatase. A control diet (casein) and three experimental diets were prepared TVP, TVP + 0.4% $CaCO_3$, TVP + 0.3% $CaCl_2$. The experimental animals were screened every 4, 8 and 12 wk for observed changes in growth and in the circulating levels of Ca, phosphorus (P) and alkaline phosphatase. The results at 8 wk showed that Ca retention and Ca absorption were 42.22 ± 6.47 and 44.37 ± 5.97 mg.d^{-1} in the TVP + 0.4% $CaCO_3$ group. This was significantly higher than in the TVP and TVP + 0.3% $CaCl_2$ groups, which were 23.12 ± 6.15 and 28.43 ± 8.05 mg.d^{-1} and 25.91 ± 5.94 and 30.89 ± 7.95 mg.d^{-1}, respectively. The metabolic study on rats revealed that the calcium bioavailability of TVP fortified with $CaCO_3$ was slightly higher than for the unfortified and fortified TVP with $CaCl_2$.

Keywords: calcium, bioavailability, absorption, retention, textured vegetable protein.

INTRODUCTION

Textured vegetable protein (TVP) is a food product made from soybeans. After the soybean oil has been extracted, the remaining soy flour is then cooked under pressure, extruded and dried. As TVP contains absolutely no meat or meat by-products, those who are on strict vegetarian diets can use this to supplement their protein intake. TVP has a long shelf life if stored properly, and is an excellent source of protein and fiber (Endres, 2001). It is claimed that it not only meets nutritional standards but is economical and plain TVP has zero cholesterol since it is made from purely vegetable sources (All About Textured Vegetable Protein-TVP®, 2013).

Calcium is a very important mineral for humans. Human bones contain large amounts of calcium, which helps to make them firm and rigid, while calcium is also needed for many other tasks, including nerve and muscle function and blood clotting (McCarron and Heaney, 2004). These other tasks are so important for survival that, when dietary calcium is too low, calcium will be lost from bone and used for other critical functions (McCarron and Heaney, 2004). Adequate amounts of calcium are also necessary for normal bone growth and development in children (Tardoff, 2001).

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By the 1970s, medical research had begun to focus on osteoporosis. Since then, many clinical studies have emphasized the importance of calcium supplementation in the prevention of osteoporosis (Gerstner, 2003; Swaim *et al*., 2008; Fardellone *et al*., 2010). Calcium balance studies have also demonstrated that calcium requirements increase after menopause in women and this, coupled with an early epidemiological study associating increased hip fracture with low calcium intake, strongly suggested the importance of adequate calcium intake with regard to the prevention of osteoporosis, particularly in postmenopausal women (Swaim *et al*., 2008). There remains widespread interest in assuring adequate calcium intake at critical stages in a woman's life (Zhong *et al*., 2009).

The notion of bioavailability includes not only absorption or retention but also utilization for normal physiological processes or storage; thus, the bioavailability of calcium from a food source is best evaluated by measuring its effect on changes in bone mass over time (Schaafsma, 1997; Barclay, 2001). Several studies investigating the bioavailability of calcium from food sources have used male or female rats during their peak stage of growth (at age 4–8 wk) as during this stage, rats respond rapidly to changes in the calcium environment; therefore, relevant differences in the bioavailability of calcium from various sources can be detected over a short period (Peterson *et al*., 1995; Patwardhan *et al*., 2001). As a result of living longer lives, many more people are at greater risk of suffering from osteoporosis (Fardellone *et al*., 2010). Consequently, obtaining adequate amounts of bioavailable calcium throughout life is of growing importance as a major component in osteoporosis prevention. TVP was chosen as the food for calcium fortification because TVP is a favorite food for vegetarians instead of meat and milk products which are especially high in calcium (Green *et al*., 2003) but are not available options in their diet. Thus fortified levels of calcium in TVP (the addition of about 30% of the recommended daily intake of calcium) may provide one of the best ways to increase their calcium intake. No previous studies have investigated the bioavailability of calcium from textured vegetable protein fortified with various sources of calcium. Thus, the purpose of this research was to study, using a rat model, the bioavailability of calcium from textured vegetable protein (TVP) fortified with 0.4% $CaCO_3$ (calcium carbonate) or 0.3% $CaCl_2$ (calcium chloride).

MATERIAL AND METHODS

Preparation of textured vegetable protein

TVP was produced from defatted soy flour and fortified with 0.4% $CaCO_3$ or 0.3% $CaCl_2$ mixed together and then passed through a Cooker Extruder X-25 (Wenger, New York, NY, USA) to produce a textured vegetable protein.

