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Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones

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Abstract

Phosvitin was successfully purified from egg yolks by an NaCl extraction method. When 1.0% of purified phosvitin was incubated with 0.1% of Ca under ileum conditions (pH 7.0, 37 °C), 44.1% of added Ca remained soluble in the supernatant, whereas less than 15% of added Ca was found soluble in the supernatant of the control group with no phosvitin added. Purified phosvitin was subjected to tryptic hydrolysis and examined for its efficiency in enhancing Ca absorption and accumulation in bones of SD rats. The rates of intestinal Ca absorption and its accumulation in bones were significantly higher in the groups in which phosvitin peptides were added in diets, with concentrations of 0.125–0.5%, which were equivalent amounts of 25–100% of the Ca in the diets. Although no significant differences in weights or ash contents of bones were observed, higher values of Ca to ash ratios, as well as bone mineral density and bone mineral content in femurs and tibias of the phosvitin peptides groups, suggested that phosvitin peptides improved bioavailability of Ca and thus increased incorporation of Ca into bones.

Keywords: Phosvitin; Peptide; Ca; Absorption

1. Introduction

Calcium is an essential macronutrient for the body. The normal recommended dietary intake of Ca for an adult is 800–1200 mg/day. Foods high in Ca content include milk, dairy products, meat, fish with bones, oysters, and many leafy green vegetables (e.g. spinach and collard greens). Ca deficiency leads to metabolic bone disease. Although serum Ca levels can be maintained in the normal range by bone resorption, dietary intake is the only source by which the body can replenish stores of Ca in bone. Absorption of Ca is influenced by numerous factors, such as a nature of chemical matrix of food source and nutritional, metabolic, and physiological status of individuals. Because Ca metabolism is so complex, treatment for Ca deficiency consists of more than just adding Ca to diets.

Hen egg yolk phosvitin is a highly phosphorylated protein with a molecular weight of 35 kDa containing 10% phosphorus and 6.5% carbohydrates. It has been separated into two components, designated α-phosvitin and β-phosvitin, by gel filtration (Abe, Itoh, & Adachi, 1982; Itoh, Abe, & Adachi, 1983). It is known to be richer in serine residues than casein and most of them are phosphorylated. Such an extraordinary abundance of phosphoryl groups in phosvitin imparts specific biological roles for phosvitin. For instance, 95% of Fe in egg yolks are complexed together with phosvitin in a strong and stable conformation for the purpose of avoiding being used up by microbes (Greengard, Sentenac, & Mendelsohn, 1964). There has been increasing public awareness of health benefits of phosphopeptides

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in the prevention of osteoporosis. As in the case of casein phosphopeptides (CPP), phosphoserine residues in phosvitin may increase bioavialbility of Ca. In this study, we tried to isolate phosvitin effectively from egg yolk and tryptically hydrolyze it to examine effectiveness of phosvitin peptides in enhancing Ca absorption and accumulation in bones.

2. Materials and methods

2.1. Preparation of phosvitin from hen eggs

Hen egg yolk phosvitin was isolated by modifying the Losso and Nakai method (1994). Egg yolk was lightly washed with distilled water and rolled on filter paper to remove adhering albumen. The yolk membrane was punctured with a needle and the contents were collected. Then, 100 g of yolk were diluted with 0.5 l of cold water at pH 5.0 and stirred at 4 °C for 1 h. The precipitate was collected by centrifugation at 10,000*g* for 20 min at 4 °C. The pellet was dissolved in 200 ml distilled water, stirred for 1 h and centrifuged at 10,000*g* for 20 min at 4 °C. The pellet was extracted with 400 ml of hexane:ethanol (3:1, v:v) at 4 °C for 3 h and centrifuged. The resulting cake was dried and extracted with 200 ml of 1.74 M NaCl overnight at 4 °C. Then the suspension was centrifuged at 10,000*g* for 20 min at 4 °C and the supernatant was dialyzed against distilled water for 24 h at 4 °C and freeze dried.

2.2. Amino acid analysis

Amino acid compositions of standard phosvitin and phosvitin fraction were determined by the Automatic Amino Acid Analyzer (JASCO model, Hachioji-shi, Tokyo, Japan) equipped with a Hewlett Packard integrator Model 3396A.

