

Short Communication

Exploring the potential of calcium-fortified sweet potato noodles for osteoporosis prevention: Insights from in vivo rat studies

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Abstract

Calcium (Ca) deficiency is a primary contributor to osteoporosis, a condition characterized by low bone density and increased fracture risk. Fortifying widely consumed foods with calcium is one approach for addressing this insufficiency. Given the popularity of noodles, adding Ca to them offers a promising approach to enhancing Ca intake within communities. The aim of this study was to investigate the effects of Ca-fortified sweet potato noodles on osteoporosis prevention. This study used 4-month-old *Sprague-Dawley* rats. A completely randomized design was used with four treatment groups: CS (SHAM, control diet), CO (Sham, test diet), US (OVX, control diet), and UO (OVX, test diet). Both control and test diets, which included Ca-fortified sweet potato noodles, were administered to OVX (ovariectomy-induced osteoporosis model) and SHAM (control for surgical procedure) groups for two months. At the end of the experiments, serum Ca levels were collected and analyzed for Ca and alkaline phosphatase (ALP) levels and their bones were analyzed for physical properties and bone mineral density (BMD) using X-ray analysis. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan as a post hoc test. The intervention of Ca-fortified sweet potato noodles for two months significantly increased serum Ca levels and reduced ALP levels compared to controls, both in SHAM (Ca: 38.03 ± 0.877 mg/dL; ALP: 355 ± 38.0 IU/L) and OVX (Ca: 36.18 ± 2.810 mg/dL; ALP: 340 ± 5.5 IU/L) groups. The test diet maintained the ratio of bone weight to bone volume and preserved the Ca content in the rats' bones. OVX rats consuming the test diet for two months exhibited significantly higher femur bone strength than OVX rats consuming the control diet (test: 6.50 ± 0.300 kg; control: 4.83 ± 0.289 kg). There was no significant difference in BMD between the SHAM and OVX groups on the test diet, indicating that the test diet can maintain bone BMD despite accelerated aging. These findings suggest that Ca-fortified sweet potato noodles can serve as a dietary Ca source, contributing to the prevention of osteoporosis by maintaining serum Ca levels, preserving bone Ca content, as well as maintaining bone density and strength.

Keywords: BMD, Ca fortification, ovariectomy, rat, sweet potato noodle

Introduction

Osteoporosis is a common disorder among the elderly, characterized by low bone density and increased fragility, which increases the risk of fractures. Recovery of fractures can be accelerated by implants [1]. This disorder primarily affects postmenopausal women and the elderly [2,3]. This



condition often becomes a serious public health problem due to its impact on the quality of life and high healthcare expenditures related to its treatment [4,5]. Osteoporosis is also known as a silent disease because the symptoms are not specific, making it difficult to detect early [6]. Globally, the prevalence of osteoporosis is predicted at 19.7% of the population, with developing countries having a higher prevalence (22.1%) compared to developed countries (14.5%) [7]. Preventing and managing osteoporosis often involves pharmacologic treatments, nutritional supplementation, and lifestyle changes to improve bone health. Among these strategies, Ca intake plays a critical role in maintaining bone strength and avoiding bone loss [5].

Calcium is an essential mineral for maintaining bone density and preventing osteoporosis. It can be found naturally in a variety of foods and can also be added to food products through fortification. Natural sources of Ca include milk and dairy products, fish, meat, and nuts [6]. Fortified foods such as Ca-enriched milk [8], rice [9], bread, and biscuits [10] as recommended by the Recommended Dietary Allowances (RDA). Fortifying popular foods with Ca can help increase people's Ca intake. Noodles are among the most popular foods consumed worldwide. According to the World Instant Noodles Association, global instant noodles consumption is expected to reach 121.2 billion servings in 2022, with the trend continually increasing [10].

