Original Research Article

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

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 Wistar 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 (a synthetic corticosteroid) daily 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 findings from the study indicated that the injection with cortisone caused osteoporosis of rats. 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.

INTRODUCTION

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

Osteoporosis increases gradually by age to reach 21.9% age groups 40-<50 years old. Relative osteoporosis among male adolescents is 16.7% and among female adolescent is 0.9% [8]. Osteoporosis has been reported to be influenced by diet, adequate nutrition, especially calcium 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:

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[1](g)	20	20	20	20	20
Mineral mixture[2](g)	80	80	80	80	80

94 [1]***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-a-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 [2]***Minerals (per kg of diet):*** *$CaHPO_4$, 12 g; K_2HPO_4, 2.1 g; KCl, 3 g; NaCl, 3g; $MgCl_2$, 2.5 g; Fe_2O_3, 1.9 mg;*
99 *$MnSO_4$, 115 mg; $CuSO_4$ $7H_2O$, 0.2 mg; $ZnSO_4$ $7H_2O$, 90 mg; KIO_3, 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.

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

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

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

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

Each rat was weighed at the beginning, weekly and the end of the experiment while a daily record of food intake was made. At the end of experimental period (eight weeks), rats were euthanized after overnight fasting. The blood of each rat was collected and centrifuged at 250 rpm for 25 minutes to obtain the serum, which was kept at -18°C until analysis. The right femurs were harvested. Each right femur bone was carefully cleaned and the weight was recorded and then stored in formalin buffer (10% formalin) until analysis. The animal research ethics was observed in the course of this research as stipulated by the ethical committee of College of Medicine, University of Lagos, Nigeria.

3.3 BIOCHEMICAL ANALYSIS

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

3.3.2 Measurement of Bone Mineral Density and Bone Mineral Concentration

125 The bone mineral concentration (BMC) and bone mineral density (BMD) of each femur were
126 measured using the method of [21], by Dual Energy X-ray Absorption (DEXA) using Norland X-
127 R46, version 3.9.6, Peachtree City, GA, USA) equipped with dedicated software for small animal
128 measurements. This technique provided integrated measurements. These measurements by
129 DEXA bone scanner yielded total, cortical and trabecular BMD and BMC.

130 **3.3.3 Determination of calcium and phosphorus content in serum and femur**

131 Serum calcium and phosphorus were estimated to using the method described by [22, 23}
132 while calcium and phosphorus in bone were determined in right femur by colorimetric method
133 according to the method described by [24,25].

134 3.3.4 **Determination of protein:** This was done using the A.O.A.C (Association of Official
135 Agricultural Chemists) method [26].

136 **3.3.5 Biological Evaluations**

137 **3.3.5.1 Rat growth assay**

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

143 **3.3.6 Bone Histology:**

144 Right femurs were immersed in 10% formalin till when ready for analytical purposes. The bone
145 was then decalcified using a mixture of 10% of formic acid and hydrochloric acid solution
146 for a period of 45 days. The processing of the rotten tissue for light microscopy was carried out
147 and tissues were bedded in paraffin.

148 **4.0 STATISTICAL ANALYSIS**

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

152

153 **RESULTS**

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

Experimental samples	Calcium (mg/100g)	Phosphorus (mg/100g)	Protein (%)
Skimmed milk	2100 ± 0.50^{a}	1140 ± 0.20^{a}	35 ± 0.20^{a}
Tuna fish powder	1810 ± 0.20^{b}	1660 ± 0.30^{b}	71 ± 0.30^{b}

156 ***Results represented as mean±S.E.M, n=5***

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

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

162

163 **Table 3: Effect of skimmed milk and dried Tuna fish on body weight gain, feed**
164 **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±2.72a	302.40±17.12^{c}	159.20±6.76^{c}	14.58±0.59^{d}	0.178±0.63^{a}
Positive Control group	142.20±2.72^{a}	180.40±11.01^{d}	38.20±6.76^{c}	8.58±0.59^{a}	0.048±0.63^{b}
5% Skimmed milk	143.80±5.20^{a}	193.20±4.02^{a}	49.4±1.27^{d}	10.20±0.14^{c}	0.052±0.43^{b}
10% Skimmed milk	145.60±1.06^{a}	202.10±2.12^{b}	56.5±10.26^{b}	10.94±0.94^{b}	0.054±0.76^{b}
5% Tuna fish powder	143.0±5.19^{a}	191.20±11.14^{d}	48.2±8.08^{d}	10.02±0.55^{c}	0.049±1.08^{b}
10% Tuna fish powder	141.80±1.12^{a}	197.0±12.21^{b}	55.2±14.16^{b}	10.88±0.54^{b}	0.057±1.16^{b}