2.3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Hegenauer, Ripley, and Nace (1977) with 12.5% polyacrylamide gel. Gels were stained with 0.05% Coomassie Brilliant Blue R 250 in a solution of 0.1 M aluminium nitrate/25% isopropanol/10% acetic acid/1% Triton X-100 and destained in 7% acetic acid.

2.4. Preparation of phosvitin peptide

The purified phosvitin was dissolved in 6 ml of distilled water to a final concentration of 30 mg/ml and the pH of the solution was adjusted to 8.0 with NaOH prior to the addition of bovine trypsin (80 U/mg solid, Sigma Chemicals, St. Louis, MO, USA) with an enzyme:substrate ratio of 1:50 (w/w) (Goulas, Triplett, & Taborsky, 1996). The mixture was incubated at 37 °C for 24 h and the tryptic reaction was stopped by adjusting the pH to 5.0 with HCl. Then, the mixture was centrifuged at 5900*g* for 15 min at 10 °C and the supernatant was lyophilized.

2.5. Ca-solubilizing abilities of phosvitin and its peptides

Ca-solubilizing ability was determined, basically according to Sato, Noguchi, and Naito (1986) with some modifications. One hundred milligrammes of phosvitin and $CaCl_2$, added equivalent to 10, 100 and 1000 ppm of Ca, were dissolved in 10 mM sodium phosphate buffer. The mixture was incubated with shaking at 37 °C for 1 h and centrifuged at 1000 rpm for 10 min. For the phosvitin peptide samples, a mixture of 20 mM phosphate buffer (pH 7.0) and varying amounts of phosvitin peptides and CPP (Arla Foods Ingredients Co. Ltd., Tokyo, Japan) were added to 5 mM $CaCl_2$, and they were allowed to stand for 1–4 h at 37 °C. Ca contents in the supernatant, after centrifugation at 5000*g* for 5 min, were determined by a colorimetric method, using an *o*-cresolphthalein complexone Ca detection reagent (Sigma Chemicals, St. Louis, MO, USA).

2.6. Animals and diets

Three-week-old male weanling SD rats were housed in mesh-bottom plastic cages in a controlled environment and acclimatised for 1 week. Then rats were randomly divided into four groups of 10 rats each. The control group was fed with a normal diet of 0.5% Ca as shown in Table 1. In phosvitin peptides groups, phosvitin peptides were added to the normal at 0.125 (low), 0.25 (med) and 0.5% (high), equivalent to 25%, 50% and 100% of the total Ca in the normal diet, respectively. The diets and deionized water were offered ad libitum for 4 weeks. Body weights and amounts of food taken were recorded weekly. All animal experiments were performed under the guidelines of the Laboratory Animal Experiment Committee of Korea Food Research Institute.

2.7. Ca balance study

Ca balance evaluation was carried out for the last 4 days of the 4-week experiment. To determine Ca intake (V_i), the amount of food consumption was recorded, and urine and feces were collected. Urine was collected under acidic conditions of 1 ml of 6 N HCl for preventing Ca precipitation and putrefaction. All the collected urine was centrifuged at 2500*g* for 15 min immediately after collection. Collected feces were burnt to ash at 580 °C for 7 h and dissolved in 3 N HCl. Fecal (V_f)

Table 1
Composition of experimental diets (g/kg)

Ingredients	Control group	Phosvitin peptides groups		
		Low	Med	High
Phosvitin peptides[a]	0	1.25	2.5	5.0
Casein	200	198.75	197.5	195.0
DL-Methionine	3	3	3	3
Corn starch	448.0	448.5	449.0	450.0
Sucrose	200	200	200	200
Cellulose	50	50	50	50
Corn oil	50	50	50	50
AIN vitamin mix[b]	10	10	10	10
Choline bitartate	2	2	2	2
AIN mineral mix	35	35	35	35
$CaCO_3$[c]	0.25	0.19	0.13	–
KH_2PO_4[c]	1.75	1.31	0.87	–