Sweet potatoes are another popular food that is high in carbohydrates (65.41% dry basis) [12]. In addition to carbohydrates, sweet potatoes also contain several nutrients such as protein, fiber, vitamins, and minerals [13-15]. Orange-fleshed sweet potatoes contain bioactive compounds, including flavonoids, phenolic acids, anthocyanins, and beta-carotene, which can help address vitamin A deficiency [16]. Beta-carotene also serves as a natural food colorant [12]. The starch in sweet potatoes contributes to creating a pleasant texture in food products. These characteristics make sweet potatoes an ideal choice for use as an ingredient in noodle production. Sweet potato noodles without Ca fortification contain around 15.45 ± 1.12 (mg/100 g) of Ca [17]. Sweet potato noodles can be fortified with Ca during production to enhance their nutritional value and combine the health advantages of Ca with the bioactive chemicals found in sweet potatoes.

Ca-fortified sweet potato noodles are a promising approach to manage Ca deficiency, especially in populations at risk for osteoporosis. Factors such as bioavailability, reactivity, price, and toxicity need to be considered in the selection of fortifiers. Among these, Ca citrate is one kind of fortifier that is more effective than Ca carbonate, as it has high solubility and bioavailability, even in low-acid environments. This makes it especially beneficial for populations like postmenopausal women, whose Ca absorption may decrease due to low stomach acid levels [18]. The formation of Ca-citrate complexes prevents Ca salt precipitation and helps stabilize apatite nanocrystals in bone, which is important for biomineralization [19].

This study involved two groups of rats, the ovariectomy (OVX) group that simulated postmenopausal bone loss and the SHAM group as a control. The OVX treatment in this study was designed to induce osteoporosis through decreased estrogen production, resulting in decreased Ca absorption. The sham treatment (SHAM) is designed to expose the rats to the same stress so that the effects on the rats are due to differences in the intervention. In vivo, studies that comply with ethical clearance permits produce valid data and reduce animal pain [20,21]. Therefore, the aim of this study was to evaluate the efficacy of Ca-fortified sweet potato noodles in enhancing bone health in a rat model of osteoporosis.

Methods

Production of sweet potato noodles

Sweet potato noodles were made by blending sweet potato flour (85%) and sago starch (15%) to make a dry dough, followed by Ca fortification. First, a 1% salt solution was prepared by dissolving salt in water equal to 40% of the dry dough's weight. This salt solution was slowly added to the dry dough until the mixture was homogeneous. The moist dough was then processed into an extruder (Forming-Cooking Extruder type LE 25-30/C, Thailand) with a screw speed of 130 rpm and a barrel extrusion temperature of 95°C. After extrusion, the noodles were dried at 40°C for 5 hours [22]. After that, the fortification process was done with 30% Ca citrate added to fortify the diet as the RDA. The Ca was then gradually mixed into the dry dough until completely homogeneous. This was done by adding Ca to 1/5 of the dry dough and mixing until

homogeneous, then adding another 1/5 of the dry dough and mixing. This process was repeated until all of the dry dough was thoroughly mixed with the Ca.

Study design of in vivo experiment

The experimental animals used in this study were female *Sprague Dawley* rats, aged four months and weighing approximately 200 g. This study involved two groups of rats: the ovariectomy (OVX) group as a model for postmenopausal bone loss, and the SHAM group as a control. The OVX treatment was used to induce osteoporosis through decreased estrogen production, resulting in decreased Ca absorption. The SHAM group (SHAM) had a similar surgical procedure to confirm that any observed effects were purely due to the differences in the intervention, not procedural stress. Twelve rats were ovariectomized, while twelve others underwent SHAM treatment. After the surgery, the rats were given a three-week recovery period and a one-week adaptation period before starting the diet intervention. The intervention period in the rats was carried out for two months, starting when the rat was four months old.

This study used a Completely Randomized Design (CRD), which included four treatment groups: CS (control diet, SHAM), CO (control diet, OVX), US (test diet, SHAM), and UO (test diet, OVX), each group containing six rats. The feed was provided *ad libitum* in pellet form at approximately 20 g/day. The control diet formula followed AIN-93M standards, while the test diet formula was a modified version of AIN-93M by replacing the starch source with Ca-fortified sweet potato noodles. Rats were housed in a pollution-free laboratory under controlled conditions, with a room temperature of 21–24°C, a 12-hour light/12-hour dark cycle, 50% relative humidity, and adequate ventilation [23]. Rat cages were coated with wood shavings and changed every two days to keep the cage bedding dry. Data were collected at baseline (time 0), one month after the intervention, and at the end of the two-month intervention period.

