

# **Ameliorative Effects of Skimmed Milk and Tuna Fish in the Dietary Management of Osteoporotic Female Rats; A Comparative Study**

## **ABSTRACT**

**Objective:** This study was carried out to comparatively investigate the ameliorative effects of skimmed milk and whole dried Tuna fish in osteoporotic female rats.

**Method:** Sixty six female albino rats were divided into two main groups. The first group (ten rats) fed basal diet and was maintained as negative control group. The second main group (fifty rats) was injected with 2mg dexamethasone sodium phosphate daily<sup>[DLH1]</sup> (a synthetic corticosteroid) for 5 weeks after which the second main group was divided into five groups. Group 1 osteoporotic group fed basal diet, while groups from 2 to 5 osteoporotic rats fed basal diet supplemented with 5% and 10% skimmed milk, 5% and 10% Tuna fish powder respectively.

**Result:** The results indicated that the injection with cortisone caused osteoporosis of rats.<sup>[DLH2]</sup> Induced osteoporosis caused decreases in body weight gain (BWG), serum osteocalcin, Estradiol (E2), calcium (Ca) and phosphorous (P) concentration in serum and femur bone. Bone mineral density (BMD), Bone mineral concentration (BMC) and increases in weight of liver and Parathyroid hormone (PTH) compared to negative control group (healthy rat). However, supplementation diet of osteoporotic rat with 5% and 10% skimmed milk and 5% and 10% dried whole dried Tuna fish respectively led to increases in body weight gain, serum osteocalcin, E2, Ca, P in serum and femur, BMD, BMC and decreases in weight of liver and PTH. Positive control group (osteoporotic rats) resulted to changes in the histological structure of bone compared to negative control group. The best results were observed of rats fed diet supplemented with 10% skimmed milk.

**Conclusion:** Administration of diet supplemented with 10% skimmed milk is more effective in the dietary management of dexamethasone induced osteoporosis in female rats than when supplemented with 5% or 10% powdered Tuna fish as a supplemental source of calcium.

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## INTRODUCTION

34 Osteoporosis (OA) is a disease predominantly involving deterioration of articular cartilage which  
35 is marked clinically by a gradual onset of pain, stiffness and loss of mobility in synovial (weight-  
36 bearing) joints. The therapeutic approach is mainly at the relief of symptoms. Treatment  
37 options, including pharmacological and non-pharmacological measures have been  
38 recommended [1] It is the most common articular disease in the elderly and has variable clinical  
39 presentations which often carry significant disability [2]. Osteoporosis is considered a public  
40 health problem of major concern and it is characterized by decreased bone density which  
41 results in skeletal fragility and fractures [3]. Osteoporosis is a major complication in patients  
42 who requires chronic glucocorticoid treatment [4], and Osteoporosis is more common in  
43 women than in men, partly because of the hormonal changes that occur at the menopause  
44 [5]. Osteoporosis also negatively affects the quality of life of elderly patients. This disease is  
45 characterized by a marked reduction in bone mass and deterioration of bone tissue which  
46 results in skeletal fragility and susceptibility to fractures [6]. In the United State, it is estimated  
47 that about 44 million Americans or 55 % of people 50 years and older are both osteoporosis and  
48 low bone mass [7].

49 Osteoporosis increases gradually by age to reach 21.9% age groups 40-<50 years old. Relative  
50 osteoporosis among male adolescents is 16.7% and among female adolescent is 0.9% [8].  
51 Osteoporosis has been reported to be influenced by diet, adequate nutrition, especially calcium  
52 intake, plays a major role in the prevention and treatment of osteoporosis [9]. Calcium intake

influences peak bone mass achieved in early adulthood by influencing skeletal role in preventing bone loss and osteoporotic fractures in later life. Low calcium intake is widespread problem across countries [10].

Numerous studies have indicated that intake of calcium is associated with bone mineral density [11]. Dairy products are good sources of calcium but plant calcium may be also important in populations that do not consume a large amount of milk [12]. Dried skimmed milk is made by removing fat and almost all moisture from milk followed by pulverization. Dried skimmed milk is constitutes more than 96% milk solid, 4% or less moisture and little fat (about 0.6%). It also contains about 36% good quality protein and about 51% lactose and is high in vitamin B complex, especially riboflavin. Diet is an equally important factor in bone health. Fish are very beneficial for bone health. Anchovies, caviar, herring, salmon, sardine, tuna fish with bone are high in usable calcium. Small fish with bone may be an important source of calcium in human diet [13]. Fish such as salmon and tuna fish that include bones is considered non-dairy good sources of calcium [14, 15].

Consumption of a diet adequate with respect to the supply of calcium and bone mineralization, principally in infancy and adolescence, is the most efficient way of preventing osteoporosis [16]. Calcium absorption is restricted to only 30% of the amount in the diet and its absorption depends on factors such as gastric acidity which has been known to be as an indispensable factor in the liberation of the mineral in the soluble form, when bound to other food constituents [17]. In situations of inadequate ingestion for prolonged periods, the ion is removed from the bone matrix to maintain the biological functions.

The objective of this study was to investigate the comparative efficacies of skimmed milk and Tuna fish powder on osteoporosis in female Wistar Rats

## 2.0 MATERIALS

Dexamethasone Sodium Phosphate (synthetic corticosteroid) Injections were purchased from Maruti Futuristic Pharma Pvt. Ltd, India. Skimmed milk powder (Chellarams brand) and Fresh Tuna fish were purchased from Oshodi local market, Lagos, Nigeria. The kit for bone marker was purchased from Sigma Aldrich Chemise GmbH, Germany. The experimental animals were procured from the animal house, College of Medicine, University of Lagos, Nigeria.

## 3.0 METHODS

**3.1 Preparation of Tuna Fish Powder:** Fresh Tuna fish (without head) was thoroughly cleaned and washed. After that, the Tuna fish including bone was cooked with pressure for 40 minutes, then dried in oven at 60°C for 3 hours and subsequently milled to obtain the powder.

### 3.2 Experimental Design: [DLH3]

Sixty female albino rats weighting 150±15 g were housed individually in ventilated cages under controlled condition at constant temperature (25°C) and lighting (12hrs light - dark cycle) and given free access to food and water ad libitum. The rats were divided into two main groups. The first group (10 rats) fed on basal diet and served as a control negative group. Basal diet preparation was formulated as shown below:

93 **Table 1: Composition (g/kg) of the basal diet (BD)**

| Ingredients                      | Diet groups |         |         |         |         |
|----------------------------------|-------------|---------|---------|---------|---------|
|                                  | Group 1     | Group 2 | Group 3 | Group 4 | Group 5 |
| Casein(g)                        | 200         | 200     | 200     | 200     | 200     |
| Skimmed milk(g)                  | 0           | 15      | 30      | 0       | 0       |
| Tuna fish powder(g)              | 0           | 0       | 0       | 15      | 30      |
| Vitamin mixture <sup>1</sup> (g) | 20          | 20      | 20      | 20      | 20      |
| Mineral mixture <sup>2</sup> (g) | 80          | 80      | 80      | 80      | 80      |

94 <sup>1</sup>**Vitamins (per kg of diet):** thiamin, 15 mg; riboflavin, 10 mg; pyridoxin, 5 mg; nicotinamide, 95 mg;  
 95 calcium panthotenate, 65 mg; folic acid, 2 mg; biotin, 0.1 mg; cyanocobalamin, 0.03 mg; retinyl  
 96 palmitate, 1.5 mg; dl- $\alpha$ -tocopheryl acetate, 115 mg; cholecalciferol, 0.15 mg; menadione, 1.2 mg;  
 97 ascorbic acid, 40 mg; myo-inositol, 90 mg; carrier wheat starch, 1.24 g.