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

166 *Values with the same superscripts across the same column are not significantly different*
167 *(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% 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 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 samples	Weight of liver (g)	Weight of kidney (g)	Weight of heart (g)	Weight of spleen (g)	Weight of right femur (g)
Negative Control group	4.280±0.145[b]	1.270±0.020[a]	0.620±0.074[a]	0.732±0.167[a]	1.420±0.015[f]
Positive Control group	6.480±0.645[a]	1.310±0.070[a]	0.640±0.114[a]	0.750±0.114[a]	0.7696±0.034[d]
5% Skimmed milk	5.760±1.12[c]	1.340±0.161[a]	0.650±0.061[a]	0.760±0.151[a]	0.9460±0.018[c]
10% Skimmed milk	5.690±0.573[c]	1.380±0.327[a]	0.660±0.089[a]	0.760±0.134[a]	1.3293±0.106[a]
5% Tuna fish powder	5.580±0.342[c]	1.320±0.083[a]	0.630±0.070[a]	0.740±0.130[a]	0.9273±0.013[e]
10% Tuna fish	5.240±0.585[c]	1.36±0.044[a]	0.620±0.109[a]	0.650±0.109[b]	1.1643±0.06

powder					1[b]

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

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

Experimental samples	**Osteocalcin (ng/ml)**	**Parathyroid hormone (PTH) (pg/ml)**	**Estradiol (E2) (pg/ml)**
Negative Control group	29.60±1.61[g]	34.10±1.179[b]	27.23±1.74[a]
Positive Control group	10.33±0.67[f]	69.50±1.439[a]	15.0±0.09[e]
5%			

Skimmed milk	23.27±1.28[a]	55.46±1.90[c]	19.33±2.25[c]
10% Skimmed milk	25.60±1.61[a]	35.13±3.70[e]	28.33±3.14[a]
5% Tuna fish powder	16.70±0.36[e]	59.66±1.72[c]	18.33±2.25[c]
10% Tuna fish powder	21.30±0.80[a]	49.30±0.97[d]	22.33±1.37[b]

Results represented as mean±S.E.M

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

Table 5 shows the effect of skimmed milk and dried tuna fish on bone marker of osteoporotic female rats. There was a significant increase ($p<0.05$) in the concentration of osteocalcin of all the group administered with skimmed milk or tuna fish powder with respect to the positive control group. There was a significant decrease ($p<0.05$) in the concentration of osteocalcin of the positive control group with respect to the negative control group. The concentration of osteocalcin of the positive control group increased significantly when compared to the negative control group. There was no significant difference in the concentration of osteocalcin among the 5% skimmed, 10% skimmed and 10% tuna fish powder respectively. There was a significant decrease ($p<0.05$) in the concentration of parathyroid hormone of all the group administered with skimmed milk or tuna fish powder supplemented basal diets with respect to the positive control group. There was no significant difference between the concentration of parathyroid hormone in the group administered with 5% skimmed milk and 5% tuna fish powder. There was a significant increase ($p<0.05$) in the concentration of estradiol of all the group administered with skimmed milk or tuna fish powder supplemented basal diets with respect to the positive control group. However there was no significant difference ($p>0.05$) between the estradiol concentration of the 10% skimmed milk supplemented group compared to the negative control. There was no significant difference between the concentration of estradiol in the group administered with 5% skimmed milk and 5% tuna fish powder.

238

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

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

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

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

Table 6 shows the effect of skimmed milk and dried tuna fish powder on calcium and phosphorus levels in serum of osteoporotic female rats. T

here 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 [a]	12.166±0.242[a]

Positive Control group	10.833±1.351[d]	6.816±0.473[d]
5% skimmed milk	15.600±0.473[c]	9.70±1.236[c]
10% skimmed milk	18.933±0.334 [a]	12.133±0.895[a]
5% Tuna fish powder	15.166±0.900 [c]	10.760±0.374[b]
10% Tuna fish powder	18.733±0.338 [a]	11.366±0.232[f]

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.

