Research Article

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Cow milk and its dairy products ameliorate bone toxicity in the Coragen-induced rat model

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Abstract: Coragen is an insecticide that stimulates calcium release from intracellular stores of muscle cells causing death to sensitive species. The present study aimed to evaluate the bone toxic effect of Coragen and the potential therapeutic effect of cow milk, yogurt, and soft cheese in rats. Toxicity was induced by Coragen administration with different doses of 1/20 or 1/40 LD50 in rats. Groups of rats (n = 6) were treated with either 5 g milk, 5 g yogurt, or 1.5 g cheese. Coragen administration elevated alkaline and acid phosphatases activity and reduced the calcium and phosphorus level in urine and serum of rats administered with Coragen. Femur and tibia length, thickness, weight, and breaking force were decreased by Coragen administration and femur Ca and P contents as well. Bone mineral area (BMA), bone mineral content (BMC), bone mineral density (BMD), protein profile (total, albumin, and globulin), and antioxidant system (TAC, GSH, GPx, GST, and SOD) were decreased by Coragen. All these parameters were improved on the treatment with milk and milk products. The results showed that yogurt treatment was significantly superior to the other treatments in increasing BMD (27%), breaking force (9%), femur Ca (41%), serum Ca (14%), and serum P (16%) and in reducing acid phosphatases (14%) and urine Ca and P by 8 and 10%, respectively. It can be concluded that the treatment with milk and milk products may provide treatment against osteoporosis and toxicity caused by Coragen.

Keywords: osteoporosis, Coragen, cow milk, yogurt, soft cheese

1 Introduction

Pesticides have long been used to improve the agricultural yield and control various pests [1,2]. Toxicological studies reported that pesticide exposure can alter the bone composition and that may lead to bone diseases such as osteoporosis [3–7]. Osteoporosis is a bone disease that causes bone density loss and increases the risk of bone fractures [5]. Chlorantraniliprole (trade name Coragen) is a new compound that belongs to a new class of selective insecticides (anthranilic diamides) and acts as a ryanodine receptor modulator. It stimulates the release of calcium from intracellular stores of muscle cells causing impaired muscle regulation, paralysis, and ultimately death of sensitive species. Coragen is used in agriculture against pests of the order Lepidoptera and Isoptera, also Diptera and Coleoptera species, in a wide variety of crops [8].

Pesticides can be toxic to other organisms such as birds, beneficial insects, fish, and soil microorganisms. Beneficial insects such as bees showed symptoms of apathy, slow movements, and lethargy after exposure to Coragen [9,10]. Coragen was highly toxic to fish such as Channa punctatus [11]. The fish (Channa punctatus) showed behavioral changes such as hyperactivity, erratic swimming, posture imbalance, and excess secretion of mucus overall the body surface after exposure to Coragen [11]. Many animal studies have reported that Coragen causes bodyweight reduction, elevation in liver enzyme activity, hemato-toxicity, and histopathological changes in liver, lung, and spleen [12–14]. Hassan et al. [15] reported that Coragen caused thrombocytopenia, leukocytosis, microcytic anemia, kidney dysfunction, hyperuricemia, and elevated level of sex hormone and thyroid hormone in rats. Coragen is classified as a non-carcinogenic and non-toxic agent for humans; however, a 26-old woman had a cardiac manifestation after exposure to
Coragen [16]. Exposure to Coragen has been reported to cause blood calcium reduction in rats [13]. Calcium is essential for cellular activation and responsible for bone rigidity [17]. Calcium deficiency is a key cause of osteoporosis [18]. Calcium reduction as a result of Coragen exposure may lead to bone loss. To the best of our knowledge, no previous research has yet investigated the effect of Coragen on bone mineralization or its potential toxic effect on bones.