Female rat ovariectomy and SHAM surgery

Ovariectomy surgery was performed to reduce estrogen levels, thereby inducing a decrease in bone density. This surgery was carried out aseptically by dissecting the midline part of the rat. Before surgery, the rats were sedated with an intraperitoneal injection of 2% xylazine (5 mg/kg body weight) and 10% ketamine (50 mg/kg body weight). The rat's abdomen was then shaved and cleaned with alcohol and betadine. During the procedure, the uterus and ovaries were removed, and the incision was then sutured [23]. During the recovery period, analgesics (ketoprofen) and antibiotics (amoxicillin) were given for seven days. SHAM surgery was performed to ensure that all groups of rats experienced similar surgical stress, making any differences between groups attributed to the effect of the intervention. SHAM surgery was performed aseptically, using the same procedure as the ovariectomy, except that the ovaries and uterus were not removed. During the recovery period, the rats were fed a standard diet. After that, the rats began an adaptation period in a metabolic cage. During this period, both SHAM and OVX groups were divided into two subgroups: one received the control diet (standard diet), and the other received the test diet (standard diet supplemented with Ca-fortified sweet potato noodles). During the adaptation period, the control diet group will continue to receive the standard diet, while the test diet group will gradually switch from the standard diet to the test diet to ensure proper adaptation. Following the adaptation period, a two-month intervention phase was initiated, during which the dietary treatments were continued. Blood and bone samples were collected from rats.

Blood samples were collected from rats through the cardiac puncture without adding an anticoagulant agent [24]. After that, the blood samples were allowed to clot at room temperature at 25°C for about 30 minutes. The serum was separated from the red blood cells by centrifugation (3,000 rpm, 10 minutes), and then stored at -20°C until analysis. Bone samples were collected using surgical procedures. Surgery was carried out through an incision to expose the femur, which was carefully removed. The femur bone was then cleaned and rinsed to remove any connected muscles. The bone was soaked in a saline solution to remove any remaining muscle residues. After that, the right femur bone was dried at room temperature. Blood and bone samples were collected at three different times: before treatment began (baseline), at the end of the first month (midpoint of intervention), and at the end of the second month (end of intervention) [24].

Measurement of calcium (Ca) and alkaline phosphatase (ALP) levels in the serum of rat

Ca levels in serum were measured using an automatic autoanalyzer (Mindray BA-88A, China) by adding a reagent kit (Calcium Arsenazo III, Linear Chemicals, Spain). The absorbance was measured at a wavelength of 650 nm. Furthermore, the same instrument was used to measure ALP levels in serum. A reagent kit (Alkaline Phosphatase BR, Linear Chemicals, Spain) was added to the serum. After that, the absorbance was measured at a wavelength of 405 nm [25].

Measurement of rat bone size

Bone length, width, and thickness were measured using a micrometer screw and caliper. Moreover, weight was measured using a scale, and bone volume was measured using a syringe [26]. The cleaned femur bones of rats were inserted into an open 5 ml syringe filled with ion-free water. The syringe was placed in a desiccator (vacuum chamber) for 90 minutes to remove air bubbles and allow trapped air to escape. Subsequently, bone volume was determined by measuring the increase in volume after inserting the bone into the syringe.

Measurement of bone mass density and bone strength

Bone mass density was calculated by dividing bone weight by bone volume. Bone strength was measured using a hardness tester (KIYA SEISAKUSHO. LTD, Tokyo Japan). The bone was placed on a work table, and the indenter was lowered until the bone broke. The force required to break the bone was indicated on the dial of the hardness tester [25].