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

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

Results represented as mean±S.E.M

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

Table 8 shows the effect of skimmed milk and dried Tuna fish on bone mass density (BMD) and bone mass concentration (BMC) of osteoporotic female rats. The bone mass density of the

skimmed milk, Tuna fish powder and the positive control group all decreased significantly ($p<0.05$) with respect to the negative control group. There was no significant difference ($p>0.05$) in the bone mineral density of the 5% Tuna fish supplemented group compared with the 5 % skimmed milk supplemented group. The bone mass concentration of the skimmed milk, Tuna fish powder and the positive control group all decreased significantly ($p<0.05$) with respect to the negative control group.

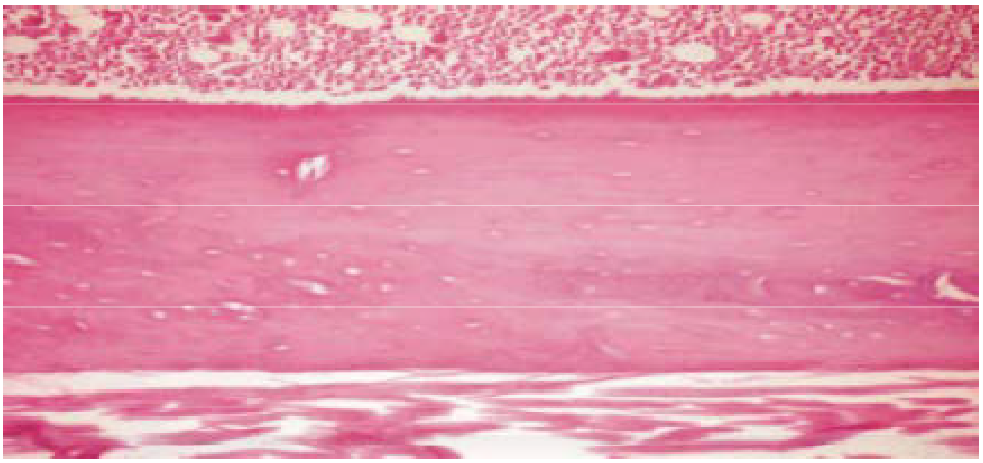
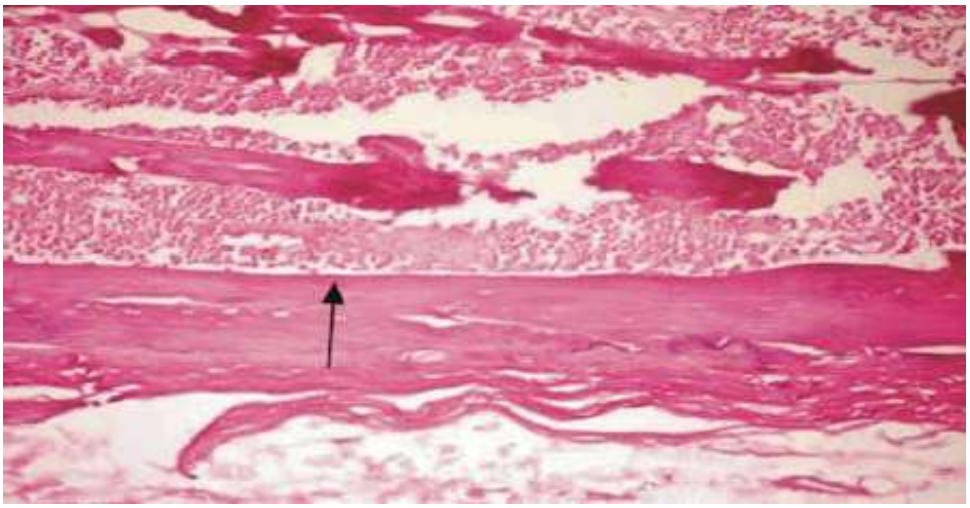


Figure 1: Photomicrograph of femur of experimental rats from negative control group showing no histopathological changes (H and E x 200).