Nutritional intervention may be a potential therapeutic approach to tackle Coragen toxicity. Milk and functional dairy products have been associated with health benefits of their constituents. Milk contains proteins, bioactive peptides, oligosaccharides, omega-3 fatty acids, conjugated linoleic acids, calcium, and vitamins. Fermented dairy products such as yogurt and soft cheese provide essential nutrients and probiotic bacteria [19,20]. Probiotics are live microorganisms that provide a health benefit to the host [21]. Products containing live probiotic bacteria have several health benefits such as blood cholesterol reduction and immunity improvement [22]. Also, it has been reported that milk and its functional dairy products have biological effects such as neuro-modulatory, immune-modulating, anti-inflammatory, anti-microbial, bone protective, and cardio-protective [23]. Milk and milk products have the antioxidant capacity and have the potential to protect against oxidative stress [23]. Skimmed milk (17%), yogurt (17%), and whey protein (6%) enhanced the bone mineral content and bone mineral density in ovariectomized rats [24]. Numerous in vitro studies showed that yogurt starter and probiotic lactobacilli can reduce pesticide load [25–28]. Probiotics showed an antioxidant, anti-inflammatory, and anti-fibrotic effect in ethephon-treated rats [29]. However, no studies have yet evaluated the effect of milk and milk products on pesticide toxicity.

In the light of the previously mentioned evidence and with the scarcity of data regarding the effect of Coragen on bone properties, the present study aimed to study the effect of Coragen on bone mineralization, bone mineral density, and biochemical parameters. This study also assessed the potential therapeutic effects of cow milk and milk products (yogurt and soft cheese) against the potential bone toxic effects of Coragen in rats.

2 Materials and methods

2.1 Chemical

Coragen 20% SC was obtained from the Central Agricultural Pesticide Laboratory (CAPL). The pesticide chlorantraniliprole with commercial name Coragen and IUPAC name is 3-bromo-5-[4-chloro-2-methyl-6-(methyl-carbamoyl)phenyl]-1-(3-chloro-2-pyridine-2-yl)-1H-pyrazole-5-carboxamide with structural formula of C₉H₇BrClN₅O₂. Its chemical class is anthranilic diamide insecticide, and its LD₅₀ >5,000 mg/kg body weight of male albino rats [30].

2.2 Preparation of yogurt and soft cheese and chemical analysis

2.2.1 Yogurt preparation

Cow milk samples were collected from the herds of the Faculty of Agriculture, Cairo University. The cow milk was heated up to 55°C, subsequently normalized and pasteurized at 71°C, then cooled to 40°C for the fermentation process. The starter culture for yogurt preparation was Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus (Mecin Lab Faculty of Agriculture, Ain Shams University). The starter cultures were added and incubated according to the manufacturer's recommendations until pH 5.2 [31]. The samples were refrigerated at 4 ± 1°C.

2.2.2 Soft cheese preparation

Calcium chloride (0.01 g/5 L of milk) was added to warm cow milk (42°C) 30 min prior to the addition of the starter culture. Cooled milk was inoculated with a starter culture. Diluted liquid camel chymosin was added after inoculation of culture depending on the accomplishment of pH value. The milk was allowed to coagulate for 2 h after the addition of camel chymosin. The coagulated curd was cut and left to stand for 10 min and then was poured into a plastic mold lined with a cheesecloth, thereafter the whey was drained off from the curd. The cheese samples were collected in sterile containers and weighed immediately using a digital weighing balance, prior to storage in the refrigerator at 4 ± 1°C [32]. The weight of the cheese sample was recorded, and the yield of the cheese was calculated as follows:

\[
\text{Cheese yield (\%) = \left( \frac{\text{Weight of cheese}}{\text{Weight of milk}} \right) \times 100.}
\]
in milk, yogurt, and soft cheese were estimated by the method described in AOAC [33]. Vitamins (A, C, D, E, B1, and B2) were determined according to procedures outlined in AOAC [33]. Mineral contents (Na, K, Ca, Mg, Fe, Zn, and Cu) were determined using atomic absorption spectrometry (Pye Unicum model SP 192 instrument) according to the method of Murthy and Rhea [34]. All samples were analyzed in triplicate.

### 2.3 Animal experiment

#### 2.3.1 Diet and animals

A total of 54 male healthy Sprague-Dawley rats (150–160 g) were obtained from the animal house of the National Research Center, Dokki, Cairo, Egypt. The animals were housed under controlled environmental conditions (23 ± 1°C, 55 ± 5% humidity, and 12 h light: 12 h dark cycle). The animals were fed with a basal diet composed of 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamins mixture, and 65% starch. Food and water were given *ad libitum* during the experimental period (90 days) [35].