98 <sup>2</sup>**Minerals (per kg of diet):** CaHPO<sub>4</sub>, 12 g; K<sub>2</sub>HPO<sub>4</sub>, 2.1 g; KCl, 3 g; ~~NACl~~NaCl, 3g; MgCl<sub>2</sub>, 2.5 g; Fe<sub>2</sub>O<sub>3</sub>, 1.9  
 99 mg; MnSO<sub>4</sub>, 115 mg; CuSO<sub>4</sub> 7H<sub>2</sub>O, 0.2 mg; ZnSO<sub>4</sub> 7H<sub>2</sub>O, 90 mg; KIO<sub>3</sub>, 0.2 mg.

100  
 101 The second group (fifty rats) fed on basal diet and injected with 2mg dexamethasone sodium  
 102 phosphate (synthetic corticosteroid) to induce osteoporosis for 5 weeks. Thereafter 5 rats were  
 103 taken from each group to test for the onset of osteoporosis, the second control group (forty  
 104 five rats) divided into five groups containing nine rats each and fed on different diets for eight  
 105 weeks as described below:

106 Group 1: Osteoporotic rats fed on basal diet preserved as positive control group.

107 Group 2: Osteoporotic rats fed on basal diet containing 5% skimmed milk

108 Group 3: Osteoporotic rats fed on basal diet containing 10% skimmed milk

109 Group 4: Osteoporotic rats fed on basal diet containing 5% Tuna fish powder

110 Group 5: Osteoporotic rats fed on basal diet containing 10% Tuna fish powder

111 Each rat was weighed at the beginning, weekly and the end of the experiment while a daily  
112 record of food intake was made. At the end of experimental period (eight weeks), rats were  
113 euthanized after overnight fasting. [DLH4] The blood of each rat was collected and centrifuged at  
114 250 rpm for 25 minutes to obtain the serum, which was kept at -18°C until analysis. The right  
115 femurs were harvested. Each right femur bone was carefully cleaned and the weight was  
116 recorded and then stored in formalin buffer (10% formalin) until analysis.

### 117 3.3 BIOCHEMICAL ANALYSIS

118 3.3.1 Bone Marker: The serum estradiol E2 was evaluated according to the method described  
119 by [20]. [DLH5] Bone Marker: Serum osteocalcin concentrations [18], parathyroid hormone (PTH)  
120 levels [19], and estradiol E2 titres [19] were determined according previously described  
121 methods. [ 18-20].

### 122 3.3.2 Measurement of Bone Mineral Density and Bone Mineral Concentration

123 The bone mineral concentration (BMC) and bone mineral density (BMD) of each femur were  
124 measured using the method of [21][DLH6], by Dual Energy X-ray Absorption (DEXA) using Norland  
125 X-R46, version 3.9.6, (Peachtree City, GA, USA) equipped with dedicated software for small

animal measurements. This technique provided integrated measurements. These measurements by DEXA bone scanner yielded total, cortical and trabecular BMD and BMC.

### **3.3.3 Determination of calcium and phosphorus content in serum and femur**

Serum calcium and phosphorus were estimated to using the method described by [Baginsk et al. \[22\]](#) and [Patricia AD \[23\]](#)~~[22, 23]~~ while calcium and phosphorus in bone were determined in **right** femur by colorimetric method according to the method described by [Ginder EM & King JD \[24\]](#) and [Goldenberg H \[24, \[25\]](#).

**3.3.4 Determination of protein:** This was done using the A.O.A.C (Association of Official Agricultural Chemists) method [26].

### **3.3.5 Biological Evaluations**

#### **3.3.5.1 Rat growth assay**

Total feed efficiency ratio (FER) was obtained by total increased weight divided by total consumed food of each rat during test period in each group. FER is equal with food conversion ratio (FCR). Generally, the amount of every other day consumed food and body weight gain by each rat was noted and was calculated by the following equation:  $\text{FER} = \frac{\text{body weights gain (g)}}{\text{consumed food (g)}}$  [27].

#### **3.3.6 Bone Histology:**

**Right femurs were immersed in 10% formalin till when ready for analytical purposes. The bone was then decalcified using a mixture of 10% of formic acid and hydrochloric acid solution**

for a period of 45 days. The processing of the rotten tissue for light microscopy was carried out and tissues were bedded in paraffin.

#### 4.0 STATISTICAL ANALYSIS

The results obtained were analyzed using SPSS program (version 18.0) and expressed as mean and standard error of measurements (SEM). Statistical significance ( $p < 0.05$ ) among the groups were determined by one-way ANOVA followed by Duncan's multiple range test.

## RESULTS

**Table 2: Calcium and phosphorus concentration of skimmed milk and dried Tuna fish powder.**

| Experimental samples | Calcium (mg/100g)            | Phosphorus<br>(mg/100g)      | Protein (%)                |
|----------------------|------------------------------|------------------------------|----------------------------|
| Skimmed milk         | 2100 $\pm$ 0.50 <sup>a</sup> | 1140 $\pm$ 0.20 <sup>a</sup> | 35 $\pm$ 0.20 <sup>a</sup> |
| Tuna fish powder     | 1810 $\pm$ 0.20 <sup>b</sup> | 1660 $\pm$ 0.30 <sup>b</sup> | 71 $\pm$ 0.30 <sup>b</sup> |

*Results represented as mean $\pm$ S.E.M, n=5*

*Values with the same superscripts across the same column are not significantly different ( $P > 0.05$ )*

Table 2 shows the calcium, phosphorus and protein concentrations of skimmed milk and dried tuna fish powder. There was a significant decrease ( $P < 0.05$ ) in the concentration of calcium of tuna fish powder compared to skimmed milk. However, there was a significant increase ( $P < 0.05$ ) in the concentrations phosphorus and protein of tuna fish compared to skimmed milk.