Analysis of X-ray absorption and bone mineral density (BMD) in rat bones

Bone mineral density (BMD) was measured using X-ray absorption with the Mobile X-ray system POX-100 BT (POSKOM Co., Ltd. Korea) with settings of 55 kV potential, 80 mA current, and 3.20 sec/mAS. Measurements were taken at the neck, diaphysis, and metaphysis of the femur. In this study, BMD was assumed to be proportional to X-ray absorption [27].

Data analysis

Data were presented in mean with standard deviation (SD). Data were analyzed one-way analysis of variance (ANOVA) test using the SPSS version 25 followed by Duncan's post hoc test with a confidence level of 95% ($p < 0.05$).

Results

Serum Ca levels

Levels of serum Ca ranged from 32.73 ± 1.25 to 38.03 ± 0.87 mg/dL, as presented in **Figure 1A**. The group that received the test diet (US, UO) had Ca levels that did not differ significantly from the baseline group ($p > 0.05$), as presented in **Figure 1A**. In contrast, the groups that received the control diet (CS, CO) showed significantly lower Ca levels compared to baseline ($p < 0.05$). During the two-month intervention period, there was no significant difference in blood Ca levels in the OVX test diet group compared to the SHAM test diet and baseline groups ($p > 0.05$). Additionally, there was no significant difference in blood Ca levels between the SHAM and OVX groups within the control diet group ($p > 0.05$).

Serum ALP levels

The ALP levels in the SHAM and OVX groups that received test diet intervention had levels similar to those of the baseline group ($p > 0.05$) in both the control diet and test diet groups (**Figure 1B**). After a two-month intervention, ALP levels were significantly higher in the control diet group (SHAM: 588 ± 97 IU/L; OVX: 622 ± 15 IU/L) compared to baseline groups ($p < 0.05$), which could be due to increased Ca turnover in the bone. However, in this study, SHAM and OVX treatments did not affect ALP levels, as indicated by the fact that ALP values did not significantly differ between SHAM and OVX in each group diet ($p > 0.05$).

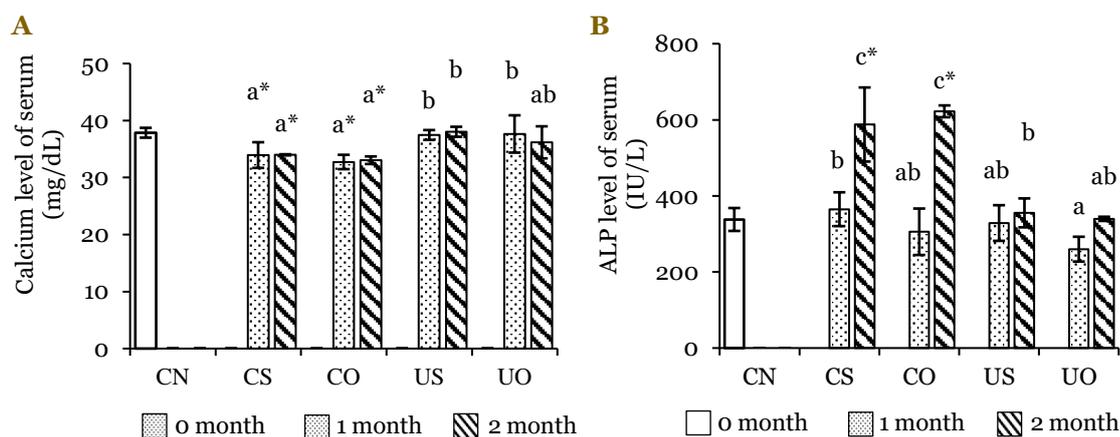


Figure 1. A) Serum calcium (Ca) levels and B) serum alkaline phosphatase (ALP) levels. Value: mean \pm SD; n=3. Data with different superscripts indicate significant differences ($p<0.05$) between groups (CS, CO, US, UO). Data with * indicates significantly different from the baseline ($p<0.05$). CN: baseline; CO: ovariectomy (OVX) with control diet; CS: SHAM with control diet; UO: OVX with test diet; US: SHAM with test diet.