#### 2.3.2 Experimental design

The animals were fed on a basal diet for 14 days as an adaptation period. After the adaptation period, rats were divided randomly into nine groups (six rats for each group). All the groups were fed on a basal diet. Group 1 (normal control group) was administered water orally three times per week. Group 2 (Coragen control group 1/20 LD50) was orally administered with Coragen (1/20 LD50) at a dose of 250 mg/kg body weight three times per week. Groups 3, 4, and 5 were orally administered with Coragen (1/20 LD50) at a dose of 250 mg/kg body weight, and each of the three groups was treated with cow milk (5 g/kg), yogurt (5 g/kg), or soft cheese (1.5 g/kg), respectively, three times per week. Group 6 (Coragen control group 1/40 LD50) was orally administered with Coragen (1/40 LD50) at a dose of 125 mg/kg body weight three times per week. Groups 7, 8, and 9 were orally administered with Coragen (1/40 LD50) at the same dose of group 6, and each group was treated with cow milk (5 g/kg), yogurt (5 g/kg), or soft cheese (1.5 g/kg), respectively, three times per week for 90 days. At the end of the experiment, body weight was recorded and 24 h urine samples were collected for mineral content determination using standard methods. Blood samples were obtained from fasted, anesthetized rats, and serum was separated for the estimation of elements content (Na, K, Ca, Mg, and P), protein profile (total, albumin, globulin) content, reduced glutathione (GSH), total antioxidant capacity, phosphatases (acid and alkaline) activity, glutathione peroxidase activity (GPx), glutathione-S-transferase activity (GST), and superoxide dismutase activity (SOD) according to the methods of Gregor et al. [36], Bergmeyer et al. [37], Kind and King [38], Koracevic et al. [39], Belfield and Golberg [40], Rotruck et al. [41], Grant and Matsumura [42], and Kakkar et al. [43], respectively. The biochemical test kits were obtained from Bio-diagnostic Company (Cairo, Egypt). The right femur and tibia of bones were separated, cleaned, and weighed. The length and thickness of the femur and tibia were measured using an ABS digimatic solar caliper (Tri-State Instrument Service, Fort Wayne, TX) [44]. The breaking force of the femur and tibia was measured using the Digital Force Gauge model, FGN-50, Japan [45]. The bone mineral parameters were measured by using a dual-X-ray absorptiometry (DXA) model, Norland XRE-46 [44].

**Ethical approval:** The research related to animal use has been complyed with all the relevant national regulations and institutional policies for the care and use of animals, and has been approved by the Ethics Committee of the Cairo University, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

### 2.4 Statistical analysis

Statistical analysis was performed with SPSS software (version 17). Values are expressed as mean ± standard error (SE). Comparisons between groups were performed with one-way analysis of variance (ANOVA) followed by Tukey’s test. *P* values were compared for all experimental groups, and *P* < 0.05 was considered to be statistically significant.

### 3 Results

#### 3.1 Chemical composition of cow milk and its products

Cow milk and its products (yogurt and soft cheese) have an important role in human nutrition. The compositions
of cow milk, yogurt, and soft cheese are summarized in Table 1. The result showed that milk and its products had good nutritive essential constituents. Milk and yogurt were comparable in their content of protein, fat, lactose, ash, and moisture. The protein and fat content of soft cheese were higher than milk and yogurt. Soft cheese mineral content (Na, Ca, Mg, Fe, Zn, and Cu) was significantly higher than milk and yogurt except for potassium content was higher in yogurt. The yield of soft cheese was 30% from cow milk.