**Table 3: Effect of skimmed milk and dried Tuna fish on body weight gain, feed intake and feed efficiency ratio of osteoporotic female rats**

| Experimental samples   | IBW (g)                  | FBW (g)                   | BWG (g)                  | FI(g)                   | FER                     |
|------------------------|--------------------------|---------------------------|--------------------------|-------------------------|-------------------------|
| Negative Control group | 143.20±2.72 <sup>a</sup> | 302.40±17.12 <sup>c</sup> | 159.20±6.76 <sup>c</sup> | 14.58±0.59 <sup>d</sup> | 0.178±0.63 <sup>a</sup> |
| Positive Control group | 142.20±2.72 <sup>a</sup> | 180.40±11.01 <sup>d</sup> | 38.20±6.76 <sup>c</sup>  | 8.58±0.59 <sup>a</sup>  | 0.048±0.63 <sup>b</sup> |
| 5% Skimmed milk        | 143.80±5.20 <sup>a</sup> | 193.20±4.02 <sup>a</sup>  | 49.4±1.27 <sup>d</sup>   | 10.20±0.14 <sup>c</sup> | 0.052±0.43 <sup>b</sup> |
| 10% Skimmed milk       | 145.60±1.06 <sup>a</sup> | 202.10±2.12 <sup>b</sup>  | 56.5±10.26 <sup>b</sup>  | 10.94±0.94 <sup>b</sup> | 0.054±0.76 <sup>b</sup> |
| 5% Tuna fish powder    | 143.0±5.19 <sup>a</sup>  | 191.20±11.14 <sup>d</sup> | 48.2±8.08 <sup>d</sup>   | 10.02±0.55 <sup>c</sup> | 0.049±1.08 <sup>b</sup> |
| 10% Tuna fish powder   | 141.80±1.12 <sup>a</sup> | 197.0±12.21 <sup>b</sup>  | 55.2±14.16 <sup>b</sup>  | 10.88±0.54 <sup>b</sup> | 0.057±1.16 <sup>b</sup> |

Results represented as mean± S.E.M

Values with the same superscripts across the same column are not significantly different (P>0.05)

**IBW: Initial Body Weight, FBW: Final Body Weight, BWI: Body weight Gain, FI: Feed Intake, FER: Feed Efficiency Ratio**

Table 3 shows the effect of skimmed milk and dried tuna fish powder on body weight gain, feed intake and feed efficiency ratio of osteoporotic female rats. There were no significant difference ( $p>0.05$ ) in the initial weights of all the group administered with skimmed milk or tuna fish powder with respect to the positive control group. There were no significant difference ( $p>0.05$ ) in the initial weights of positive control group with respect to the negative control group. The final weight of the positive control group and all skimmed milk and tuna fish supplemented group were significantly lower than the negative control group. ( $p<0.05$ ) The final weight of the 5% ~~sardine-tuna~~ fish powder supplemented dietary group had no significant changes ( $p>0.05$ ) in value when compared with the positive control group (non-supplemented). The final weight of the 10% skimmed milk powder supplemented dietary group had no significant changes ( $p>0.05$ ) in value when compared with the 10% tuna fish powder supplemented group. However, there was a significant increase ( $p<0.05$ ) in the final weights of the 5% skimmed milk powder, 10% skimmed milk powder and 10% tuna fish powder respectively compared with the positive control group (non-supplemented). Also, the experimental animals of all treatment groups gained significant weights ( $p<0.05$ ) when compared to the positive control group whereas there was no significant weight gain ( $p>0.05$ ) between the experimental animals in 5% skimmed powder and 5% tuna fish treatment groups respectively with similar trend observed in the 10% skimmed powder and 10% tuna fish powder treatment groups respectively. There were also significant weight gain ( $p<0.05$ ) in experimental rats of the negative control group compared to the positive control group and all skimmed milk and tuna fish supplemented group.

Furthermore, the feed intakes of the experimental animals of all treatment groups were significantly increased ( $p<0.05$ ) when compared to the positive control group but were significantly reduced ( $p<0.05$ ) when compared to the negative control group. However there was no significant changes ( $p>0.05$ ) in the feed intake between the experimental animals in 5% skimmed powder and 5% Tuna fish treatment groups respectively with similar trend observed in the 10% skimmed powder and ~~55-Sardine~~5% tuna fish powder treatment groups respectively.

There was no significant difference in the Feed Efficiency Ratio of all diet supplemented groups with respect to the positive control group.

**Table 4: Effect of skimmed milk and dried Tuna fish on weight of organs and weight right femur of osteoporotic female rats**

| Experimental | Weight of | Weight of | Weight of | Weight of | Weight of |
|--------------|-----------|-----------|-----------|-----------|-----------|
|              |           |           |           |           |           |

| samples                | liver (g)                | kidney (g)               | heart (g)                | spleen (g)               | right femur (g)                      |
|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------------------|
| Negative Control group | 4.280±0.145 <sup>b</sup> | 1.270±0.020 <sup>a</sup> | 0.620±0.074 <sup>a</sup> | 0.732±0.167 <sup>a</sup> | 1.420±0.015 <sup>f</sup>             |
| Positive Control group | 6.480±0.645 <sup>a</sup> | 1.310±0.070 <sup>a</sup> | 0.640±0.114 <sup>a</sup> | 0.750±0.114 <sup>a</sup> | 0.7696±0.03 <sup>4<sup>d</sup></sup> |
| 5% Skimmed milk        | 5.760±1.12 <sup>c</sup>  | 1.340±0.161 <sup>a</sup> | 0.650±0.061 <sup>a</sup> | 0.760±0.151 <sup>a</sup> | 0.9460±0.01 <sup>8<sup>c</sup></sup> |
| 10% Skimmed milk       | 5.690±0.573 <sup>c</sup> | 1.380±0.327 <sup>a</sup> | 0.660±0.089 <sup>a</sup> | 0.760±0.134 <sup>a</sup> | 1.3293±0.10 <sup>6<sup>a</sup></sup> |
| 5% Tuna fish powder    | 5.580±0.342 <sup>c</sup> | 1.320±0.083 <sup>a</sup> | 0.630±0.070 <sup>a</sup> | 0.740±0.130 <sup>a</sup> | 0.9273±0.01 <sup>3<sup>e</sup></sup> |
| 10% Tuna fish powder   | 5.240±0.585 <sup>c</sup> | 1.36±0.044 <sup>a</sup>  | 0.620±0.109 <sup>a</sup> | 0.650±0.109 <sup>b</sup> | 1.1643±0.06 <sup>1<sup>b</sup></sup> |

200 Results represented as mean±S.E.M

201 Values with the same superscripts across the same column are not significantly different  
202 ( $P>0.05$ )

203 IBW: Initial Body Weight, FBW: Final Body Weight, BWI: Body weight Gain, FI: Feed Intake, FER:  
204 Feed Efficiency Ratio

205 Table 4 shows the effect of skimmed milk and dried tuna fish on weight of organs and weight  
206 right femur of osteoporotic female rats. There was a significant decrease ( $p>0.05$ ) in the weights  
207 of liver of experimental animals in all skimmed milk and tuna fish powder supplemented groups  
208 compared with the positive control (non-supplemented group). There was no significant  
209 difference ( $p>0.05$ ) in the weights of kidney, heart and spleen respectively of experimental  
210 animals in all skimmed milk and Tuna fish powder supplemented groups with respect to the  
211 positive control group (non-supplemented group). The weights of the right femur in all skimmed  
212 milk and Tuna fish powder supplemented group increased significantly ( $p<0.05$ ) with respect to  
213 the positive control group.

