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*Research Article*

# Effect of calcium compound obtained from fish by-product on calcium metabolism in rats

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## Abstract

Calcium compound was prepared by sintering muscle-free fish bone of *Priacanthu tayenus* at $1300^0$C. Four groups of six male Wistar rats were fed a basic diet with marginal calcium content for seven weeks. Three of these groups received the sintered calcium compound as a supplement to gain 11, 22, or 44 mg Ca/day. The fourth group was used as control. Effects of the sintered calcium compound on metabolism of calcium in rats were monitored. Supplementation of calcium increased mean body weight of rats whereas a 9% loss of mean body weight of the control group was observed by week seven. Absorption and retention rate of the supplemented calcium increased significantly ($p<0.05$) with increase of calcium intake. Femur weight of rats was increased by increasing the amount of calcium supplement with respect to that of the control rats. In contrast, the femur length of the rats was shortened following feeding with the sintered calcium compound, compared with the length of the control rats. Abnormal trabecular conformation was noticed in the control rats whereas thick and narrow inter-trabecular spaces were observed in the calcium supplement groups. The results thus revealed that high bioavailability of calcium compound could be prepared by sintering process from fish bone.

**Keywords:** sintering, *Priacanthu tayenus,* food waste, waste recovery, dietary supplement, osteoporosis, Thailand.

## Introduction

A considerable amount of the total marine fish catch is discarded as processing leftovers, including trimming, fins, bones, head, skin and viscera. One estimate suggests that current discards from the world's fisheries exceeds 20 million tons, equivalent to 25% of the total production of marine capture fisheries [1]. Fish bones are the main solid by-product of the frozen fish processing industry. The bones account for 25-30% of fish weight.

Inadequate intake of calcium in the human diet is one factor in the etiology of several disorders [2]. Adequate calcium intake during growth is critical to the achievement of peak bone mass that may reduce the risk of osteoporosis [3]. Osteoporosis has become an important degenerative disease in the world especially in Asia. Osteoporosis related fractures can occur in any of the bones, but mainly occur at the hip. In Thailand, an epidemiological survey has indicated that the incidence of hip fracture is 162/100,000 population over the age of 50 [4]. Nutrition interventions to increase calcium intake are the consumption of high calcium food and the use of calcium supplements.

In Thailand, most of the fish waste generated from processing of frozen fish fillets is used for animal feed production. However, with regard to its chemical composition, fish bone could be transformed into a high value product. For instance fish bone phosphopeptide with high affinity to calcium had been isolated from hoki (*Johnius belengerii*) skeletons [5]. Microcrystalline hydroxyapatite has been successfully prepared from fish bone by sinter treatment [5, 6]. Hydroxyapatie is the principle calcium compound existing in bone and is high in calcium. Bioavailability of this compound has been proposed [7, 8, 9]. To date, there has been no study aimed at clarifying the metabolism of hydroxyapatite derived from fish bone. Thus, the objective of this study was to evaluate the bioavailability of calcium compound from fish bone and to compare the effect of supplement levels.

## Materials and Methods

### *Materials*

Bones from purple-spotted bigeye (*Priacanthus tayenus*) were collected as a by-product from a local frozen fish processing plant in Songkhla, Thailand. All chemicals and reagents were food grade.

### *Calcium compound processing*

The fish bones or skeletons were manually cleaned to remove muscle residue. Calcium compounds were produced from the cleaned fishbone by sintering at 1300ºC for 1 hour. The sintered compounds obtained were composed of calcium triphosphate and calcium hydroxyapatite as measured by XRD.

### *Animals and diet*

Male Wistar rats weighing about 200-230 g were purchased from the Laboratory Animal Centre of the Science Faculty, Prince of Songkla University, Thailand. They were housed at the Primate Research Unit of the university in a room at 23 $\pm$ 2°C with 12-h light/12-h dark cycles. The rats were fed with a standard rat diet (C. P. 082, Lot No. 17, S. W. T. Co., Ltd, Thailand) during two

weeks in this habitat. During the experimental period, rats were fed with the basic diet, AIN-93M, advised by NRC [10]. Water was supplied *ad libitum*. Before commencing the experiment, the experimental protocol was approved by the Animal Ethical Committee in accordance with the guidelines for care and use of laboratory animals prepared by Prince of Songkla University.

***Experiment protocol***

Weaned 5-week-old male rats were randomly divided into 4 groups of 6 animals/group and fed with the basic diet. The calcium compounds were mixed with Gum Arabic with a calcium/gum ratio of 1:1 (w/w). The mixture was prepared freshly and administered orally to the rats to receive a calcium supplement of 0, 11, 22 or 44 mg calcium/day for 7 weeks. Animals were allowed tap water *ad libitum*.