3.2 Mineral content and osteoporosis evaluation

K, Mg, Ca, and P contents were determined in serum and urine of rats (Table 2). Serum and urine contents of K and Mg were not affected by the administration of Coragen (1/20 LD$_{50}$ or 1/40 LD$_{50}$). However, Coragen (1/20 or 1/40 LD$_{50}$) administration significantly decreased the level of Ca and P in serum and significantly increased their level in urine when compared with normal control rats. Treatments with cow milk, yogurt, and soft cheese ameliorated the harmful effect of Coragen. Milk and milk products significantly increased the concentration of Ca in the serum of rats administered with Coragen at a dose of 1/20 LD$_{50}$. Only yogurt and soft cheese treatments significantly increased serum Ca and P levels in Coragen-induced rats with a dose of 1/40 LD$_{50}$. However, the curative effect of yogurt was more effective than cow milk and soft cheese treatments on the reduction of Ca and P in the urine of rats administered with Coragen at a dose of 1/20 LD$_{50}$.

The result showed that administration of Coragen at a dose of either 1/20 or 1/40 LD$_{50}$ significantly decreased the length, thickness, and weight of femur and tibia when compared with normal control rats (Table 3). The treatments with cow milk, yogurt, and soft cheese significantly increased the length and weight of femur in Coragen-induced rats (1/20 LD$_{50}$ or 1/40 LD$_{50}$). Only yogurt treatment significantly increased the thickness of femur in the Coragen (1/40 LD$_{50}$) administration group. The thickness of the tibia significantly increased after treatment of milk, yogurt, and cheese for both doses of Coragen-administered groups. In addition, yogurt treatment significantly increased the length of the tibia in the Coragen-administered (1/20 LD$_{50}$) group. The result showed that yogurt treatment was superior to the other treatments.

Administration of Coragen significantly decreased the bone strength of the femur and tibia when compared with normal control rats (Table 4). Cow milk and its products increased the breaking force in Coragen-induced rats at a dose of 1/40 LD$_{50}$. However, only yogurt significantly increased the breaking force of femur and tibia in Coragen-induced rats at a dose of 1/20 LD$_{50}$. Coragen significantly reduced femur Ca and P content when compared with normal control rats (Table 4). Milk, yogurt, and soft cheese treatments significantly increased Ca content in the femur of Coragen-induced rats with both doses but all treatments did not affect femur P content.

Bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) of the total, proximal, and distal bone were presented in Table 5. The total, proximal, and distal bone parameters (BMA, BMC, and BMD) were significantly decreased in Coragen-administered rats when compared with normal control rats. Treatment with milk and its products reversed the

### Table 1: Chemical composition of cow milk, yogurt, and soft cheese

|          | Milk          | Yogurt        | Soft cheese  |
|----------|---------------|---------------|--------------|
| Total    | 12.5 ± 1.02b  | 13.1 ± 1.00b  | 46.0 ± 2.88a |
| solids (%) | 87 ± 6.66a    | 87 ± 5.97a    | 53.0 ± 4.01b |
| Water (%) | 3.3 ± 0.27b   | 3.4 ± 0.21b   | 16.3 ± 1.02a |
| Lactose (%) | 4.6 ± 0.31ab  | 5.5 ± 0.33a   | 3.2 ± 0.22b  |
| Fat (%)   | 3.7 ± 0.24ab  | 3.2 ± 0.19b   | 19.8 ± 1.12a |
| Ash (%)   | 0.8 ± 0.04b   | 0.9 ± 0.06b   | 7.3 ± 0.46a  |
| Na (mg/L) | 37 ± 2.41b    | 40 ± 2.12b    | 2769 ± 109a  |
| K (mg/L)  | 102 ± 9.21b   | 1080 ± 7.11a  | 115 ± 7.21b  |
| Ca (mg/L) | 110 ± 8.71b   | 118 ± 3.12b   | 456 ± 21.72a |
| P (mg/L)  | 90 ± 4.01b    | 101 ± 6.26b   | 273 ± 15.55a |
| Mg (mg/L) | 13.10 ± 1.00b | 16 ± 1.00b    | 45 ± 2.76a   |
| Fe (µg/mL)| 0.35 ± 0.02a  | 0.2 ± 0.01b   | 0.2 ± 0.01b  |
| Zn (µg/mL)| 1.30 ± 0.07b  | 0.2 ± 0.01c   | 1.9 ± 0.10a  |
| Cu (µg/mL)| 0.10 ± 0.01b  | 0.12 ± 0.01b  | 0.31 ± 0.02a |
| Vit. A    | 0.36 ± 0.02b  | 0.24 ± 0.01c  | 0.70 ± 0.04a |
| Vit. C    | 5.0 ± 0.33a   | 0.11 ± 0.01b  | 0.02 ± 0.001c|
| Vit. B1   | 0.02 ± 0.001c | 0.06 ± 0.004b | 0.08 ± 0.005a|
| Vit. B2   | 0.04 ± 0.02c  | 0.18 ± 0.01b  | 0.37 ± 0.02a |
| Vit. D    | 0.08 ± 0.05b  | 0.10 ± 0.01b  | 0.15 ± 0.01a |
| Vit. E    | 0.10 ± 0.01b  | 0.09 ± 0.01b  | 0.13 ± 0.01a |