Size of rat bones

After two months of intervention, substantial changes in the size of femur bones were found between groups, as presented in **Table 1**. The results showed that the OVX group had a higher bone volume compared to the SHAM group ($p<0.05$), both in groups fed with control and test diets. Similar results were also observed in the length and width of bones. In the control diet group, differences in bone volume were not accompanied by differences in bone weight. In the control diet group, there was no significant difference in bone weight between the SHAM group (0.51 ± 0.03 g) and OVX group (0.54 ± 0.03 g) ($p>0.05$), both at one and two-month intervention. In contrast, in the test diet group, there was a significant difference in bone weight, with the SHAM group (0.51 ± 0.035 g) having a lower bone weight compared to the OVX group (0.56 ± 0.017 g) ($p<0.05$).

Table 1. Measurement results of rat femur bone size (number of repetitions=3)

Code	Duration (month)	Bone volume, mean \pm SD (mL)	Bone length, mean \pm SD (mm)	Bone width, mean \pm SD (mm)	Bone thick, mean \pm SD (mm)	Bone weight, mean \pm SD (g)
CN	0	0.40 \pm 0.00	32.18 \pm 0.13	4.25 \pm 0.04	3.29 \pm 0.09	0.46 \pm 0.03
CS	1	0.43 \pm 0.06 ^a	33.42 \pm 0.89 ^a	4.26 \pm 0.03 ^a	3.54 \pm 0.24 ^a	0.49 \pm 0.02 ^a
	2	0.43 \pm 0.06 ^a	34.42 \pm 0.73 ^{ab*}	4.12 \pm 0.69 ^a	4.49 \pm 0.25 ^{d*}	0.51 \pm 0.03 ^{ab}
CO	1	0.50 \pm 0.00 ^{ab*}	35.48 \pm 0.80 ^{bcd*}	5.00 \pm 0.26 ^{bc*}	3.80 \pm 0.16 ^{abc}	0.55 \pm 0.01 ^{bcd*}
	2	0.53 \pm 0.06 ^{b*}	35.97 \pm 0.84 ^{cd*}	5.31 \pm 0.05 ^{c*}	4.38 \pm 0.45 ^{cd*}	0.54 \pm 0.03 ^{abcd*}
US	1	0.43 \pm 0.06 ^a	34.57 \pm 0.48 ^{abc*}	4.56 \pm 0.10 ^{ab}	3.72 \pm 0.10 ^{ab}	0.50 \pm 0.03 ^a
	2	0.43 \pm 0.06 ^a	34.18 \pm 1.27 ^{ab*}	4.03 \pm 0.60 ^a	4.30 \pm 0.57 ^{bcd*}	0.51 \pm 0.03 ^{ab*}
UO	1	0.50 \pm 0.00 ^{ab*}	36.52 \pm 0.38 ^{d*}	5.23 \pm 0.37 ^{bc*}	4.15 \pm 0.35 ^{abcd*}	0.57 \pm 0.02 ^{d*}
	2	0.53 \pm 0.06 ^{b*}	36.18 \pm 0.60 ^{d*}	5.29 \pm 0.19 ^{c*}	4.26 \pm 0.27 ^{bcd*}	0.56 \pm 0.02 ^{cd*}

CN: baseline; CO: OVX with control diet; CS: SHAM with control diet; UO: OVX with test diet; US: SHAM with test diet

*Significantly different from the baseline ($p<0.05$)

Data in one column with different superscripts indicates differences significantly between groups ($p<0.05$)

Density and strength of bone

The density showed that there was no significant difference among groups with the baseline group ($p>0.05$), as presented in **Figure 2A**. Furthermore, there were no significant differences observed between the SHAM and OVX groups ($p>0.05$). After two months of intervention, the SHAM test diet group showed the highest bone density trend (1.18 ± 0.08 g/cm³). The femur bone strength of rats ranged from 4.83 ± 0.28 to 7.47 ± 0.115 kg (**Figure 2B**). Changes occurred in the femur bone after a two-month intervention. Compared to the baseline, the femur bone strength in the OVX control diet group was significantly lower ($p<0.05$). Meanwhile, in the SHAM group, both the test and control diets showed higher bone strength ($p<0.05$). The OVX test group did not differ significantly ($p>0.05$) compared to the baseline. After two months of intervention,

the OVX group had significantly lower femur strength than the SHAM group ($p < 0.05$). This study shows that the OVX group who received the test diet significantly had higher femur strength compared to OVX rats who received the control diet ($p < 0.05$).