[a] Phosvitin peptides contain 3% of Ca and 8.2% phosphorus.
[b] Mineral mix (g/kg): $CaHPO_4$ 500.0, NaCl 74.0, $K_3C_6H_5O_7 \cdot H_2O$ 220.0, K_2SO_4 52.0, MgO 24.0, $MnCO_3 \cdot H_2O$ 3.5, $FeC_6H_5O_7$ 6.0, $ZnCO_3 \cdot H_2O$ 1.6, $CuCO_3$ 0.3, KIO_3 0.01, $Na_2SeO_3 \cdot H_2O$ 0.01, $CrK(SO_4)_2 \cdot 12H_2O$ 0.55, sucrose, finely powdered to make 1000.
[c] $CaCO_3$ and KH_2PO_4 were added to adjust the amounts of Ca and P in added phosvitin peptides.

and urine (V_u) Ca excretions were measured by a colorimetic method (Sigma diagnostic calcium reagent). This reagent contains Arsenazo III (2,2′-[1,8-dihydroxy-3-6-disulfonaphthylene-2,7-bisazo]-bis-benzenearsonic acid), which specifically complexes with Ca to form a purple-coloured complex. The intensity of the purple-coloured complex was measured at 600 nm. Then, apparent intestinal Ca absorption (Vad: $V_i - V_f$) and Ca accumulation (Vac: $V_{ad} - V_u$) were calculated.

2.8. Determination of bone Ca

The left femurs and tibias of the test rats were removed, cleaned of muscles and connective tissues and dried at 105 °C in an oven for 4 h. The dry weight was recorded and the length was measured on the axis between the greater trochanter and the top of the external condyle. After the bone was heated at 580 °C for 8 h, ash was dissolved to 10 ml of 6 N HCl. The suspension was filtered through Whatman No. 41 filter paper. Ca content of the solution was determined by a Sigma Diagnostics Ca Reagent.

2.9. Bone mineral density and bone mineral content

Bone mineral density (BMD) and bone mineral content (BMC) values for femurs and tibias of test rats were determined by dual energy X-ray absorptiometry (DXA: Lunar PIXImus densitometer, Lunar Corp., Madison, WI, USA). The tibia and the femur were both placed with the proximal ends to the left on the Delrin attenuation block provided with the instrument. The bones were directly scanned three times with repositioning between scans. All DXA scans were conducted by the same personnel.

2.10. Statistical analysis

The Statistical Analysis System (SAS) software ver. 6.11 was used to perform data analysis. All analyses were determined by Duncan's multiple range test at $p < 0.05$. The results shown were expressed in means ± SD.

3. Results and discussion

3.1. Purification of phosvitin

Fig. 1 shows electrophoretic patterns of standard phosvitin (left), whole egg yolk proteins (middle) and the phosvitin fraction from egg yolk, by the NaCl extraction method (right). From the appearance on SDS–PAGE, extracting with NaCl seemed successful and selective in isolating phosvitin from whole egg yolk. Similarity in amino acid compositions between standard phosvitin and the fraction from egg yolk further confirmed that this fraction was mostly composed of phosvitin (Table 2). One notable feature was the high concentration of serine residues. Serine in standard and purified phosvitin made up 37.3% and 34.9% of

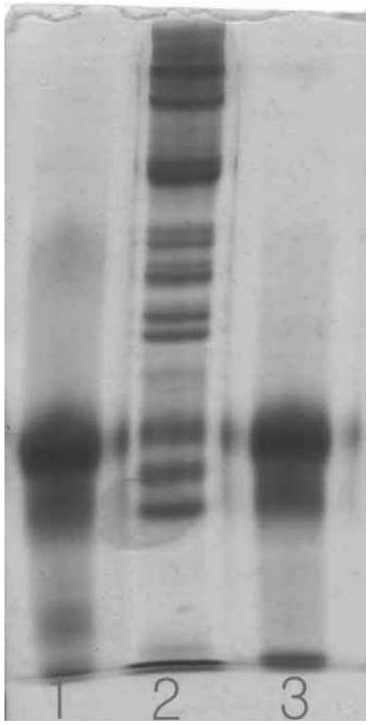

Fig. 1. Polyacrylamide-gel electrophoretic patterns of standard phosvitin (1), whole egg yolk protein (2) and purified phosvitin (3).