Values are mean ± SE; the same letter in each row is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.
Table 2: Effect of milk, yogurt, and soft cheese on mineral concentration in serum and urine of Coragen-induced rats

|                | Serum (mg/dL) | Urine (mg/dL) |
|----------------|---------------|---------------|
|                | K             | Mg            | Ca            | P             | K             | Mg            | Ca            | P             |
| G1 (control)   | 18.08 ± 1.34a | 10.34 ± 0.80a | 10.44 ± 0.78a | 25.21 ± 1.42a | 118.03 ± 6.66a | 11.00 ± 0.61a | 10.66 ± 0.61c | 101.11 ± 6.61c |
| G2 (control 1/20 LD₅₀ Coragen) | 19.01 ± 1.73a | 11.00 ± 0.94a | 8.01 ± 0.66c  | 19.76 ± 1.37b | 126.61 ± 9.14a | 10.98 ± 0.54a | 12.76 ± 0.77a | 124.02 ± 7.23a |
| G3 (1/20 LD₅₀ Coragen + 5 g milk) | 18.77 ± 1.62a | 10.43 ± 0.78a | 9.10 ± 0.72c  | 21.01 ± 1.72b | 122.71 ± 7.16a | 11.01 ± 0.73a | 12.00 ± 0.69ab | 119.47 ± 6.41a |
| G4 (1/20 LD₅₀ Coragen + 5 g yogurt) | 18.00 ± 1.77a | 10.66 ± 0.89a | 9.71 ± 0.74a  | 23.21 ± 1.66a | 119.99 ± 7.18a | 10.87 ± 0.81a | 11.66 ± 0.59b  | 111.51 ± 7.77b |
| G5 (1/20 LD₅₀ Coragen + 5 g cheese) | 17.97 ± 1.62a | 10.74 ± 0.69a | 9.65 ± 0.76a  | 22.22 ± 1.74ab| 120.27 ± 8.74a | 10.98 ± 0.98a | 11.81 ± 0.57ab | 115.07 ± 6.97ab |
| G6 (control 1/40 LD₅₀ Coragen) | 18.61 ± 1.41a | 10.81 ± 0.94a | 8.49 ± 0.69b  | 20.16 ± 1.59b | 121.27 ± 6.21a | 11.00 ± 0.087a| 12.16 ± 0.81ab | 115.61 ± 8.00ab |
| G7 (1/40 LD₅₀ Coragen + 5 g milk) | 18.12 ± 1.21a | 11.00 ± 0.59a | 9.34 ± 0.80b  | 22.00 ± 1.56ab| 119.71 ± 6.21a | 10.26 ± 0.78a | 11.21 ± 0.69bc | 109.00 ± 6.21bc |
| G8 (1/40 LD₅₀ Coragen + 5 g yogurt) | 18.00 ± 1.32a | 10.61 ± 0.71a | 9.91 ± 0.82a  | 23.99 ± 1.99a | 120.11 ± 7.14a | 10.99 ± 0.91a | 11.2 ± 0.69bc  | 109.00 ± 6.21bc |
| G9 (1/40 LD₅₀ Coragen + 5 g cheese) | 18.21 ± 1.41a | 10.43 ± 0.82a | 9.82 ± 0.82a  | 23.12 ± 2.00a | 112.00 ± 6.21a | 11.10 ± 0.72a | 11.47 ± 0.62bc | 110.52 ± 5.91bc |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