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

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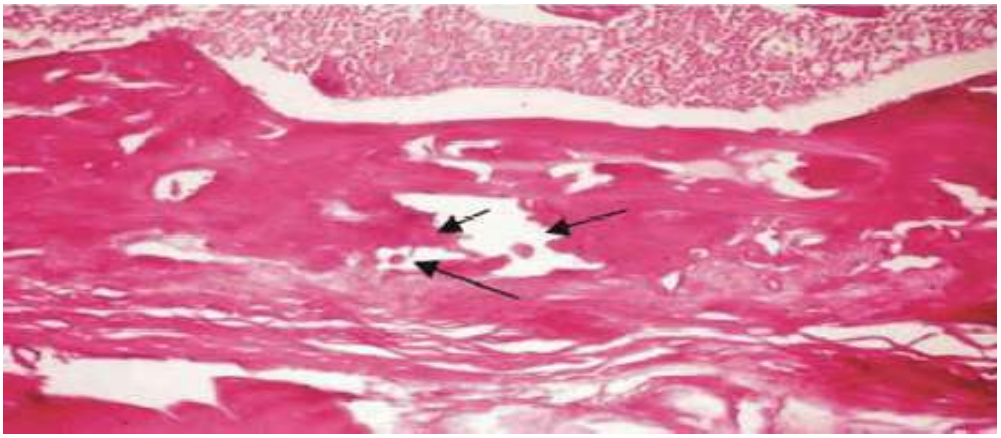

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308 **Figure 3: Photomicrograph of femur of rats from positive control group**
309 **showing cracks and necrosis in bony cortex (H and E x 200).**

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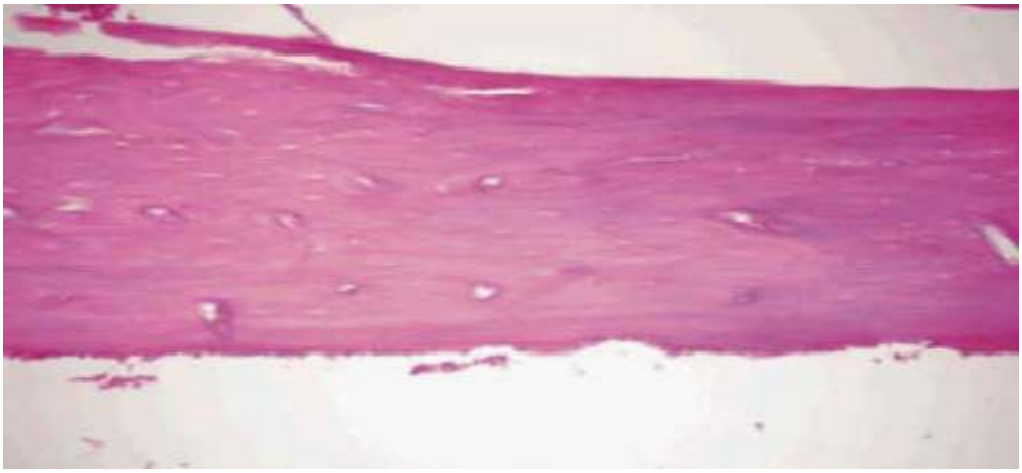

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312 **Figure 4: Photomicrograph of femur of rats from group supplemented with 5%**
313 **skimmed milk showing normal bone cortex (H and E x 200).**

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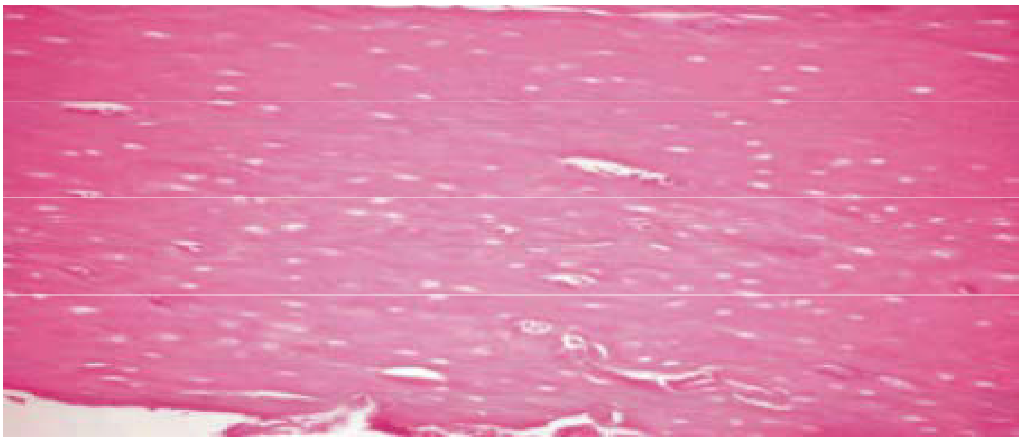

Figure 5: Photomicrograph of femur of rats from group control supplemented with 10% skimmed milk showing thick cortical bone (H and E x 200).