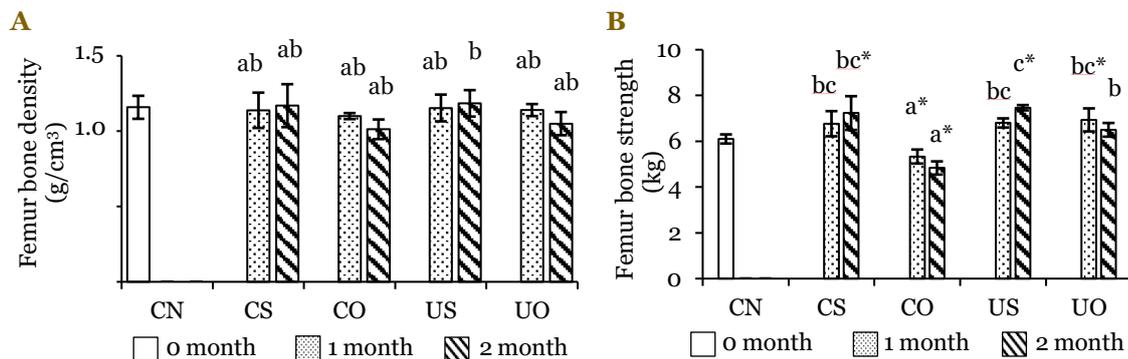


Figure 2. (A) Femur bone density and (B) femur bone strength. Value: mean \pm SD; n= 3. Data with different superscripts indicate significant differences ($p < 0.05$) between groups (CS, CO, US, UO). Data with * indicates significantly different from the baseline ($p < 0.05$). CN: baseline; CO: ovariectomy (OVX) with control diet; CS: SHAM with control diet; UO: OVX with test diet; US: SHAM with test diet.

Bone mineral density of rat

BMD values ranged from 56.18 ± 3.69 to 84.79 ± 5.56 AU, as presented in **Figure 2A**. At the end of the two-month intervention, the OVX control diet had the lowest BMD values (56.18 ± 3.69 AU), whereas the SHAM test diet group had the highest BMD values (84.79 ± 5.563 AU). In the OVX group, the test diet group (81.391 ± 2.251 AU) had significantly higher BMD values compared to the control diet group (56.185 ± 3.693 AU) ($p < 0.05$). These results indicated that both the SHAM and OVX test diet groups had BMD values that were not significantly different ($p > 0.05$).