Table 3: Femur and tibia length, thickness, and weight of the different experimental groups

|                | Femur bone | Tibia bone |
|----------------|------------|------------|
|                | Length (mm) | Thickness (mm) | Weight (g) | Length (mm) | Thickness (mm) | Weight (g) |
| G1 (control)   | 18.55 ± 1.11a | 1.68 ± 0.090a | 0.51 ± 0.032a | 25.21 ± 1.76a | 1.11 ± 0.063a | 0.44 ± 0.033a |
| G2 (control 1/20 LD₅₀ Coragen) | 15.20 ± 0.99b | 1.36 ± 0.071b | 0.28 ± 0.022e | 19.12 ± 1.47c | 0.71 ± 0.042d | 0.30 ± 0.021b |
| G3 (1/20 LD₅₀ Coragen + 5 g milk) | 17.23 ± 1.00a | 1.47 ± 0.081ab | 0.34 ± 0.021d | 20.67 ± 1.84bc | 0.84 ± 0.056b | 0.36 ± 0.027b |
| G4 (1/20 LD₅₀ Coragen + 5 g yogurt) | 17.88 ± 1.03a | 1.52 ± 0.081ab | 0.40 ± 0.031c | 21.72 ± 1.53b | 0.89 ± 0.056b | 0.37 ± 0.030ab |
| G5 (1/20 LD₅₀ Coragen + 5 g cheese) | 17.37 ± 1.01a | 1.48 ± 0.090b | 0.35 ± 0.032d | 20.71 ± 1.62bc | 0.85 ± 0.055bc | 0.35 ± 0.028b |
| G6 (control 1/40 LD₅₀ Coragen) | 16.88 ± 0.97b | 1.42 ± 0.071b | 0.31 ± 0.023d | 20.31 ± 1.50bc | 0.82 ± 0.053c | 0.36 ± 0.025b |
| G7 (1/40 LD₅₀ Coragen + 5 g milk) | 17.90 ± 0.98a | 1.50 ± 0.082ab | 0.40 ± 0.032c | 22.00 ± 2.00b | 0.90 ± 0.051b | 0.38 ± 0.030ab |
| G8 (1/40 LD₅₀ Coragen + 5 g yogurt) | 18.01 ± 1.01a | 1.58 ± 0.101a | 0.45 ± 0.030b | 22.61 ± 1.87ab | 0.96 ± 0.067b | 0.40 ± 0.030ab |
| G9 (1/40 LD₅₀ Coragen + 5 g cheese) | 17.87 ± 1.00a | 1.49 ± 0.081ab | 0.41 ± 0.021bc | 21.97 ± 1.79b | 0.91 ± 0.071b | 0.38 ± 0.029ab |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.
Table 4: Femur and tibia breaking force and femur mineral content of different experimental groups

|                | Breaking force                     | Femur mineral content |
|----------------|-----------------------------------|-----------------------|
|                | Femur (N)                         | Tibia (N)             | Calcium (g/100 g) | Phosphorus (g/100 g) |
| G1 (control)   | 105.5 ± 9.74a                     | 81.32 ± 5.12a         | 95.27 ± 5.47a     | 11.00 ± 0.62a         |
| G2 (control 1/20 LD<sub>50</sub> Coragen) | 71.17 ± 4.13d | 65.47 ± 4.34b | 49.74 ± 3.24c | 8.91 ± 0.54b |
| G3 (1/20 LD<sub>50</sub> Coragen + 5 g milk) | 76.00 ± 4.11cd | 69.01 ± 5.00b | 78.71 ± 4.26b | 9.46 ± 0.71b |
| G4 (1/20 LD<sub>50</sub> Coragen + 5 g yogurt) | 78.42 ± 3.99c | 71.32 ± 5.27ab | 85.71 ± 5.71ab | 10.01 ± 0.58ab |
| G5 (1/20 LD<sub>50</sub> Coragen + 5 g cheese) | 75.89 ± 5.01cd | 69.34 ± 4.99b | 77.77 ± 4.27b | 10.23 ± 0.57ab |
| G6 (1/40 LD<sub>50</sub> Coragen) | 76.89 ± 4.74cd | 68.42 ± 5.01b | 51.11 ± 3.11c | 10.12 ± 0.60ab |
| G7 (1/40 LD<sub>50</sub> Coragen + 5 g milk) | 85.12 ± 4.44b | 71.62 ± 5.24ab | 81.12 ± 5.12b | 10.25 ± 0.58ab |
| G8 (1/40 LD<sub>50</sub> Coragen + 5 g yogurt) | 90.14 ± 6.16b | 74.31 ± 5.46ab | 87.22 ± 5.22ab | 10.56 ± 0.61a |
| G9 (1/40 LD<sub>50</sub> Coragen + 5 g cheese) | 86.00 ± 5.67b | 72.01 ± 5.96ab | 81.78 ± 5.55b | 10.78 ± 0.71a |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