Diets

The experimental diet given to the animals was an AIN 93G formula (a mineral mix used for growth, pregnancy and lactation phases of rodents) with a semi-synthetic diet containing textured vegetable protein which had two formulae: 0.4% calcium carbonate and 0.3% calcium chloride. Two control diets were used as positive (casein) and negative (TVP) controls. The chemical compositions of the TVP diets and casein appear in Table1 and the two control diets are shown in Table 2.

Animals

Three-week-old weanling male Sprague-Dawley rats were obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The experimental protocol was developed according to the guidelines of the Committee on the Care and Use of Animals for Scientific Purposes (National Research Council of Thailand, 1999). A total of 84 rats were used and were divided into four groups (21 rats per group) namely, casein, TVP, TVP + 0.4%$CaCO_3$ and TVP + 0.3% $CaCl_2$. The animals were separately housed in shoebox cages and kept at a temperature

of 21–23 °C in a light-controlled room (12 hr day/night cycle).

Animals had *ad libitum* access to water and their diet. Animals were fed the test and control diets for up to 12 wk. At the end of weeks 4, 8 and 12 wk, animals from each group were placed in metabolic cages for overnight urine and feces collection. Blood was then collected via heart puncture, after which the animals were euthanized.

Calcium balance studies

Feed intake was measured and urine and feces collected for further analysis. Urine samples were kept under dark conditions and measured and filtered for calcium analysis. Feces were weighed, dried and ground for calcium content analysis. Food, urine, feces and bone were determined for calcium content and measured by an atomic emission spectrophotometer (induced couple plasma model DV 2000; Perkin-Elmer Inc.; Watsonville, CA, USA) according to the standard method (Association of Official Analytical Chemists, 2000). Triplicate measurements were performed from each treatment. Blood samples were tested for calcium, phosphorus and alkaline phosphatase by an automated analyzer for clinical chemistry (Vita Lab Flexor E; Vital Scientific; Berkhamsted, UK).

The calcium balance studies were calculated as follows:

Calcium retention = Calcium intake – Fecal Ca – Urinary Ca

Calcium absorption = Calcium intake – Fecal Ca

True absorption coefficient = (Ca intake – Fecal Ca) / Ca intake

Statistical analysis

The results were analyzed using analysis of variance and Duncan's new multiple range test, in which $P < 0.05$ was considered to be significant. Statistics were calculated using the SPSS software package (version 15.0; SPSS Inc.; Chicago IL, USA). Animal groups were compared using analysis of variance. Values were expressed as the mean ± SD.

RESULTS AND DISCUSSION

Chemical composition

The compositions of the TVP and casein diets are summarized in Table 1. The calcium bioavailability of TVP fortified with 0.4% $CaCO_3$ or 0.3% $CaCl_2$ was studied in rats in terms of the weight gain, calcium retention, calcium absorption, true absorption coefficients (TAC), Ca in bone, and Ca in serum (that is, phosphorus and alkaline phosphatase). The results of these indicators showed that there were significant differences among the sample groups. Rats fed a diet of TVP fortified with 0.4% $CaCO_3$ compared to casein showed higher values of Ca absorption, Ca retention and TAC in weeks 8 and 12 (Tables 6, 7 and 8).

The protein content in the TVP samples varied from 53.7 to 54.0%, while the protein content in the casein was 81.3% (Table 1). The calcium content in the TVP samples varied from 334 to 471 mg per 100 g; however, the calcium content in the casein was comparatively low at only 109 mg per 100 g. Proximate analysis of the experimental diets (Table 3) showed that the protein content in the TVP diets varied from 11.4 to 11.7 g per 100 g, while protein in the casein diet was 17.7%. These diets contained more than 10% of the recommended minimum protein requirement for rats (Association of Official Analytical Chemists, 2000).

The calcium content in the diets varied from 487 to 549 mg per 100 g and was lower in the TVP diet than in the TVP + 0.3% $CaCl_2$ and TVP + 0.4% $CaCO_3$ diets. Table 4 shows the weight gain among rats in the experimental groups; the TVP + 0.3% $CaCl_2$ groups showed a higher weight gain in week 4, which was significantly different from the other two TVP groups. However, no significant differences in body weight gain were observed among the TVP groups in week 12, which showed that the protein quality among the TVP groups was almost the same.