Table 2
Amino acids composition of standard phosvitin and the phosvitin fraction (unit: mg%)

Amino acid	Standard phosvitin	Phosvitin fraction
Cya[a]	1804	2206
Asx[b]	4282	3944
Glx[c]	4848	4470
Ser	19,968	16,368
His	3979	3315
Gly	4848	4470
Arg	4375	3717
Thr	1188	1086
Ala	4375	3717
Pro	1142	970
Tyr	667	721
Val	923	806
Met	292	328
Ile	557	522
Leu	759	708
Phe	676	602
Lys	5513	4779
Total	53,529	46,937

[a] Sum of cystein and cystine.
[b] Sum of asparagine and aspartic acid.
[c] Sum of glutamine and glutamic acid.

Table 3
Mineral compositions of standard phosvitin and the phosvitin fraction (unit: mg%)

Minerals	Standard phosvitin	Purified phosvitin
Ca	199	2939
P	8699	8218
Mg	3788	158
K	332	566
Na	2433	2461

total amino acids, respectively. Although contents of phophorus between the two phosvitins were similar, Ca in purified phosvitin made up 2939 mg%, being 15 times higher than that in standard phosvitin (199 mg%) (Table 3), whereas the content of Mg in standard phosvitin (3788 mg%) was 24 times higher than that in purified phosvitin (158 mg%). Considering the fact that standard phosvitin was obtained by the Mecham and Olcottmethod (1949), using $MgSO_4$ for phosvitin extraction, differences in mineral compositions may stem from differences in extracting methods.

3.2. Ca-solubilizing abilities of phosvitin and phosvitin peptides

Minute concentrations of trace minerals are essential elements for maintaining the health of the whole body. As mentioned Section 1, Ca absorption is controlled by the nutritional and physiological status of the body. To be absorbed properly into the body, it is prerequisite for Ca to be soluble under ileum conditions (pH 7.0, 37 °C). When $CaCl_2$ was incubated with phosvitin under ileum conditions, the amounts of solubilized Ca ions in the supernatant were increased by phosvitin addition (Fig. 2). Even considering the fact that purified phosvitin already contained 3000 mg% (300 ppm) of Ca, as shown in Table 2, purified phosvitin could solubilize more than 400 ppm of Ca ions out of 1000 ppm of added Ca. Less than 15% of total Ca ions were solubilized in the supernatant of the control group in which no phosvitin was added.

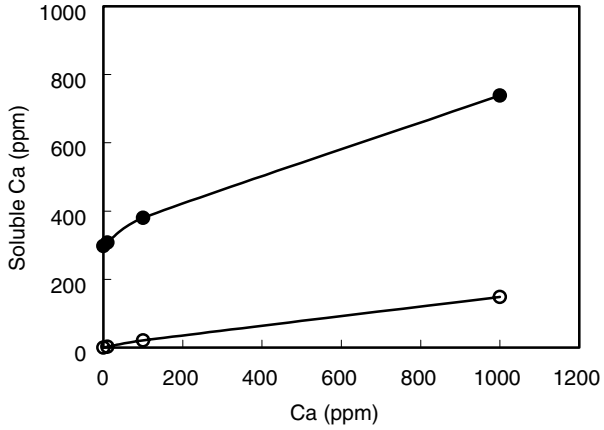

Fig. 2. Amounts of soluble Ca in the supernatant after incubating $CaCl_2$ (○) and $CaCl_2$ with purified phosvitin (●) at an ileum condition (pH 7.0, 37(°C) for 1 h).

Families of casein-derived peptides, enriched in phosphoseryl groups and named casein phosphopeptides (CPP), are mineral carriers and prevent precipitation of cations, such as Ca and Fe, thus improving their bioavailabilities. The early work of Mellander (1950) was the first indication of a potential physiological role for CPP that enhanced bone calcification of rachitic children in the absence of vitamin D. Other studies also reported increased Ca absorption in rats by oral intake of CPP (Mykkanen & Wasserman, 1980; Sato et al., 1986). Chelation of Ca with phophoseryl groups in CPP plays a key role in enhancing bioavailability of Ca (West, 1986). Since phosvitin is richer in phosphoserin than is casein, we tried to produce phosvitin peptides and compare their Ca-solubilizing abilities with commercial CPP. Pepsin and other proteases of microbial origins were tried but were not successful in properly hydrolyzing purified phosvitin (except trypsin). The tryptic digestions of purified phosvitin were done at 37 °C for 24 h and electrophoresed on SDS–PAGE (Fig. 3). Phosvitin was cleaved by trypsin into two large peptides and several smaller ones. A similar result was reported by Jiang and Mine (2000) with two large distinct peptides and three small ones. They also reported that only one visible peptide band was found, with a low molecular weight, when phosvitin was alkaline-dephosphorylated. According to this information, two distinctive peptide