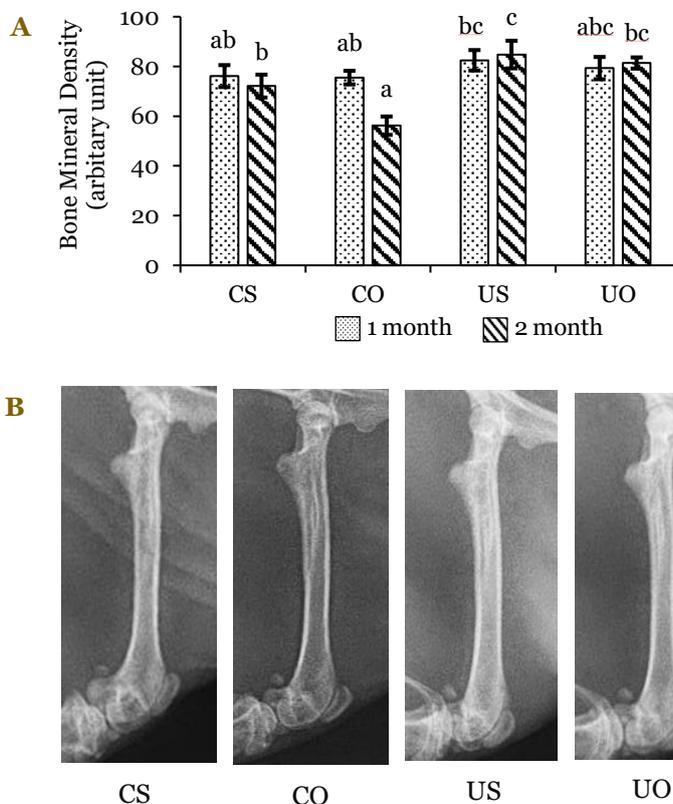


Figure 3. A) Bone mineral density (BMD) of rats and B) X-ray photos of the femur bones. Value: mean \pm SD; n=3. Data with different superscripts indicate significant differences ($p < 0.05$) between groups (CS, CO, US, UO). CO: ovariectomy (OVX) with control diet; CS: SHAM with control diet; UO: OVX with test diet; US: SHAM with test diet.

Discussion

The findings of this study indicated that the test diet effectively maintained serum Ca levels in osteoporosis rats. This finding is consistent with the study by Nakada *et al.*, which found that Ca citrate intervention had positive effects on the increase of serum Ca levels [28]. The absence of significant differences in serum Ca levels between SHAM and OVX groups within the control diet could be due to the body's ability to manage Ca homeostasis via mechanisms such as absorption, excretion, secretion, and Ca storage processes in bones. It is difficult to use serum Ca levels as an indicator of osteoporosis because the body naturally maintains blood Ca levels within normal concentrations [29]. Ca citrate supplements offer several advantages. Its higher solubility and bioavailability than Ca carbonate make it an efficient Ca maintainer. Ca citrate creates a soluble complex formation between Ca and citrate that prevents precipitation of Ca salts when the intestinal pH increases, resulting in more effective absorption. Furthermore, citrate also plays an important role in stabilizing apatite nanocrystals in bone which is necessary for bone biomineralization.

ALP is an enzyme that helps break down phosphate in cells for various biological functions, including bone formation. To encourage bone mineralization, ALP biomarkers help in the hydrolysis of pyrophosphate and give inorganic phosphate. Increased ALP levels indicate increased bone remodeling because of a negative balance in bone resorption [30]. In this study, the test diet effectively maintained bone turnover conditions. Conversely, after two months of intervention, the control diet group showed increased bone Ca turnover, as evidenced by elevated ALP levels. Surprisingly, there were no significant differences in ALP between the SHAM and OVX treatments in each group. This could be because serum ALP is not only derived from bones but also from other organs such as the liver, intestines, and kidneys. Therefore, ALP has been used as a biomarker for various diseases, including liver disease and brain damage [31]. Although ALP levels in the test diet group increased with age, the difference was not statistically significant, indicating that the diet was successful in reducing age-related increases in bone turnover. In the OVX groups, ALP levels after two months of intervention demonstrated that the test diet group had considerably lower ALP concentrations than the control diet group. This finding shows the test diet's ability to decrease excessive bone remodeling in estrogen-deficient situations, ultimately supporting better bone health.

The findings indicated that the OVX group had a higher femur bone volume than the SHAM group. Similar results were found in femur length and width measures, indicating that OVX treatment affects bone dimensions. In the control diet group, significant differences in femur bone volume between SHAM and OVX were not accompanied by differences in femur weight. In contrast, in the test diet group, differences in femur bone volume between SHAM and OVX were accompanied by corresponding changes in femur weight, indicating that the test diet may influence bone composition and mineral deposition. Interestingly, bone density, calculated as the ratio of the femur weight to its volume, remained consistent across all groups and did not differ from the baseline. This suggests that the intervention may have preserved bone density despite structural changes, particularly in the OVX groups.