Table 1 Chemical composition of textured vegetable proteins and casein.

Sample	Protein	Moisture	Fat	Ash	Fiber	Carbohydrate	Energy (kcal)	Calcium (mg per 100 g)
	(all measured in grams per 100 gm)							
Casein	81.3	10.4	1.0	3.9	1.6	1.6	340	109
TVP	53.8	2.7	4.6	6.9	2.0	29.8	375	334
TVP + $CaCO_3$	53.7	3.1	4.5	7.1	1.9	29.8	374	409
TVP + $CaCl_2$	54.0	2.8	4.5	7.2	2.0	29.5	374	471

TVP = Textured vegetable protein.

Table 2 Composition (g) of AIN 93-G (mineral mix used for growth, pregnancy and lactation phases of rodents) experimental diets.

Ingredient	Casein (positive control)	TVP (negative control)	TVP + 0.4% $CaCO_3$	TVP + 0.3% $CaCl_2$
Sample	-	200	200	200
Casein	200	-	-	-
L-cystine	3.0	3.0	3.0	3.0
Corn starch	529.5	529.5	529.5	529.5
Sucrose	100	100	100	100
Cellulose	50	50	50	50
Soybean oil	70	70	70	70
AIN-93VX Vitamin Mix[1]	10	10	10	10
AIN-93G-Mx Mineral Mix[2]	35	35	35	35
Choline chloride	2.5	2.5	2.5	2.5
Total	1,000	1,000	1,000	1,000

TVP = Textured vegetable protein.

[1] = AIN-93VX vitamin mix is a component of the AIN-93G diet used for rodents, composed of ($g.kg^{-1}$): niacin 3, calcium pantothenate 1.6, pyridoxine HCl 0.7, thiamine HCl 0.6, riboflavin 0.6, folic acid 0.2, biotin 0.02, vitamin E acetate (500 international units (IU).g^{-1}) 15, vitamin B12 (0.1%) 2.5, vitamin A palmitate (500,000 IU.g^{-1}) 0.8, vitamin D3 (400,000 IU.g^{-1}) 0.25, vitamin K1 dextrose mix (10 mg.g^{-1}) 7.50, sucrose 967.23; total 1,000 g.

[2] = AIN-93G-MX mineral mix diet used for rodents, composed of ($g.kg^{-1}$): calcium carbonate 357, potassium phosphate monobasic 196, potassium citrate H_2O 70.78, sodium chloride 74, potassium sulfate 46.6, magnesium oxide 24, ferric citrate (United States Pharmacopeia) 6.06, zinc carbonate 1.65, manganous carbonate 0.63, cupric carbonate 0.3, potassium iodate 0.01, sodium selenate 0.01025, ammonium paramolybdate $4H_2O$ 0.00795, sodium metasilicate $9H_2O$ 1.45, chromium potassium sulfate $12H_2O$ 0.275, lithium chloride 0.0174, boric acid 0.0815, sodium fluoride 0.0635, nickel carbonate 0.0318, ammonium vanadate 0.0066, sucrose (finely powdered) 221.026; total 1,000 g.

Table 3 Chemical composition of experimental diet.

Diet	Protein	Moisture	Fat	Ash	Fiber	Carbohydrate	Energy (kcal)	Calcium (mg per 100g)
	(all measured in grams per 100 gm)							
Casein	17.7	7.9	7.8	2.6	2.9	61.1	385	487
TVP	11.7	6.7	8.8	3.7	3.1	65.9	390	532
TVP + $CaCO_3$	11.5	7.3	8.9	3.8	3.2	65.2	387	547
TVP + $CaCl_2$	11.4	7.8	8.4	3.8	2.8	65.7	384	549

TVP = Textured vegetable protein.

Table 4 Average ± SD body weight of experimental rats fed the different diets.

Sample	Initial body weight (g)	4 wk / n = 21 (g)	8 wk / n = 12 (g)	12 wk / n = 6 (g)
Casein diet	80.90±4.32[a]	297.17±15.64[c]	433.48±25.73[b]	519.79±32.24[b]
TVP diet	80.95±2.65[a]	234.69±19.92[a]	360.02±26.65[a]	437.20±28.46[a]
TVP + $CaCO_3$ diet	80.96±3.79[a]	242.69±17.13[a]	361.92±19.34[a]	437.59±49.10[a]
TVP + $CaCl_2$ diet	80.80±4.45[a]	255.11±17.70[b]	374.32±28.44[a]	462.16±37.41[a]

TVP = Textured vegetable protein.