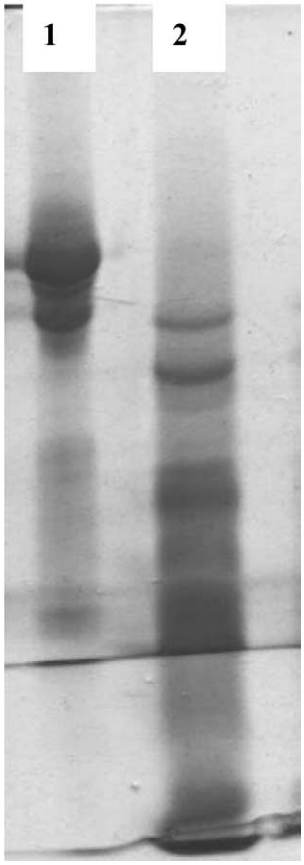

Fig. 3. SDS–PAGE patterns of tryptic hydrolysates of purified phosvitin. Purified phosvitin (1), tryptic hydrolysates of phosvitin (2).

bands might be high in phosphorylated serine residues that were resistant to tryptic hydrolysis.

After phosvitin was hydrolyzed by trypsin, the Ca-solubilizing ability of phosvitin peptides was compared with commercial CPP (Fig. 4). Ca-solubilizing ability of phosvitin peptides was much higher than that of commercial CPP at a ratio of phosvitin peptides/Ca (w/w) above 1.0. This indicated that phosvitin peptides might be much more effective than commercial CPP in solubilizing Ca ions under ileum conditions and, thus, phosvitin peptides enhance bioavailability of Ca. Concentrations of soluble Ca ions in phosvitin peptide-treated groups did not decrease as the incubation time increased. This meant that phosvitin peptides and Ca ions had formed stable complexes. In the case of CPP treated groups, however, the Ca-CPP complexes were becoming unstable, and this resulted in precipitation of complexes as incubation time increased.

3.3. Effects of phosvitin peptides on enhancing Ca accumulation in bones

Body weight gains and amounts of food intake in the control and phosvitin peptides groups are shown in Table 4. No statistical differences in amounts of food intake and body weight gains were observed between the control and phosvitin peptides groups. Table 5 shows the Ca intake and fecal and urinary Ca excreted for the last 4 days in the Ca balance experiment. The amounts of Ca intake in the control group were 119 mg/day, showing no statistical difference from the phosvitin peptide groups (121–138 mg/day). On the other hand, more Ca was excreted into feces in the control group (53.4 mg/day) than phosvitin peptide groups (45.4–51.3 mg/day), whereas almost the same amounts of Ca were excreted into urine in all groups (0.16–0.20 mg/day). These differences in the amounts of fecal Ca between the control group and phosvitin peptides groups represented higher rates of intestinal Ca absorption in the groups with phosvitin peptides addition. Although the correlation between Ca absorption rate and amounts of phosvitin peptides fed was not clearly established, it was certain that feeding rats with a small amount of phosvitin peptides (less than 2.5% of total protein fed) increased Ca absorption into their bodies.

To evaluate effectiveness of phosvitin peptides for enhancing incorporation rates of Ca into bones, weights, ash and Ca contents of tibias and femurs of all experimental rats were measured and compared. Although statistically not significant, average wet weight of tibias in the control group (531.13 mg) was lower than that of

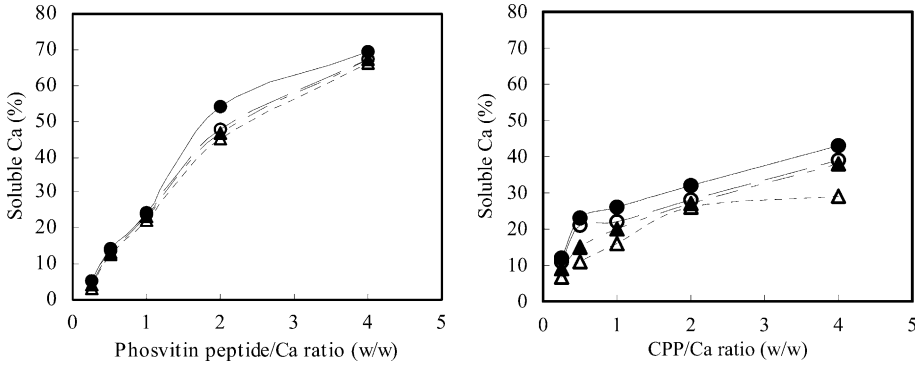

Fig. 4. Effect of phosvitin peptides and commercial CPP on Ca-solubilizing abilities at different ratios between phosvitin peptides/CPP and Ca. (●) 1; (○) 2; (▲) 3; (△) 4 h.