Body weight of rats was monitored on a daily basis. During the last week each group of rats was placed individually in metabolic cages to collect urine and fecal samples for a 24-hour period. Fecal weight and urine volume were measured and stored at 4ºC until used for analysis. At the end of the experiment, rats were sacrificed by cervical dislocation and thereafter blood of each rat was collected. Their femurs were de-fleshed from adjacent tissues, wrapped in saline-soaked gauze bandages to prevent dehydration and stored frozen at -20°C in small Ziploc freezer bags until the histopathological features were measured.

***Analysis***

*Calcium adsorption and absorption*

Calcium content of basic feed, feces and urine were measured by inductive coupled plasma atomic emission spectrometer (PERKIN'ELMER, Analytical Instrument Co.) according to the method described by Yoon [11]. Calcium concentration was presented as $\mu g\ g^{-1}$ (ppm) of tissue on a dry weight basis. Analytical limits of detection were determined as 0.01 $\mu g\ g^{-1}$ dry weight. Calcium adsorption was calculated by subtraction of total calcium in fecal samples from total calcium intake according the method of Choi [12]. The absorption rate of calcium was calculated according to the method described by Cui [13], while retention rate of calcium was calculated according to the method described by Alam [14].

*Femur weight and length*

Femoral lengths were measured with a caliper made in England and bones weighed on a precision balance made by China Instrument Works.

*Bone histology*

After scarification, the right femur of the rats was selected for histopathological study according to the method of Miao and Scutt [15] with slight modification. The bones were de-fleshed and placed in 10% (w/v) phosphate-formalin buffer for at least 72 hours. The cleaned flesh-free bone was cut to a small size and then decalcified in EDTA-G solution (EDTA disodium salt 14.50 g, NaOH 1.25 g, glycerol 15 ml and distilled water 100 ml) for 3 weeks by changing the solution every week. After three weeks, the decalcified bone was dehydrated in a series of ethanol gradients and clearing in xylene. It was then embedded in paraffin, cut into sections of 5 $\mu$m thickness and stained with Hematoxylin and Eosin. The slides were analyzed under a light microscope (ZEISS: Axiostar plus) and photographed using a digital camera (SONY: DSC-S85).

*Statistics*
Experimental values are presented as the mean ± SD. Number of experiments was indicated in the legends as appropriate. Significance was assessed by using Oneway –ANOVA ($P<0.05$ as significant).

## Results and Discussion

### *Effect of sintered calcium compound on body weight of Wistar rats*

Figure 1 shows the weekly mean body weight of rats in each feeding regime over the seven weeks of the feeding period. The average body weight of rats receiving the basic feed and without a calcium supplement were not significantly different during the first four weeks. Thereafter, a decrease in the average body weight was observed. By the seventh week, body weight was about 9% lower than the initial value. Since calcium content of the basic feed was 2.64 mg/kg, thus habitual feed intake (22 mg/day) would provide calcium equivalent to 12% of required calcium/day [10]. The insufficient intake of calcium might cause abnormal growth of rats as observed by the decrease in body weight. In contrast to the control group, rats receiving the calcium supplement showed an increase in average body weight from the first week of the experiment. By the end of feeding period the average body weight of rats receiving calcium supplement of 11, 22 or 44 mg/day were increased by about 7.77 %, 10.4% and 9.85% from their initial average body weight, respectively. However, there is no significant difference in the average body weight among the treated groups receiving different levels of calcium supplement. Thus, the results revealed, at least partially, that calcium supplement had not drastically affected the growth of rats.

These findings were in line with other research mentioning the abnormal growth of rats receiving an appropriate amount of calcium. For instance, rats fed with low calcium (10 mg/d) showed inactivity and the hair lacked luster and consequently became coarse [16]. Whereas, overfeeding of calcium (1.8% of feed) caused less development as measured by gaining of body weight compared to development of rats receiving lower calcium content (0.2% or 0.6% of feed) .