[a, b, c] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 5 Average ± SD Ca intake per rat for experimental rats fed the different diets.

Sample	4 wk ($mg.day^{-1}$)	8 wk ($mg.day^{-1}$)	12 wk ($mg.day^{-1}$)
Casein diet	83.44±3.85[a]	88.72±4.16[a]	101.10±6.84[a]
TVP diet	82.71±7.71[a]	95.91±5.94[b]	100.02±4.44[a]
TVP + $CaCO_3$ diet	88.89±8.81[a]	99.37±1.80[b]	106.49±8.55[a]
TVP + $CaCl_2$ diet	97.77±6.52[b]	100.89±7.95[b]	109.73±10.99[a]

TVP = Textured vegetable protein.

[a, b] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 6 Average ± SD Ca absorption per rat ($mg.day^{-1}$) of experimental rats fed the different diets.

Sample	4 wk ($mg.day^{-1}$)	8 wk ($mg.day^{-1}$)	12 wk ($mg.day^{-1}$)
Casein diet	53.44±3.85[b]	43.60±5.04[b]	21.20±10.49[a]
TVP diet	35.43±8.04[a]	25.91±5.94[a]	20.02±9.74[a]
TVP + $CaCO_3$ diet	38.89±8.81[a]	44.37±5.97[b]	31.49±10.30[a]
TVP + $CaCl_2$ diet	41.11±4.20[a]	30.89±7.95[a]	25.58±10.99[a]

TVP = Textured vegetable protein.

[a, b] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 7 Average ± SD Ca retention per rat of experimental rats fed the different diets.

Sample	4 wk ($mg.day^{-1}$)	8 wk ($mg.day^{-1}$)	12 wk ($mg.day^{-1}$)
Casein diet	48.88±4.07[b]	41.93±5.70[b]	18.28±11.09[a]
TVP diet	31.31±7.39[a]	23.12±6.15[a]	17.66±9.86[a]
TVP + $CaCO_3$ diet	31.41±8.32[a]	42.22±6.47[b]	27.72±10.48[a]
TVP + $CaCl_2$ diet	35.72±4.14[a]	28.43±8.05[a]	26.29±11.06[a]

TVP = Textured vegetable protein.

[a, b] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 8 True absorption coefficient ± SD of experimental rats fed the different diets.

Sample	4 wk	8 wk	12 wk
Casein diet	0.64±0.01[b]	0.49±0.05[b]	0.20±0.09[a]
TVP diet	0.43±0.05[a]	0.26±0.04[a]	0.20±0.10[a]
TVP + $CaCO_3$ diet	0.43±0.06[a]	0.44±0.05[b]	0.29±0.07[a]
TVP + $CaCl_2$ diet	0.42±0.03[a]	0.30±0.05[a]	0.26±0.07[a]

TVP = Textured vegetable protein.

[a, b] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 9 Average ± SD Ca in bone of experimental rats fed the different diets.

Sample	4 wk (mg per bone weight)	8 wk (mg per bone weight)	12 wk (mg per bone weight)
Casein diet	143.37±3.04[a]	165.63±3.48[a]	162.90±2.38[a]
TVP diet	163.52±3.07[c]	180.61±4.27[c]	175.92±2.28[b]
TVP + $CaCO_3$ diet	162.02±4.09[c]	175.83±3.05[b]	173.41±4.09[b]
TVP + $CaCl_2$ diet	155.36±3.08[b]	172.60±3.15[b]	166.03±10.60[a]

TVP = Textured vegetable protein.

[a, b, c] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 10 Phosphorus ± SD in blood of experimental rats fed the different diets.

Sample	4 wk ($mg.dL^{-1}$)	8 wk ($mg.dL^{-1}$)	12 wk ($mg.dL^{-1}$)
Casein diet	8.07±1.37[a]	7.08±0.63[a]	5.46±0.75[a]
TVP diet	7.41±0.57[a]	6.71±0.48[a]	5.51±0.46[a]
TVP + $CaCO_3$ diet	7.15±0.72[a]	6.66±0.59[a]	5.15±0.70[a]
TVP + $CaCl_2$ diet	8.01±1.94[a]	6.31±0.66[a]	5.23±0.32[a]

TVP = Textured vegetable protein.

[a] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Table 11 Alkaline phosphatase ± SD in blood of experimental rats fed the different diets.