Table 4
Food intake and body weight gain in the control and phosvitin peptides groups (g/4 weeks)

Group	Total food intake^{NS}	Final body weight^{NS}	Weight gain^{NS}	FER^{NS} (WG/FI)
Control	496.91 ± 14.14	305.96 ± 17.68	161.70 ± 5.90	0.32 ± 0.01
Phosvitin petides				
Low	494.67 ± 22.32	307.25 ± 12.95	158.92 ± 7.44	0.32 ± 0.01
Med	505.76 ± 20.49	303.63 ± 11.52	159.62 ± 14.47	0.31 ± 0.02
High	483.31 ± 25.43	303.65 ± 24.10	156.99 ± 26.94	0.33 ± 0.06

NS: not significant by F test.

Table 5
Results of Ca balance experiment for the control and phosvitin peptides groups

Groups	Ca intake (mg/day)	Fecal Ca (mg/day)	Urinary Ca (mg/day)	Ca absorption (%)	Ca accumulation (%)
Control	119.1 ± 5.41^{a}	53.4 ± 2.20^{b}	0.16 ± 0.02^{a}	55.1 ± 0.80^{b}	55.0 ± 0.79^{b}
Phosvitin peptides					
Low	120.9 ± 2.91^{a}	45.4 ± 2.09^{a}	0.13 ± 0.04^{a}	62.4 ± 2.25^{a}	62.3 ± 2.28^{a}
Med	129.1 ± 16.24^{a}	50.2 ± 7.50^{ab}	0.20 ± 0.10^{a}	61.2 ± 2.06^{a}	61.1 ± 2.10^{a}
High	137.6 ± 14.98^{a}	51.3 ± 3.05^{ab}	0.19 ± 0.09^{a}	62.6 ± 1.92^{a}	62.5 ± 1.97^{a}

Values within a column with different superscripts are significantly different at $p < 0.05$ by Duncan's multiple-range test.

Table 6
Contents of Ca and ash, and ratios of ash/wt, Ca/wt and Ca/ash of tibias of the control and phosvitin peptides groups

Groups	Wet weight (mg)	Ash (mg)	Ca (mg)	Ash/wt (%)	Ca/wt (%)	Ca/ash (%)
Control	531.13 ± 9.26^{a}	166 ± 2.98^{c}	46.0 ± 1.73^{c}	31.2 ± 0.16^{c}	8.66 ± 0.38^{c}	27.9 ± 0.96^{b}
Phosvitin peptides						
Low	541.38 ± 16.88^{ab}	175 ± 2.70^{b}	51.0 ± 1.59^{b}	32.5 ± 0.97^{bc}	9.43 ± 0.25^{b}	29.1 ± 0.95^{ab}
Med	559.72 ± 17.02^{a}	189 ± 8.97^{a}	55.3 ± 2.39^{c}	33.7 ± 1.15^{ab}	9.89 ± 0.25^{ab}	29.4 ± 0.83^{a}
High	536.05 ± 13.54^{ab}	184 ± 5.99^{ab}	54.6 ± 2.90^{c}	34.4 ± 1.14^{a}	10.2 ± 0.43^{a}	29.6 ± 0.79^{a}

Values within a column with different superscripts are significantly different at $p < 0.05$ by Duncan's multiple-range test.