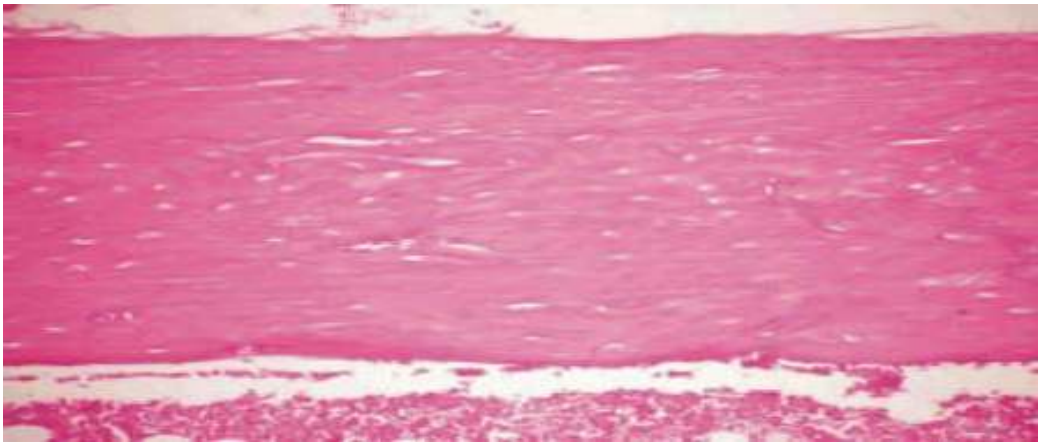

Figure 6: Photomicrograph of femur of rats from group supplemented with 10% skimmed milk showing proliferation of osteoblasts (H and E x 200).

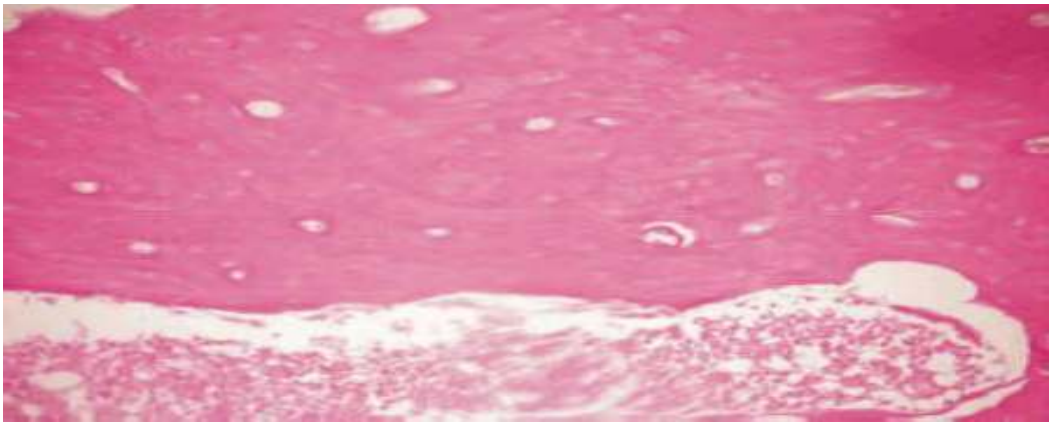

Figure 7: Photomicrograph of femur of rats from group supplemented with 5% Tuna fish showing thick cortical bone (H and E 200).