Sample	4 wk ($units.L^{-1}$)	8 wk ($units.L^{-1}$)	12 wk ($units.L^{-1}$)
Casein diet	241.00±23.65[a]	183.16±29.86[a]	116.66±40.72[a]
TVP diet	374.77±57.99[b]	300.50±26.26[b]	179.50 ±31.41[bc]
TVP + $CaCO_3$ diet	378.66±54.03[b]	253.83±50.96[b]	215.00±64.64[c]
TVP + $CaCl_2$ diet	332.66±57.13[b]	277.83±44.26[b]	147.33±25.00[ab]

TVP = Textured vegetable protein.

[a, b, c] = Different lowercase superscripts in the same column indicate a significant difference ($P \leq 0.05$).

Calcium intake (Table 5) is a reflection of the food intake and the amount of calcium present in the diet. The highest calcium intake was found in week 4 in animals fed TVP + 0.3% $CaCl_2$. However, in weeks 8 and 12, there were no significant differences in the Ca intake among the TVP, TVP + 0.4% $CaCO_3$ and TVP + 0.3% $CaCl_2$ groups. In week 4, the calcium absorption (Table 6) and calcium retention (Table 7) in absolute values were higher in rats fed a casein diet than the other groups, though in week 8, calcium absorption and calcium retention were higher in the casein and TVP + 0.4% $CaCO_3$ groups, which were significantly different from the other two groups. However, in week 12, there were no significant differences in calcium absorption and calcium retention among the rats from the four groups. The absorption values of the casein and TVP + 0.4% $CaCO_3$ groups were higher at the beginning of the experiment and lower at the end because rats fed these two experimental diets acquired their calcium requirement more rapidly than rats fed TVP and TVP + 0.3% $CaCl_2$.

It has been reported (Léon and Pointillart, 2000) that the greater the intake of animal protein, the greater the urinary secretion of calcium, because calcium is separated and removed from bones to neutralize the sulfur-containing amino

acids in animal protein after it is metabolized in the body. The mechanism for increased calcium retention is unknown, but has been related to enhanced absorption efficiency (Singh *et al* 2007). Growth in animals should be considered in making valid assessments of mineral bioavailability. Bioavailability is defined as the absorption and utilization of a nutrient (Krebs, 2001). Limitations of existing methods to describe the bioavailability of dietary calcium in rats have led to the alternative suggestion of using the true absorption coefficient (TAC) defined earlier. Table 8 shows the TAC values of the experimental rats. The higher true absorption of calcium reflected a greater TAC value for rats in the TVP + 0.4% $CaCO_3$ group at week 8 (0.44), which was significantly higher than those in the other experimental groups studied. This result agrees with the studies by Zikán *et al*. (2001) and Green *et al*. (2003), which showed that $CaCO_3$ is more rapidly absorbed. The average amount of calcium in bone (Table 9) showed significant differences among the test groups after 4, 8 and 12 wk. The amount of phosphorus in the blood (Table 10) showed no significant differences throughout the experiment. Alkaline phosphatase (ALP) is an enzyme localized on cell membranes that hydrolyzes phosphate esters liberating inorganic phosphate, and has an optimal pH of about 10.0. Serum alkaline phosphatase activity is elevated in hepatobiliary disease, especially in obstructive jaundice, and in bone diseases with increased osteoblastic activity such as hyperparathyroidism, osteitis deformans, and bone cancer. The liver and bone tissue each produce a distinct isoenzyme (Sogabe *et al*., 2000). In the present experiment (Table 11), the ALP activity was assessed at 4, 8 and 12 wk and there was a significant difference in the activity of alkaline phosphatase, which is a biological marker of bones. The results showed a progressive fall in the total ALP activity with increasing rat age. The reason for the fall in the bone isoenzyme with age is unclear, but may have been related to a reduction in bone growth.

CONCLUSION

The results from the present study indicated that over a period of 8 wk, TVP fortified with 0.4% $CaCO_3$ showed higher calcium bioavailability than TVP alone or TVP with 0.3% $CaCl_2$. A good mineral balance is important for both animals and humans. Deficiency, overdose or imbalances in inorganic nutrients have a negative effect on health. It is not the size of the dose of a mineral that is important to maintain a proper balance, but rather the amount of the mineral that is bioavailable. Hence, subjects who wish to increase their dietary Ca need to be aware of differences that may exist in the biological availability and/or usefulness of a particular Ca supplement. Further studies are required to determine the appropriate intake level of TVP for effective calcium metabolism.

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