214 **Table 5: Effect of skimmed milk and dried Tuna fish on bone marker of**  
215 **osteoporotic female rats**

| Experimental samples   | Osteocalcin (ng/ml)     | Parathyroid hormone (PTH) (pg/ml) | Estradiol (E2) (pg/ml)  |
|------------------------|-------------------------|-----------------------------------|-------------------------|
| Negative Control group | 29.60±1.61 <sup>g</sup> | 34.10±1.179 <sup>b</sup>          | 27.23±1.74 <sup>a</sup> |
| Positive Control group | 10.33±0.67 <sup>f</sup> | 69.50±1.439 <sup>a</sup>          | 15.0±0.09 <sup>e</sup>  |
| 5% Skimmed milk        | 23.27±1.28 <sup>a</sup> | 55.46±1.90 <sup>c</sup>           | 19.33±2.25 <sup>c</sup> |
| 10%                    |                         |                                   |                         |

|                            |                         |                         |                         |
|----------------------------|-------------------------|-------------------------|-------------------------|
| Skimmed milk               | 25.60±1.61 <sup>a</sup> | 35.13±3.70 <sup>e</sup> | 28.33±3.14 <sup>a</sup> |
| 5%<br>Tuna fish<br>powder  | 16.70±0.36 <sup>e</sup> | 59.66±1.72 <sup>c</sup> | 18.33±2.25 <sup>c</sup> |
| 10%<br>Tuna fish<br>powder | 21.30±0.80 <sup>a</sup> | 49.30±0.97 <sup>d</sup> | 22.33±1.37 <sup>b</sup> |

216 Results represented as mean±S.E.M

217 Values with the same superscripts across the same column are not significantly different  
218 ( $P>0.05$ ).

219 | Table 5 shows the effect of skimmed milk and dried ~~sardine~~-tuna fish on bone marker of  
220 osteoporotic female rats. There was a significant increase ( $p<0.05$ ) in the concentration of  
221 osteocalcin of all the group administered with skimmed milk or tuna fish powder with respect  
222 to the positive control group. There was a significant decrease ( $p<0.05$ ) in the concentration of  
223 osteocalcin of the positive control group with respect to the negative control group. The  
224 concentration of osteocalcin of the positive control group increased significantly when compared to the  
225 negative control group. There was no significant difference in the concentration of osteocalcin  
226 among the 5% skimmed, 10% skimmed and 10% tuna fish powder respectively. There was a  
227 significant decrease ( $p<0.05$ ) in the concentration of parathyroid hormone of all the group  
228 administered with skimmed milk or tuna fish powder supplemented basal diets with respect to  
229 the positive control group. There was no significant difference between the concentration of  
230 parathyroid hormone in the group administered with 5% skimmed milk and 5% tuna fish  
231 powder. There was a significant increase ( $p<0.05$ ) in the concentration of estradiol of all the  
232 group administered with skimmed milk or tuna fish powder supplemented basal diets with  
233 respect to the positive control group. However there was no significant difference ( $p>0.05$ )  
234 between the estradiol concentration of the 10% skimmed milk supplemented group compared  
235 to the negative control. There was no significant difference between the concentration of  
236 estradiol in the group administered with 5% skimmed milk and 5% tuna fish powder.

237

238 **Table 6: Effect of skimmed milk and dried Tuna fish on calcium and phosphorus**  
 239 **levels in serum of osteoporotic female rats**

| Experimental samples    | Calcium (mmol/l)         | Phosphorus (mmol/l)      |
|-------------------------|--------------------------|--------------------------|
| Negative Control group  | 4.201±0.12 <sup>a</sup>  | 3.953±0.165 <sup>a</sup> |
| Positive Control group  | 2.058±0.354 <sup>c</sup> | 2.083±0.085 <sup>e</sup> |
| 5%<br>skimmed milk      | 3.141±0.83 <sup>b</sup>  | 2.687±0.248 <sup>d</sup> |
| 10%<br>skimmed milk     | 4.127±0.62 <sup>a</sup>  | 3.034±0.107 <sup>c</sup> |
| 5%<br>Tuna fish powder  | 3.023±0.309 <sup>b</sup> | 3.237±1.890 <sup>b</sup> |
| 10%<br>Tuna fish powder | 3.253±0.98 <sup>b</sup>  | 3.963±0.135 <sup>a</sup> |

240 *Results represented as mean±S.E.M*

241 *Values with the same superscripts across the same column are not significantly different*  
 242 *(P>0.05)*

243 Table 6 shows the effect of skimmed milk and dried sardine fish powder on calcium and  
 244 phosphorus levels in serum of osteoporotic female rats. There was a significant increase

( $p < 0.05$ ) in the concentration of calcium in the serum of all the experimental groups administered with skimmed milk or tuna fish powder with respect to the positive control group. There was a no significant difference ( $p > 0.05$ ) in the concentration of calcium in the serum of the experimental groups administered with 10% skimmed milk to the negative control group. However, there was no significant difference ( $p > 0.05$ ) in the concentration of calcium in the serum of all the experimental groups administered with 5% skimmed milk, 5% tuna fish powder and 10% tuna fish powder. Furthermore, there was a significant increase ( $p < 0.05$ ) in the concentration of phosphorus in the serum of all the experimental groups administered with skimmed milk or tuna fish powder with respect to the positive control group. However there was no significant difference ( $p > 0.05$ ) in the concentration of phosphorus in the serum of the experimental group administered with 10% tuna fish powder com to the negative control group.

**Table 7: Effect of skimmed milk and dried Tuna fish on calcium and phosphorus in bone (femur) of osteoporotic female rats**

| Experimental samples   | Calcium (mg/100g)         | Phosphorus (mg/100g)      |
|------------------------|---------------------------|---------------------------|
| Positive Control group | 18.971±0.124 <sup>a</sup> | 12.166±0.242 <sup>a</sup> |
| Positive Control group | 10.833±1.351 <sup>d</sup> | 6.816±0.473 <sup>d</sup>  |
| 5%                     |                           |                           |

|                      |                           |                           |
|----------------------|---------------------------|---------------------------|
| skimmed milk         | 15.600±0.473 <sup>c</sup> | 9.70±1.236 <sup>c</sup>   |
| 10% skimmed milk     | 18.933±0.334 <sup>a</sup> | 12.133±0.895 <sup>a</sup> |
| 5% Tuna fish powder  | 15.166±0.900 <sup>c</sup> | 10.760±0.374 <sup>b</sup> |
| 10% Tuna fish powder | 18.733±0.338 <sup>a</sup> | 11.366±0.232 <sup>f</sup> |

*Results represented as mean±S.E.M*

*Values with the same superscripts across the same column are not significantly different (P>0.05)*

Table 7 shows the effect of skimmed milk and dried Tuna fish on concentrations of calcium and phosphorus in bone (femur) of osteoporotic female rats. There was a significant increase ( $p<0.05$ ) in the concentration of calcium in the bone (femur) of all the experimental groups administered with skimmed milk or Tuna fish powder supplemented basal diets with respect to the non-supplemented positive control group. The concentrations of calcium in the bone (femur) of 5% skimmed milk and 5% Tuna fish supplemented basal diets showed no significant difference ( $p>0.05$ ) with respect to the negative control group. There was a significant increase ( $p<0.05$ ) in the concentration of phosphorus in the bone (femur) of all the experimental groups administered with skimmed milk or Tuna fish powder supplemented basal diets with respect to the non-supplemented positive control group.