reduction of total BMA, BMC, and BMD in Coragen-induced rats (1/20 LD<sub>50</sub>). Only yogurt significantly altered the total BMD in Coragen-induced rats (1/40 LD<sub>50</sub>). All treatments significantly increased distal bone BMC and BMD in either Coragen-induced rats at a dose of 1/20 LD<sub>50</sub> or Coragen-induced rats at a dose of 1/40 LD<sub>50</sub>. Distal bone BMA was significantly increased after treatment with milk and its products in Coragen-induced rats at a dose of 1/20 LD<sub>50</sub>. However, only yogurt significantly elevated distal bone BMA in Coragen-induced rats at a dose of 1/40 LD<sub>50</sub>. Proximal bone BMC was significantly improved after treatment with milk and its products in both doses of Coragen-induced rats. Cow milk, yogurt, and cheese significantly elevated the proximal bone BMA in Coragen-induced rats at a dose of 1/20 LD<sub>50</sub>. Proximal bone BMD was insignificantly improved by treatments.

3.3 Protein profile and phosphatase activity

Serum total protein, albumin, and globulin contents were altered by Coragen ingestion (Table 6). The changed values of total proteins, albumin, and globulin showed a significant decrease either by 1/20 LD<sub>50</sub> or by 1/40 LD<sub>50</sub> of Coragen ingestion, but 1/20 LD<sub>50</sub> was more effective than 1/40 LD<sub>50</sub>. Treatments with cow milk and its products (yogurt and cheese) significantly attenuated the harmful effect of Coragen (1/20 LD<sub>50</sub> or 1/40 LD<sub>50</sub>) on protein profile and globulin content in serum and improved these disturbances. Serum albumin was significantly increased after treatment with milk, yogurt, and cheese in Coragen-induced rats (1/40 LD<sub>50</sub>).

Coragen ingestion caused a highly significant stimulation in ALP and ACP activity when compared with normal control rats (Table 6). The influence of Coragen 1/20 LD<sub>50</sub> on ALP and ACP activities was more than that of Coragen 1/40 LD<sub>50</sub>. In addition, the results showed that the levels of ALP and ACP activity were improved upon treatment with cow milk and its products (yogurt and cheese). However, the treatment with yogurt was superior.

3.4 The antioxidant system

The effects of Coragen toxicity on the total antioxidant capacity (TAC), glutathione (GSH), and antioxidant enzyme activity were investigated (Table 7). Administering Coragen at a dose of 1/20 LD<sub>50</sub> and 1/40 LD<sub>50</sub> significantly decreased serum TAC level, GSH, and antioxidant enzyme activity (GST, GPX, and SOD). Yogurt treatment led to a significant elevation of GSH and TAC level and antioxidant enzyme activity in rats induced by Coragen at a dose of 1/20 LD<sub>50</sub>. However, milk and soft cheese ameliorated the harmful effect of Coragen but not to a significant level.