The test diet intervention could maintain femur strength in the osteoporosis rat model, as indicated by no decrease in bone strength compared to the baseline group. The OVX treatment, which is often used to imitate osteoporosis, significantly reduced femur strength with its known effect on bone health. After a two-month intervention, the OVX group had lower femur strength than the SHAM group, which may be the effect of estrogen deficiency. A study by Quintero-García *et al.* showed that ovariectomy significantly reduces estrogen levels in experimental animals. Estrogen is a hormone that plays a crucial role in stimulating active Ca transport in the intestines [26]. Decreased levels of this hormone can disrupt Ca absorption in the intestines, thereby triggering osteoporosis [26]. In the OVX group, the test diet resulted in stronger femurs compared to rats that received the control diet. Ca citrate positively impacts bone strength [24]. Decreased bone mass and microarchitectural damage can result in bone fragility and fractures [32].

The results of bone density measurements showed no significant differences between groups. However, in bone strength testing, some groups showed significant differences. The group of OVX rats fed the test diet had significantly higher femur strength than the OVX rats fed the control diet. This suggests that even slight differences in bone density will have a large effect

on bone strength. After two months of intervention, bone Ca measurements in the OVX group on the control diet showed a decrease in bone Ca content, but there was an increase in bone strength in this group. This shows that bone strength is not only influenced by the presence of Ca in the bone. Some other bone building blocks, such as protein, phosphorus, and magnesium, also play a role in bone strength.

The femur is one of the most prominent bones in the body that bears the heaviest weight loads [33]. Many osteoporosis studies focus on the femur bone because fractures in that area are common in humans, and the histological structure of rat femur bones is similar to that of humans. Observations of loss in the femur neck area of rats after OVX are similar to those in human hip bones [23]. In bone density measurements, differences between groups were not significantly observed. However, several groups showed significant differences in the bone strength test. This indicates that even small differences in bone density can significantly affect bone strength.

Compared to the control diet intervention, the test diet intervention was more effective in maintaining femur BMD in rats that had undergone accelerated aging due to OVX treatment. The test diet maintained femur BMD in rats under both non-accelerated and accelerated aging conditions. Additionally, the test diet helps preserve BMD in rats, improving overall bone health. Ca citrate intervention positively affects BMD values [34]. The longer the duration of Ca citrate intervention, the more significant its effect on BMD values, thereby helping to prevent osteoporosis [34].

After two months of intervention, the test diet can maintain the ratio between rat bone weight and bone volume in the femur bone measurements. The test diet can maintain femur bone strength despite the OVX treatment, this is indicated by bone strength values that are not significantly different from the SHAM control diet and baseline groups. In the OVX group, the test diet intervention significantly enhances strength compared to the control diet. Even slight differences in bone density can significantly impact bone strength. Feeding the test diet to the OVX group has BMD values that are not significantly different from the SHAM test diet group, indicating that the test diet can effectively maintain rat BMD values.

For a more comprehensive study, the intervention period of the product on rats can be extended to see the trend resulting from the intervention of the Ca-fortified sweet potato noodles. In order for this product to be ready for mass production and marketing in the community, some additional analysis is needed. Economic value calculations are also needed to provide insight into production costs and acceptable market prices. Fortification with other nutrients, such as vitamin D, which can help the body absorb Ca, can also be added to increase nutritional value.

Conclusion

Sweet potato noodles fortified with Ca have the potential to serve as Ca-rich food and contribute to the prevention of osteoporosis. The intervention of sweet potato noodles fortified with 30% AKG Ca citrate in ovariectomized rats can maintain the concentration of Ca and ALP in blood serum. This product can also maintain the Ca content in the bones of rats that experience decreased estrogen due to ovariectomy treatment. The femur bones of rats given Ca-fortified sweet potato noodles can maintain the strength of the femur bones despite ovariectomy treatment. Notably, giving this product to rats increased bone strength compared to the control diet. This product can also maintain bone mineral density (BMD) in rats. The results of this test conclude that sweet potato noodles fortified with 30% AKG Ca citrate can support Ca balance in the body and prevent osteoporosis associated with aging.

Ethics approval

The research protocol was approved by the Animal Ethics Committee of the IPB School of Veterinary Medicine and Biomedicine No: 043/KEH/SKE/X/2022.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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