283 **Table 8:** Effect of skimmed milk and dried Tuna fish on bone mass density (BMD) and bone  
 284 mass concentration (BMC) of osteoporotic female rats

| Experimental samples    | BMD (g/cm <sup>2</sup> )   | BMC (g/cm <sup>2</sup> )    |
|-------------------------|----------------------------|-----------------------------|
| Negative Control group  | 0.16010±0.003 <sup>c</sup> | 0.11993±0.028 <sup>a</sup>  |
| Positive Control group  | 0.0820±0.011 <sup>e</sup>  | 0.10256±0.034 <sup>b</sup>  |
| 5%<br>skimmed milk      | 0.12876±0.005 <sup>d</sup> | 0.10776±0.007 <sup>c</sup>  |
| 10%<br>skimmed milk     | 0.15770±0.006 <sup>c</sup> | 0.10963±0.028 <sup>d</sup>  |
| 5%<br>Tuna fish powder  | 0.12733±0.002 <sup>d</sup> | 0.10431±0.004 <sup>e</sup>  |
| 10%<br>Tuna fish powder | 0.14490±0.002 <sup>b</sup> | 0.10654±0.001a <sup>f</sup> |

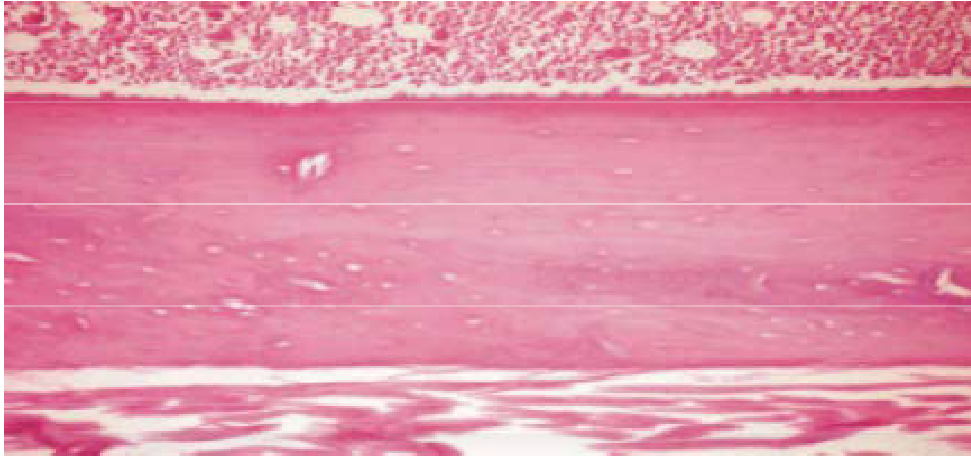
285 Results represented as mean±S.E.M

286 Values with the same superscripts across the same column are not significantly different  
 287 ( $P>0.05$ )<sub>[DLH7]</sub>

288 Table 8 shows the effect of skimmed milk and dried Tuna fish on bone mass density (BMD) and  
 289 bone mass concentration (BMC) of osteoporotic female rats. The bone mass density of the  
 290 skimmed milk, Tuna fish powder and the positive control group all decreased significantly  
 291 ( $p<0.05$ ) with respect to the negative control group. There was no significant difference ( $p>0.05$ )  
 292 in the bone mineral density of the 5% Tuna fish supplemented group compared with the 5 %  
 293 skimmed milk supplemented group. The bone mass concentration of the skimmed milk, Tuna

294 *fish powder and the positive control group all decreased significantly ( $p<0.05$ ) with respect to*  
295 *the negative control group.*

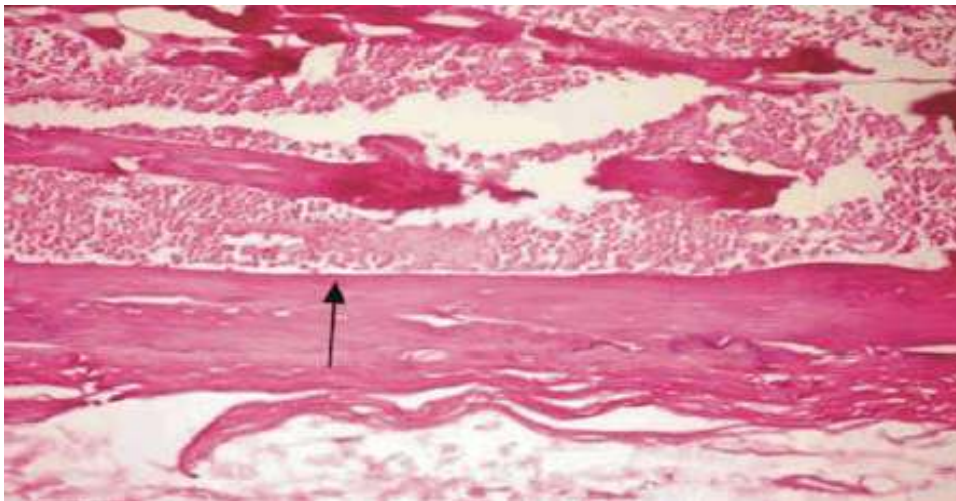
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298 **Photo 1: Photomicrograph of femur of experimental rats from negative control**  
299 **group showing no histopathological changes (H and E x 200).**

300



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302 **Photo 2: Photomicrograph of femur of experimental rats from positive control**  
303 **group showing thin bone cortex and dilatation of bone marrow cavity (H and**  
304 **E x 200).**

305

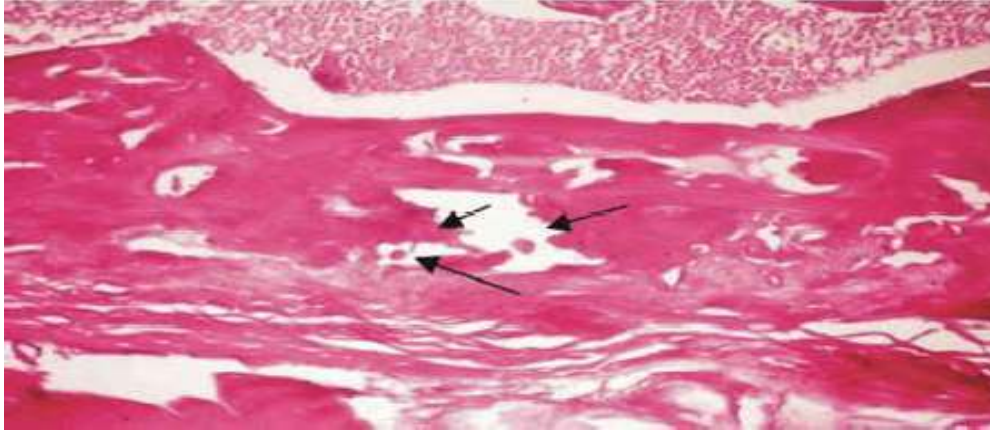


**Photo 3: Photomicrograph of femur of rats from positive control group showing cracks and necrosis in bony cortex (H and E x 200).**