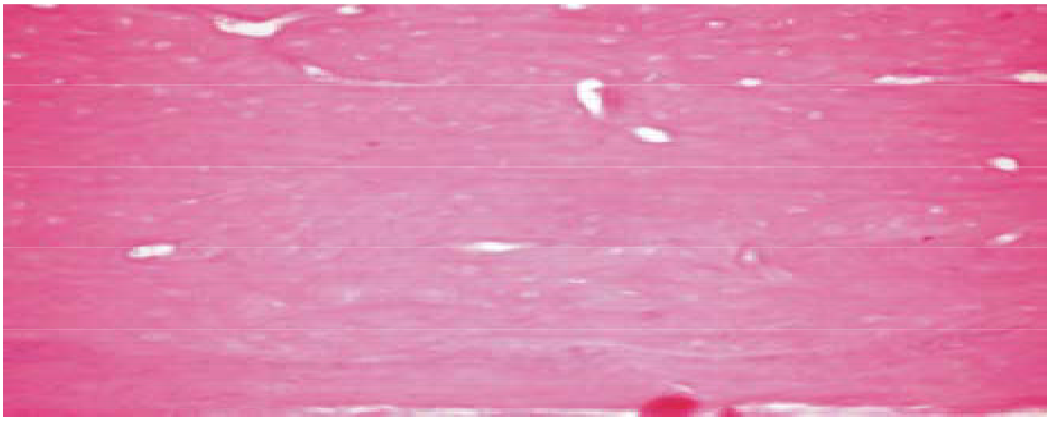

Photo 8: Photomicrograph of femur of rats from group supplemented with 10% tuna fish showing very thick cortical bone (H and E x 200)

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

341 modifies the rate of bone loss associated with aging [30]. In the present study, there was a
342 significant decrease (P<0.05) in the concentrations of calcium of Tuna fish powder compared to
343 skimmed milk. The majority of the phosphorus in the body is found as phosphate
344 (PO_4).Approximately 85% of the body's phosphorus is found in bone. Phosphorus is found in
345 most foods because it is a critical component of all living organisms. Dairy products, meat, and
346 fish are particularly rich sources of phosphorus. In the current study, there was a significant
347 increase (P<0.05) in the concentrations of phosphorus and protein of Tuna fish powder
348 compared to skimmed milk. From the current studies, it was revealed that skimmed milk
349 contains a higher calcium concentration (2100 mg/100g) as against Tuna fish with a calcium
350 concentration of 1990mg/kg. These results however differ from those obtained by Etherton,
351 PM et al. {31], who mentioned that Tuna are high in major minerals such phosphorus, calcium
352 and potassium. Protein is known to be a key component of bone tissue hence an adequate
353 dietary supply is needed [32].The majority of the research studies state that there is a positive
354 association between protein intake and bone health. There are several epidemiological studies,
355 both cross-sectional and longitudinal that has reported an association between dietary protein
356 and bone [33,34].These studies show that individuals who eat the most dietary protein have
357 the highest BMD. Furthermore, prospective studies have observed that individuals with the
358 highest protein intake have the slowest risk of bone loss [33, 35]. One of the possible
359 mechanisms by which dietary protein may contribute to the improvement of bone mass can be
360 explained by the fact that increasing dietary protein is also known to increase levels of
361 circulating insulin-like growth factor 1 (IGF-1),and conversely, a low-protein diet decreases IGF-

362 1 [36.IGF-1 is known to be a key mediator of bone growth but also has also been implicated in
363 its role in the skeletal response to anabolic Parathyroid Hormone (PTH) therapy [37].

364 The results presented in table 3 are in agreement with the findings of Oliveira et al. [38], who
365 reported that glucocorticoids induced a significant lower weight gain compared to control
366 group. Meanwhile, rat's administration of diet supplemented with 10% skimmed milk and 10%
367 Tuna fish recorded significant increase in final body weight and body weight gain compared to
368 positive control group. These results are accordance with the results obtained by Oliveira et al.
369 [38], who reported that control positive (osteoporotic rats) showed significant decrease in body
370 weight gain. The decrease in body weight gain may be due to high dose from cortisone and
371 long-term.