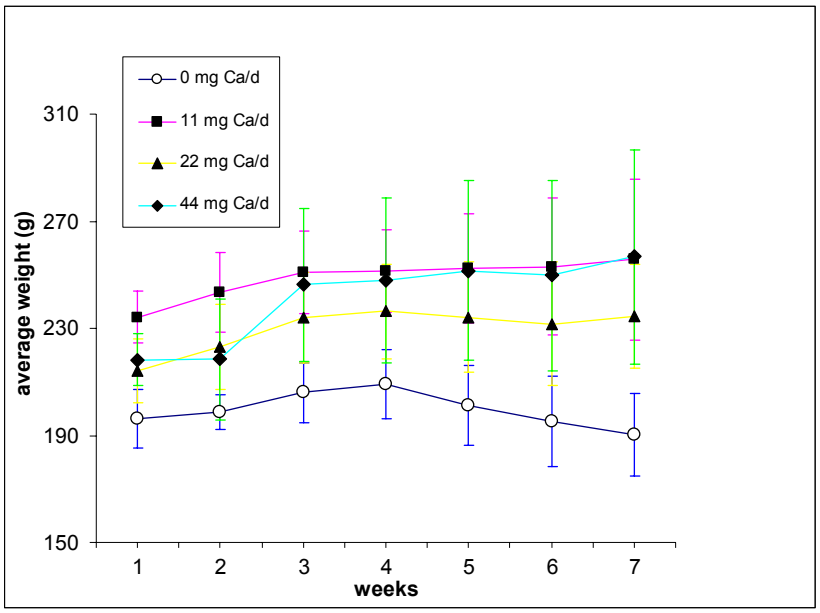


**Figure 1. Average body weight of male Wistar rats fed with different levels of calcium supplement.**

***Effect of sintered calcium compound on absorption and retention rate in Wistar rats***

Effects of sintered calcium compounds on absorption and retention rate of calcium are presented in Table 1. It is clear that each group of rats responded differently to calcium content in their feed. The lowest calcium content in faeces and urine of the control group highlighted adaptation of animals to increases of both calcium absorption and retention rates under limited calcium amounts in feed. When the calcium in feed was 11 mg/d, no significant difference in calcium retention rate with that of the control group was attained by a decrease in calcium absorption rate as noticed by increased amount of calcium in faeces. In contrast, no significant difference in total calcium absorption rate between the control group and the rats receiving 22 mg Ca/d was noted. Identical calcium retention rate among rats of these groups was however obtained by a significant increase in calcium discharged with urine of the rats receiving calcium supplement. It was an unexpected result that rats receiving 44 Ca/d showed a similar calcium absorption rate with that of the control rats. Thus, it illustrated that the supplementary calcium could be absorbed by rats even though the dose concentration was as high as two times of the recommended calcium intake from NRC [10]. The observation that there was no significant difference in retention rate of calcium among the studied groups suggested that bioavailability of calcium compounds in the sintered bone were not determined by the supplementary level of the studied range.

**Table 1. Effect of sintered calcium compound on absorption and retention rate in Wistar rats.**

| Treatment (mg Ca/d) | *n* | Calcium content in feces (mg/kg/d) | Total calcium absorption (mg/kg/d) | Absorption rate of calcium (%) | Calcium content in urine (mg/kg/d) | Retention rate of calcium (%) |
|---|---|---|---|---|---|---|
| 0 | 6 | 2.15± 1.02$^{c}$ | 21.17± 2$^{d}$ | 90.71±4.42$^{ab}$ | 0.41±0.15$^{b}$ | 88.99±3.97$^{a}$ |
| 11 | 6 | 9.87±3.01$^{b}$ | 53.48± 8.77$^{c}$ | 84.19±10.6$^{b}$ | 0.35±0.16$^{b}$ | 83.63±3.6$^{a}$ |
| 22 | 6 | 8.56±2.97$^{b}$ | 108.64± 8.56$^{b}$ | 92.76±4.32$^{a}$ | 2.16±0.29$^{a}$ | 90.96±4.50$^{a}$ |
| 44 | 6 | 16.82±3.83$^{a}$ | 186.17±18.10$^{a}$ | 91.78±2.91$^{ab}$ | 0.94±0.10$^{ab}$ | 91.3±2.87$^{a}$ |

Values in the same column followed by different superscript letters are significantly different ($P < 0.05$)

***Effect of sintered calcium compound on rat femurs weight and length***

Effects of calcium supplement levels on femur weight and length of rats are presented in Figure 2. There was a statistically significant effect of sintered calcium compound on these bone parameters. Rats in the control group showed the highest femur bone length (A) but expressed the lowest bone weight (B). This might be associated with insufficient intake of calcium. Calcium deficiency reduced maturation related gains in wet weight of femurs in the study of Iwamoto [17]. Administration of sintered calcium compounds altered the development of femur bone with respect to those of the control group. In general, the calcium supplemented groups exhibited shorter femur bone length relative to that of the control group. The administration of sintered calcium compound at 11 mg Ca/d significantly decreased femur length of rats with respect to those of rats receiving 22 mg Ca/d. In the case of bone weight, it increased with an increase of calcium supplement. The heaviest bones were observed in the rats receiving calcium supplementation of 44 mg Ca/day. However, femur weight and length in rats with calcium compound 22 and 44 mg Ca/d were heavier and longer than rats with 0 and 11 mg Ca/d. It is clearly seen that the level of calcium supplement affected bone development and consequently altered bone morphology.