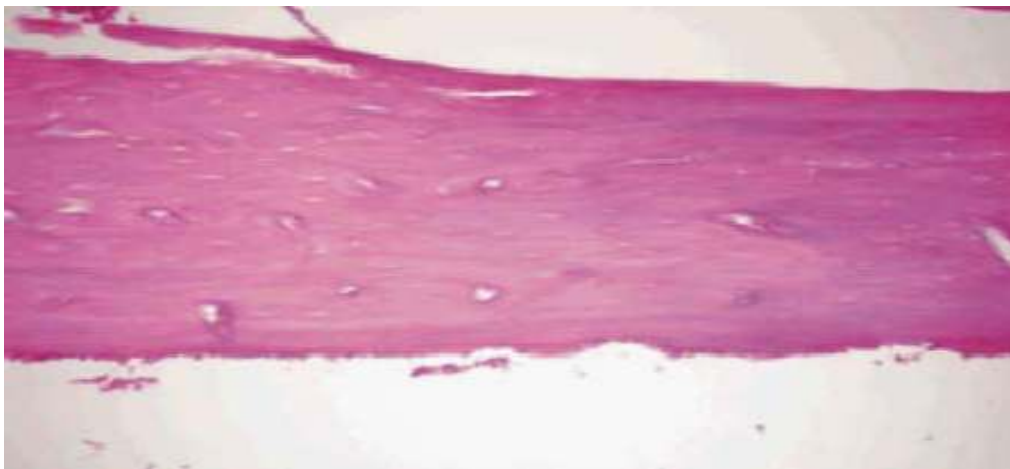
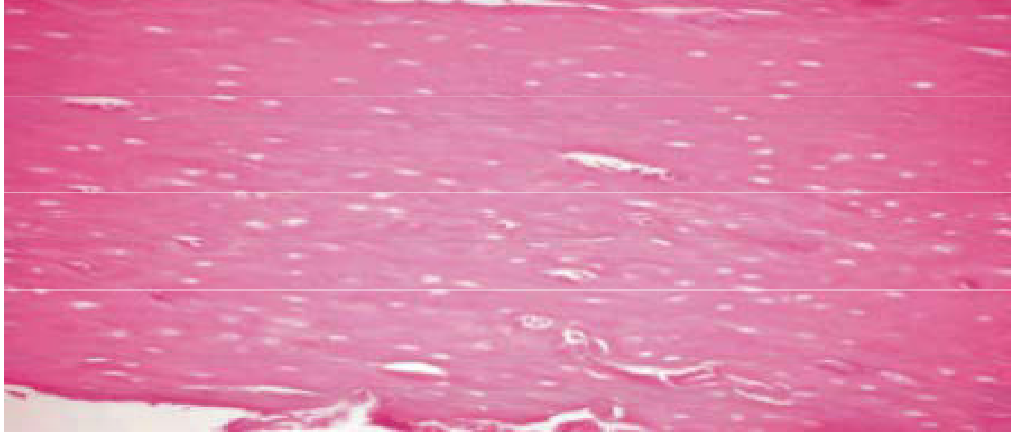


**Photo 4: Photomicrograph of femur of rats from group supplemented with 5% skimmed milk showing normal bone cortex (H and E x 200).**

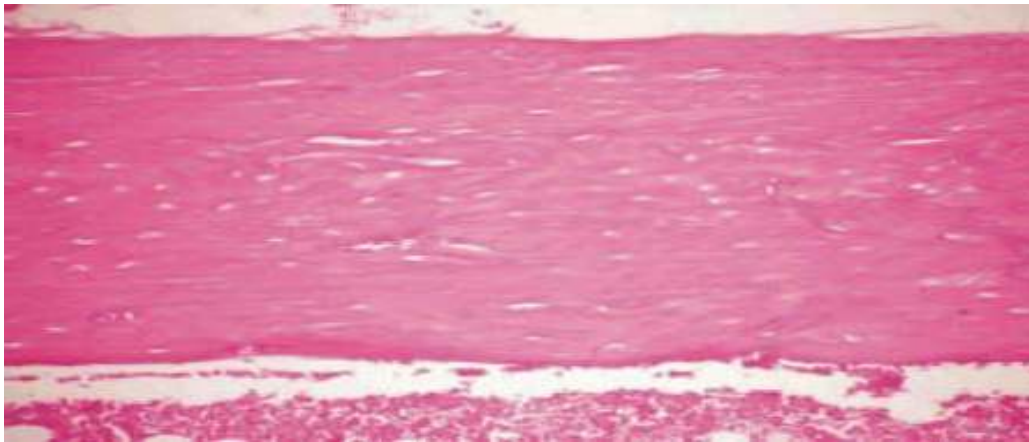


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**Photo 5: Photomicrograph of femur of rats from group control supplemented with 10% skimmed milk showing thick cortical bone (H and E x 200).**



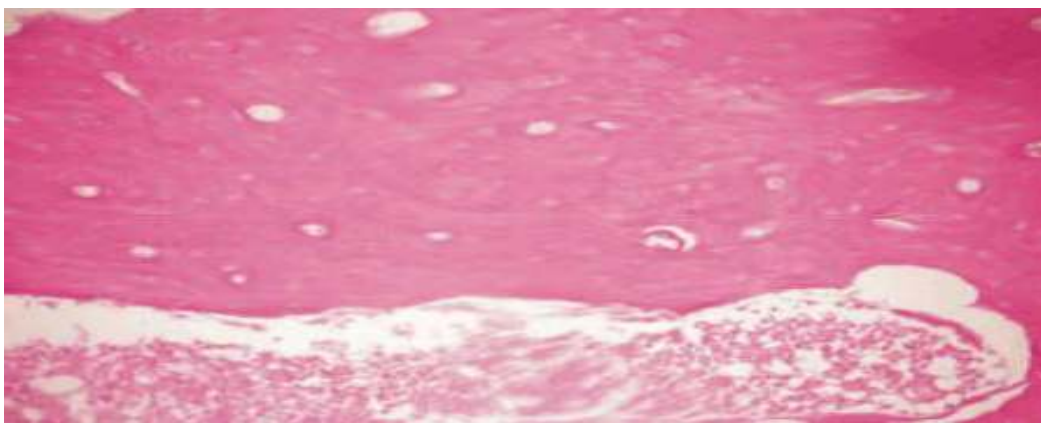
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**Photo 6: Photomicrograph of femur of rats from group supplemented with 10% skimmed milk showing proliferation of osteoblasts (H and E x 200).**

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321

**Photo 7: Photomicrograph of femur of rats from group supplemented with 5% Tuna fish showing thick cortical bone (H and E 200).**