372 Effect of skimmed milk and Tuna fish on weight organs are shown in Table 4. Results revealed
373 that there were no significant differences in weight of kidney, heart and spleen between all
374 groups. Meanwhile, positive control showed significant increase in weight of liver and
375 decreases in weight of femur bone compared to the diet supplemented groups with skimmed
376 milk and Tuna fish, injection with cortisone for 5 week led to increases in liver weight and
377 decrease in weight of femur bone. These results were collaborated by Gouda, SMH [39] and
378 Stanley, LE et al. [40], who reported that cortisone caused the increase in size and weight of
379 cells of regeneration liver through the increased infiltration of lipid and decreases in femur
380 bone weight. In addition, Gouda, SMH [39] reported that mean value ±SD of liver weight/body
381 weight of positive control (rats administration cortisone) increased as compared to negative
382 control group. However, Howard C et al. [41] reported that administration of cortisone to rats

383 increased the protein content and relative weight of the liver of animal. Group of rats fed with
384 diet supplemented with the 5% skimmed milk powder, 10% skimmed milk powder and 10%
385 Tuna fish powder respectively showed a significant increase ($p<0.05$) in their final weights
386 compared with the positive control group (non-supplemented). Tuna fish at high concentration
387 (10%) led to decrease in weight of liver. The decrease in liver weight of rats fed diet
388 supplemented sardine fish may be due to omega-3 present in tuna fish. These results were
389 corroborated Also, [42], mentioned that rats administration omega-3 fatty acids decrease
390 weight of liver (5.76 g) which increased (6.43 g) by treated with paracetamol.[43] reported that
391 omega-3fatty acid have benefit effect on liver disease.

392 Effects of skimmed milk and Tuna fish on bone marker of osteoporotic rats are represented in
393 Table 5. The concentration mean of serum osteocalcin, a parameter of bone formation in
394 positive control group decreased significantly ($P<0.05$) in comparison to all the group
395 administered with skimmed milk or Tuna fish powder and negative control group. Osteocalcin
396 is chiefly deposited in the extracellular matrix of bone, but a small amount enters the blood.
397 Serum osteocalcin is sensitive and specific marker of osteoblastic activity and its serum level
398 thus reflect the rate of bone formation [44]. The osteoporotic rats fed with diet supplemented
399 with 10% skimmed milk showed no significant difference ($p>0.05$) compared with positive
400 control group. The increase in osteocalcin may be due to skimmed milk and Tuna fish having
401 higher calcium content. These results are in agreement with Kruger MC [45], who reported that
402 supplementation of the diet with skimmed milk high calcium for 16 week led to increase in total
403 osteocalcin compared to control group that received diet no supplementation..PTH recreation
404 contributes to an increase in bone resorption and osteoporosis [46], over production of PTH led

405 to an increase in bone resorption compared with bone formation and contributes to general
406 skeletal demineralization. However, rats feeding on skimmed milk and Tuna fish revealed
407 significantly increased in E2 and significantly decreased in PTH. The reduction in PTH in rats fed
408 on diet supplemented with 10% skimmed milk was higher compared to other groups. These
409 results are collaborated by Micheal, P et al. [47], who reported that supplementation diet with
410 calcium showed a decrease in PTH.

411 Data in Table 6 illustrated the effect of skimmed milk and dried Tuna fish powder on calcium
412 and phosphorus levels in serum of osteoporotic female rats. There was a significant increase
413 ($p<0.05$) in the concentration of calcium in the serum of all the experimental groups
414 administered with skimmed milk or Tuna fish powder with respect to the positive control
415 group. However, there was no significant difference ($p>0.05$) in the concentration of calcium in
416 the serum of all the experimental groups administered with 5% skimmed milk, 5% Tuna fish
417 powder and 10% Tuna fish powder. Furthermore, there was a significant increase ($p<0.05$) in
418 the concentration of phosphorus in the serum of all the experimental groups administered with
419 skimmed milk or Tuna fish powder with respect to the positive control group. The increase in
420 calcium may be due to cortisone decrease leading to limited calcium absorption from
421 gastrointestinal tract. Glucocorticoid affects mineral homeostasis by reducing calcium
422 absorption and causing secondary hyper parathyrodism. Glucocorticoid directly inhibit
423 osteoblastic bone formation, impair intestinal calcium excretion absorption and promote renal
424 calcium excretion [48]. All treatment showed significant increase $p<0.05$ in serum calcium and
425 phosphorus, the highest concentration of calcium was observed in serum rats fed on diet
426 supplemented with 10% skimmed milk. These results may be due to skimmed milk a have

427 higher calcium content and easy absorbed by body. Also, lactose present in skimmed milk
428 increase calcium absorption. These results were collaborated by Napoli N et al. [49], who stated
429 that the best dietary of calcium is dairy products because of the favorable element calcium
430 content and absorption ability of calcium was higher. Larson T et al. [50] reported calcium from
431 fish would be absorbed by body and the intake of small fish with bones could increase calcium
432 bioavailability.