Table 7
Contents of Ca and ash, and ratios of ash/wt, Ca/wt and Ca/ash of femurs of the control and phosvitin peptides groups

Groups	Wet weight (mg)	Ash (mg)	Ca (mg)	Ash/wt (%)	Ca/wt (%)	Ca/ash (%)
Control	652.53 ± 28.27^{a}	214 ± 7.92^{a}	74.7 ± 3.72^{b}	32.8 ± 1.05^{b}	11.5 ± 0.68^{b}	35.0 ± 1.57^{b}
Phosvitin peptides						
Low	642.15 ± 34.63^{a}	217 ± 16.0^{a}	82.7 ± 6.64^{ab}	33.8 ± 0.75^{ab}	12.9 ± 0.71^{a}	38.1 ± 2.54^{a}
Med	678.37 ± 26.79^{a}	237 ± 14.9^{a}	89.6 ± 2.88^{a}	34.9 ± 1.10^{a}	13.3 ± 0.85^{a}	38 ± 2.80^{a}
High	669.75 ± 17.77^{a}	234 ± 14.5^{a}	87.6 ± 5.72^{a}	34.9 ± 1.60^{a}	13.1 ± 0.52^{a}	37.5 ± 1.07^{a}

Values within a column with different superscripts are significantly different at $p < 0.05$ by Duncan's multiple-range test.

Table 8
Bone mineral densities (BMD) and bone mineral contents (BMC) of femurs and tibias of the control and phosvitin peptides groups

Groups	Femur		Tibia	
	BMD (g/cm^2)	BMC (g)	BMD (g/cm^2)	BMC (g)
Control	0.1130 ± 0.0059^{c}	0.2518 ± 0.0193^{b}	0.0997 ± 0.0062^{b}	0.1850 ± 0.0151^{b}
Phosvitin peptides				
Low	0.1262 ± 0.0041^{b}	0.3098 ± 0.0194^{a}	0.1110 ± 0.0058^{a}	0.2118 ± 0.0115^{a}
Med	0.1328 ± 0.0049^{a}	0.3068 ± 0.0221^{a}	0.1115 ± 0.0058^{a}	0.2198 ± 0.0226^{a}
High	0.1370 ± 0.0035^{a}	0.3165 ± 0.0164^{a}	0.1171 ± 0.0043^{a}	0.2285 ± 0.0124^{a}

Values within a column with different superscripts are significantly different at $p < 0.05$ by Duncan's multiple-range test.

phosvitin peptides groups (536.05–559.72 mg) (Table 6). Contents of both ash (175–189 mg:166 mg) and Ca (51.0–55.3 mg:46.0 mg) in tibias of phosvitin peptides groups were significantly higher than those of the control group. Similar results from femurs were also observed (Table 7). Among various parameters, ratios of Ca/bone weight and Ca/bone ash directly provide information on efficiency of Ca incorporation into bones. As shown in Tables 6 and 7, phosvitin peptides groups had higher Ca/bone weight and Ca/bone ash ratios in tibia as well as femurs than those of the control group.

The effectiveness of phosvitin peptides in enhancing incorporation of Ca into bones was further confirmed by measuring BMD and BMC of femurs and tibias in each group (Table 8). As expected from the previous results, BMD and BMC of both tibias and femurs were significantly higher in phosvitin peptides groups. Although there seemed that no significant difference existed in BMD and BMC values among phosvitin peptides-fed groups, average BMD and BMC values were getting higher as concentrations of phosvitin peptides in the diets were increased from 0.125% to 0.5%. These results indicated that addition of phosvitin peptides at 0.125–0.5% into diets increased Ca absorption and bone mineral densities in rats in a dose-dependent manner.

Phosvitin is polyanionic phosphoglycoprotein that can bind multivalent metals, such as Fe, Ca and Mg. In addition, this highly phosphorylated protein may have a potent affinity for lipid, as egg yolk has often been used as a natural emulsifier. Therefore, most studies on phosvitin have been focussed on its powerful emulsifying properties in which even antimicrobial potential for Gram-negative bacteria is included (Khan, Babiker, Azakami, & Kato, 1998; Khan et al., 2000). Jiang and Mine (2001) reported that phosvitin peptides, which were prepared by tryptic hydrolysis of phosvitin, enhanced Ca-binding capacity and inhibited the formation of insoluble Ca phosphate. We also proved the excellent Ca-solubilizing abilities of phosvitin, as well as phosvitin peptides, in this study. In addition, as shown through the animal study, we found that the diets fortified with phosvitin peptides significantly enhanced Ca incorporation into bones. This information seems to be valuable in respect to future application of egg phosvitin as a functional food ingredient.

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