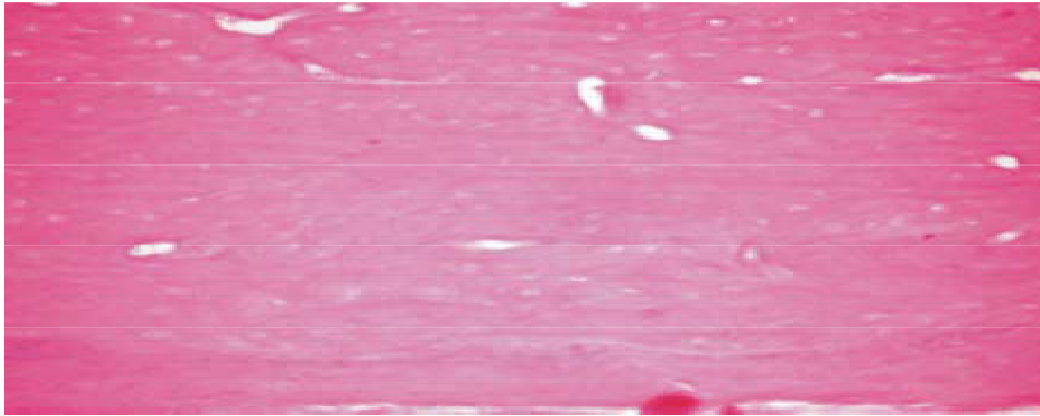


**Photo 8: Photomicrograph of femur of rats from group supplemented with 10% tuna fish showing very thick cortical bone (H and E x 200)**

## DISCUSSION

Table 2 shows calcium, phosphorus and protein concentrations in skimmed milk and dried Tuna fish. The mineral calcium plays a major role in bone strength and is of prime nutritional importance in osteoporosis, being essential for bone health throughout life [28]. The primary role of calcium in the body is structural, providing the rigidity necessary for the skeleton and teeth to function mechanically. Bone contains about 99% of the body's calcium. Calcium in body fluids also exerts critical metabolic functions, binding to proteins, and operating as a signal transmitter and protein activator within cells. Muscle contraction and nerve transmission are two of the many body functions that rely on calcium for activation. Additionally, calcium is also involved in blood clotting [29]. Calcium is required for normal growth and development of the skeleton [30]. Adequate calcium intake is critical for achieving optimal peak bone mass and

modifies the rate of bone loss associated with aging [30].<sup>[DLH8]</sup>In the present study, there was a significant decrease ( $P<0.05$ ) in the concentrations of calcium of Tuna fish powder compared to skimmed milk. The majority of the phosphorus in the body is found as phosphate ( $\text{PO}_4$ ). Approximately 85% of the body's phosphorus is found in bone. Phosphorus is found in most foods because it is a critical component of all living organisms. Dairy products, meat, and fish are particularly rich sources of phosphorus. In the current study, there was a significant increase ( $P<0.05$ ) in the concentrations of phosphorus and protein of Tuna fish powder compared to skimmed milk. From the current studies, it was revealed that skimmed milk contains a higher calcium concentration (2100 mg/100g) as against Tuna fish with a calcium concentration of 1990mg/kg. These results are in agreement<sup>[DLH9]</sup> with those obtained by [Kris-Etherton, PM et al.](#)<sup>[31]</sup>, who mentioned that Tuna are high in major minerals such phosphorus, calcium and potassium. Protein is known to be a key component of bone tissue hence an adequate dietary supply is needed [32].The majority of the research studies state that there is a positive association between protein intake and bone health. There are several epidemiological studies, both cross-sectional and longitudinal that has reported an association between dietary protein and bone [33,34].These studies show that individuals who eat the most dietary protein have the highest BMD. Furthermore, prospective studies have observed that individuals with the highest protein intake have the slowest risk of bone loss [33, 35]. One of the possible mechanisms by which dietary protein may contribute to the improvement of bone mass can be explained by the fact that increasing dietary protein is also known to increase levels of circulating insulin-like growth factor 1 (IGF-1),and conversely, a low-protein diet decreases IGF-

1 [36.IGF-1 is known to be a key mediator of bone growth but also has also been implicated in its role in the skeletal response to anabolic Parathyroid Hormone (PTH) therapy [37].

Data presented in table 3 demonstrated that there was no significant difference ( $p>0.05$ ) in the initial weights of all the group administered with skimmed milk or Tuna fish powder with respect to the positive control group. There were no significant difference ( $p>0.05$ ) in the initial weights of positive control group with respect to the negative control group. Also, the experimental animals of all treatment groups gained significant weights ( $p<0.05$ ) when compared to the positive control group whereas there was no significant weight gain ( $p>0.05$ ) between the experimental animals in 5% skimmed powder and 5% Tuna fish treatment groups respectively with similar trend observed in the 10% skimmed powder and 5% Tuna fish powder treatment groups respectively. [DLH10] These results are in agreement with the findings of Oliveira et al.[54][38], who reported that glucocorticoids induced a significant lower weight gain compared to control group. Meanwhile, rat's administration of diet supplemented with 10% skimmed milk and 10% Tuna fish recorded significant increase in final body weight and body weight gain compared to positive control group. These results are accordance with the results obtained by Oliveira et al.[38], who reported that control positive (osteoporotic rats) showed significant decrease in body weight gain. The decrease in body weight gain may be due to high dose from cortisone and long-term.

Effect of skimmed milk and Tuna fish on weight organs are shown in Table 4. Results revealed that there were no significant differences in weight of kidney, heart and spleen between all groups. Meanwhile, positive control showed significant increase in weight of liver and

decreases in weight of femur bone compared to the diet supplemented groups with skimmed milk and Tuna fish, injection with cortisone for 5 week led to increases in liver weight and decrease in weight of femur bone. These results were collaborated by [Gouda, SMH](#) [39] and [Stanley, LE et al.](#)[40], who reported that cortisone caused the increase in size and weight of cells of regeneration liver through the increased infiltration of lipid and decreases in femur bone weight. In addition, [Gouda, SMH](#)[39] reported that mean value  $\pm$ SD of liver weight/body weight of positive control (rats administration cortisone) increased as compared to negative control group. However, [41] [DLH11] reported that administration of cortisone to rats increased the protein content and relative weight of the liver of animal. Group of rats fed with diet supplemented with the 5% skimmed milk powder, 10% skimmed milk powder and 10% Tuna fish powder respectively showed a significant increase ( $p < 0.05$ ) in their final weights compared with the positive control group (non-supplemented). Tuna fish at high concentration (10%) led to decrease in weight of liver. The decrease in liver weight of rats fed diet supplemented [sardine-tuna](#) fish may be due to omega-3 present in tuna fish. These results were corroborated Also, [42], mentioned that rats administration omega-3 fatty acids decrease weight of liver (5.76 g) which increased (6.43 g) by treated with ~~baracetamol~~[paracetamol](#). [43] reported that omega-3 fatty acid have benefit effect on liver disease.

Effects of skimmed milk and Tuna fish on bone marker of osteoporotic rats are represented in Table 5. The concentration mean of serum osteocalcin, a parameter of bone formation, in positive control group decreased significantly ( $P < 0.05$ ) in comparison to all the group administered with skimmed milk or Tuna fish powder and negative control group. Osteocalcin is chiefly deposited in the extracellular matrix of bone, but a small amount enters the blood.