433 Data in Table 7 also showed a decrease in calcium and phosphorus femur bone compared to
434 negative control group. Also, it increased urinary excretion of calcium and decrease bone
435 uptake of calcium. All treatment group with two concentrations (5% and 10%) of skimmed milk
436 and tuna fish showed significant increase in calcium and phosphorous femur bone as compared
437 to positive control group. The best results of femur bone calcium and phosphorous recorded of
438 osteoporotic rats fed diet supplemented with 10% skimmed milk. These results may be due to
439 higher bioavailability of calcium and phosphorous in skimmed milk and deposition of them in
440 bone.

441 Total BMD and BMC of femur bone in each group are summarized in Table 8. Results from the
442 current study showed that osteoporosis caused significant decrease of BMD and BMC of femur
443 bone. The Glucocorticoid has deleterious effects on bone density [51]. Glucocorticoid induced
444 osteoporosis and led to a suppression of bone formation by decreasing the number and
445 functioning of osteoblast and induced bone loss [52-54]. Other past studies reported decrease
446 of BMD and BMC in osteoporosis disease [55, 56]. The mean BMD and BMC of osteoporotic rats
447 fed diet supplemented with 10% skimmed milk were higher than other treatment groups. The

448 increases in BMD and BMC due to increased calcium and phosphorus levels in diet led to
449 increased amount of osteoblast cells which led to increased rate of bone formation. These
450 results are in agreement with the result of the studies of [9] and [57], who reported that
451 increasing calcium intake or dairy products is associated with a greater gain in BMD and BMC.
452 [10] reported those milk and calcium intakes are related to bone mineral accretion during
453 growth.[58] also stated that women with high intake of dark fish (Salmon) have protective
454 effect of bone loss because they increase the intake of calcium and vitamin D preventing bone
455 loss, possibly due to the effect of calcium in suppressing PTH secretion. [59] also reported that
456 combined diet supplementation with soybean and skimmed milk enhanced bone mass density
457 in Wistar rats. Calcium supplementation has also been attributed to have a positive effect on
458 bone mineral density in postmenopausal women [60].

459 Histopathologically, the femur of rat from negative control revealed no histopathology change
460 (Figure 1). Meanwhile, section of femur of rat from positive control group (osteoporotic rats)
461 showed thin bone cortex and dilatation of bone marrow cavity (Figure 2); cracks and necrosis in
462 bone cortex was shown in Figure 3. This results agreement with [39], who reported that
463 treatment of rats treated with cortisone revealed a decreased bone mass and also caused a
464 reduction in thickness of cortical bone. The femur bone of experimental rats from group
465 supplemented with 5% skimmed milk revealed normal bone cortex (Figure 4). Moreover, the
466 femur of experimental rats from group supplemented 10% skimmed milk revealed no
467 histopathological changes except thick cortical bone (Figure 5) and proliferation of osteoblasts
468 (Figure 6). These results are collaborated by that obtained by [9], who reported Chinese
469 children receiving milk supplements increased cortical bone thickness. Thick cortical bone was

470 noticed in bone rat from group supplemented with 5% tuna fish showed thick of cortical bone
471 (Figure 7), whereas section of rat from group supplemented with 10% tuna fish showed very
472 thick cortical bone (Figure 8).

473

474 **CONCLUSION**

475 In conclusion, significant improvements in osteocalcin, parathyroid hormone, estradiol, calcium
476 and phosphorus levels in serum and bone (femur) including bone mass density (BMD) and bone
477 mass concentration (BMC) were attained in all four diets supplemented groups. These findings
478 show that skimmed milk and Tuna fish are effective in positively moderate metabolic
479 dysfunctions associated with osteoporosis in DOPI rats. 10% skimmed milk dietary
480 supplementation is more therapeutically effective compared with the other three methods.
481 This may have implications for humans, which indicates for more research into this important
482 field of medicine.

483

484 CONFLICT OF INTERESTS

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

486

487

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