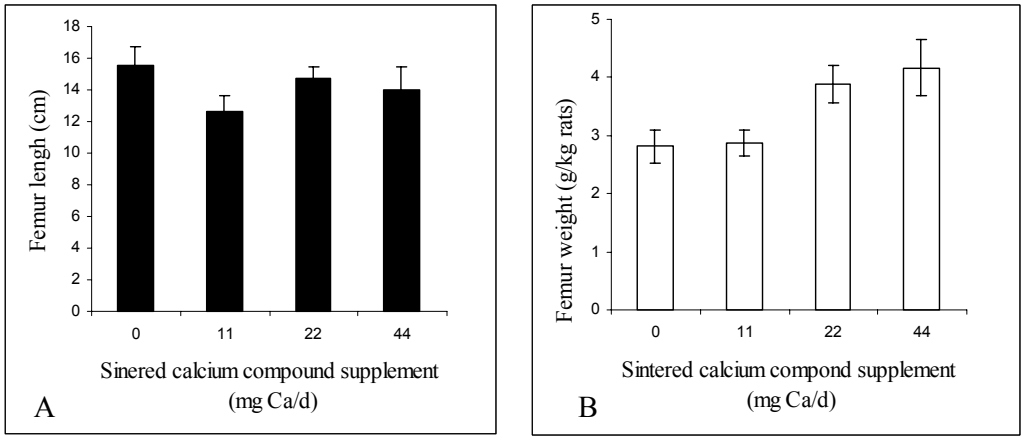


**Figure 2. Effect of sintered calcium compound supplement levels on femur length (A) and weight (B).**

***Effect of amount of sintered calcium compound on bone histology***

Histological sections revealed an abnormal trabeculae conformation in rats with 0 mg Ca/d and 11 mg Ca/d. They showed sparse and thinner trabeculae which resulted in greater inter-trabeculae spaces (Figures 3A, 3B). Calcium deficiency also reduced maturation-related cortical bone gain as a result of decreased periosteal bone gain, and enlarged marrow cavity [18]. The thicker trabeculae with high connectivity and narrowed inter-trabeculae spaces were observed in rats receiving 22 and 44 mg Ca/d (Figure 3C). The thickest trabeculae was presented in rats with 44 mg Ca/d (Figure 3D.) Bone actuation was caused by a high level of sintered calcium compound (44 mg Ca/d) treatment over seven weeks.

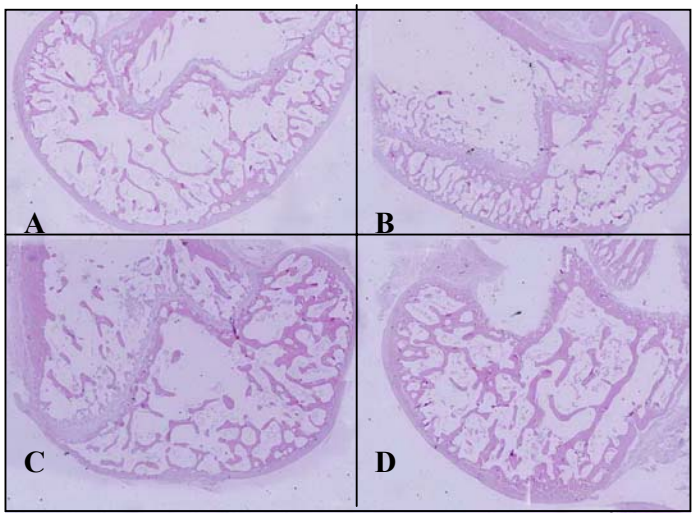


**Figure 3. Histological sections of bone from rats fed with different levels of sintered calcium compound.**

## Conclusions

The study revealed that high bioavailable calcium material could be prepared from fish bone by sintering process. Calcium supplement levels of this study supported normal growth and bone development of rats. Absorption rate and retention rate of calcium were not significantly different amongst most rats receiving different calcium supplement levels.

## Acknowledgements

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