404 Serum osteocalcin is sensitive and specific marker of osteoblastic activity and its serum level  
405 thus reflect the rate of bone formation [44]. The osteoporotic rats fed with diet supplemented  
406 with 20% skimmed milk [DLH12] showed no significant difference ( $p>0.05$ ) compared with positive  
407 control group. The increase in osteocalcin may be due to skimmed milk and Tuna fish having  
408 higher calcium content. These results are in agreement with [Kruger MC](#) [45], who reported that  
409 supplementation of the diet with skimmed milk high calcium for 16 week led to increase in total  
410 osteocalcin compared to control group that received diet no supplementation. PTH recreation  
411 contributes to an increase in bone resorption and osteoporosis [46], over production of PTH led  
412 to an increase in bone resorption compared with bone formation and contributes to general  
413 skeletal demineralization. However, rats feeding on skimmed milk and Tuna fish revealed  
414 significantly increased in E2 and significantly decreased in PTH. The reduction in PTH in rats fed  
415 on diet supplemented with 10% skimmed milk was higher compared to other groups. These  
416 results are collaborated by [Micheal, P et al.](#) [47], who reported that supplementation diet with  
417 calcium showed a decrease in PTH.

418 Data in Table 6 illustrated the effect of skimmed milk and dried Tuna fish powder on calcium  
419 and phosphorus levels in serum of osteoporotic female rats. There was a significant increase  
420 ( $p<0.05$ ) in the concentration of calcium in the serum of all the experimental groups  
421 administered with skimmed milk or Tuna fish powder with respect to the positive control  
422 group. However, there was no significant difference ( $p>0.05$ ) in the concentration of calcium in  
423 the serum of all the experimental groups administered with 5% skimmed milk, 5% Tuna fish  
424 powder and 10% Tuna fish powder. Furthermore, there was a significant increase ( $p<0.05$ ) in  
425 the concentration of phosphorus in the serum of all the experimental groups administered with

426 skimmed milk or Tuna fish powder with respect to the positive control group. The decrease in  
427 calcium may be due to cortisone decrease leading to limited calcium absorption from  
428 gastrointestinal tract.<sup>[DLH13]</sup> Glucocorticoid affects mineral homeostasis by reducing calcium  
429 absorption and causing secondary hyper parathyroidism. Glucocorticoid directly inhibit  
430 osteoblastic bone formation, impair intestinal calcium excretion absorption and promote renal  
431 calcium excretion [48]. All treatment showed significant increase  $p < 0.05$  in serum calcium and  
432 phosphorus, the highest concentration of calcium was observed in serum rats fed on diet  
433 supplemented with 10% skimmed milk. These results may be due to skimmed milk a have  
434 higher calcium content and easy absorbed by body. Also, lactose present in skimmed milk  
435 increase calcium absorption.<sup>[DLH14]</sup> These results were collaborated by [Napoli N et al.](#)[49], who  
436 stated that the best dietary of calcium is dairy products because of the favorable element  
437 calcium content and absorption ability of calcium was higher. [Larson T et al.](#)[50] reported  
438 calcium from fish would be absorbed by body and the intake of small fish with bones could  
439 increase calcium bioavailability.

440 Data in Table 7 also showed a decrease in calcium and phosphorus femur bone compared to  
441 negative control group. The decrease in calcium concentration may be due to the cortisone  
442 content led to limited calcium absorption by gastrointestinal tract.<sup>[DLH15]</sup> Also, it increased  
443 urinary excretion of calcium and decrease bone uptake of calcium. All treatment group with  
444 two concentrations (5% and 10%)<sup>[DLH16]</sup> showed significant increase in calcium and phosphorous  
445 femur bone as compared to positive control group. The best results of femur bone calcium and  
446 phosphorous recorded of osteoporotic rats fed diet supplemented with 10% skimmed milk.

These results may be due to higher bioavailability of calcium and phosphorous in skimmed milk and deposition of them in bone.

Total BMD and BMC of femur bone in each group are summarized in Table 8. Results from the current study showed that osteoporosis caused significant decrease of BMD and BMC of femur bone. The Glucocorticoid has deleterious effects on bone density [51]. Glucocorticoid induced osteoporosis and led to a suppression of bone formation by decreasing the number and functioning of osteoblast and induced bone loss [52-54]. Other past studies reported decrease of BMD and BMC in osteoporosis disease [55, 56]. The mean BMD and BMC of osteoporotic rats fed diet supplemented with 10% skimmed milk were higher than other treatment groups. The increases in BMD and BMC due to increased calcium and phosphorus levels in diet led to increased amount of osteoblast cells which led to increased rate of bone formation. These results are in agreement with the result of the studies of [9] and [57], who reported that increasing calcium intake or dairy products is associated with a greater gain in BMD and BMC. [10] reported those milk and calcium intakes are related to bone mineral accretion during growth.[58] also stated that women with high intake of dark fish (Salmon) have protective effect of bone loss because they increase the intake of calcium and vitamin D preventing bone loss, possibly due to the effect of calcium in suppressing PTH secretion. [59] also reported that combined diet supplementation with soybean and skimmed milk enhanced bone mass density in ~~Wistar~~ Wistar rats. Calcium supplementation has also been attributed to have a positive effect on bone mineral density in postmenopausal women [60].

Histopathologically, the femur of rat from negative control revealed no histopathology change (Photo 1). Meanwhile, section of femur of rat from positive control group (osteoporotic rats) showed thin bone cortex and dilatation of bone marrow cavity (Photo 2); cracks and necrosis in bone cortex was shown in Photo 3. This results agreement with [39], who reported that treatment of rats treated with cortisone revealed a decreased bone mass and also caused a reduction in thickness of cortical bone. The femur bone of experimental rats from group supplemented with 5% skimmed milk revealed normal bone cortex (Photo 4). Moreover, the femur of experimental rats from group supplemented 10% skimmed milk revealed no histopathological changes except thick cortical bone (Photo 5) and proliferation of osteoblasts (Photo 6). These results are collaborated by that obtained by [9], who reported Chinese children receiving milk supplements increased cortical bone thickness. Thick cortical bone was noticed in bone rat from group supplemented with 5% tuna fish showed thick of cortical bone photo (7), whereas section of rat from group supplemented with 10% tuna fish showed very thick cortical bone (Photo 8).<sup>[DLH17]</sup>

## CONCLUSION

In conclusion, significant improvements in osteocalcin, parathyroid hormone, estradiol, calcium and phosphorus levels in serum and bone (femur) including bone mass density (BMD) and bone mass concentration (BMC) were attained in all four diets supplemented groups. These findings show that skimmed milk and Tuna fish are effective in positively moderate metabolic dysfunctions associated with osteoporosis in **DOP**<sup>[DLH18]</sup> rats. 10% skimmed milk dietary

488 supplementation is more therapeutically effective compared with the other three methods.  
489 This may have implications for humans, which indicates for more research into this important  
490 field of medicine.

491

## 492 CONFLICT OF INTERESTS

493 The authors declare that there is no conflict of interests.

494

495 [DLH19]

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