4 Discussion

Osteoporosis is a bone metabolic disease characterized by bone mineral density reduction and bone microstructure degradation, which can increase bone fragility and fracture risk [46,47]. Toxicological studies reported that exposure to pesticides, such as organochlorine, can alter bone mineralization and composition and may lead to osteoporosis [3–7]. Chlorantraniliprole (the active ingredient of Coragen) is a ryanodine receptor activator and
Table 5: Total, proximal, and distal bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) of different experimental groups

| Group          | Total BMA (cm²) | Total BMC (g) | Total BMD (g/cm³) | Proximal BMA (cm²) | Proximal BMC (g) | Proximal BMD (g/cm³) | Distal BMA (cm²) | Distal BMC (g) | Distal BMD (g/cm³) |
|----------------|----------------|----------------|-------------------|-------------------|------------------|---------------------|----------------|---------------|------------------|
| G1 (control)   | 2.34 ± 0.161a  | 0.224 ± 0.013a | 0.120 ± 0.008a    | 0.615 ± 0.041a    | 0.161 ± 0.010a   | 0.099 ± 0.006a      | 0.653 ± 0.041a  | 0.163 ± 0.009a | 0.199 ± 0.011a   |
| G2 (control 1/20 LD₅₀ Coragen) | 1.06 ± 0.072d | 0.099 ± 0.006d | 0.012 ± 0.003d    | 0.411 ± 0.027d    | 0.045 ± 0.003d    | 0.080 ± 0.006b      | 0.410 ± 0.030d  | 0.040 ± 0.003d | 0.088 ± 0.005c   |
| G3 (1/20 LD₅₀ Coragen + 5 g milk) | 1.62 ± 0.100bc | 0.164 ± 0.007bc | 0.051 ± 0.003c    | 0.469 ± 0.030c    | 0.089 ± 0.005c    | 0.085 ± 0.007b      | 0.483 ± 0.032c  | 0.072 ± 0.004c | 0.121 ± 0.007d   |
| G4 (1/20 LD₅₀ Coragen + 5 g yogurt) | 1.88 ± 0.100bc | 0.185 ± 0.011b  | 0.060 ± 0.004bc   | 0.502 ± 0.032b    | 0.099 ± 0.006bc   | 0.098 ± 0.007ab     | 0.500 ± 0.033c  | 0.080 ± 0.005bc | 0.147 ± 0.008b   |
| G5 (1/20 LD₅₀ Coragen + 5 g cheese) | 1.60 ± 0.099bc | 0.159 ± 0.010c  | 0.050 ± 0.003c    | 0.472 ± 0.028bc   | 0.090 ± 0.006c    | 0.086 ± 0.005b      | 0.490 ± 0.032c  | 0.071 ± 0.006c | 0.119 ± 0.006d   |
| G6 (control 1/40 LD₅₀ Coragen) | 1.71 ± 0.101bc | 0.168 ± 0.012bc | 0.051 ± 0.004c    | 0.481 ± 0.031bc   | 0.051 ± 0.003d    | 0.088 ± 0.006b      | 0.489 ± 0.040c  | 0.051 ± 0.003d | 0.100 ± 0.006e   |
| G7 (1/40 LD₅₀ Coragen + 5 g milk) | 1.82 ± 0.113bc | 0.180 ± 0.012b  | 0.061 ± 0.005bc   | 0.500 ± 0.040ab   | 0.099 ± 0.007bc   | 0.090 ± 0.007ab     | 0.502 ± 0.041c  | 0.081 ± 0.005bc | 0.148 ± 0.009b   |
| G8 (1/40 LD₅₀ Coragen + 5 g yogurt) | 2.00 ± 0.0103bc | 0.189 ± 0.012b  | 0.070 ± 0.004b    | 0.589 ± 0.044a    | 0.1089 ± 0.008b   | 0.094 ± 0.007a      | 0.567 ± 0.039b  | 0.098 ± 0.006b | 0.161 ± 0.010b   |
| G9 (1/40 LD₅₀ Coragen + 5 g cheese) | 1.87 ± 0.111bc | 0.179 ± 0.011b  | 0.062 ± 0.004bc   | 0.501 ± 0.039b    | 0.100 ± 0.007bc   | 0.090 ± 0.006ab     | 0.501 ± 0.032c  | 0.082 ± 0.005bc | 0.150 ± 0.009b   |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.
### Table 6: Protein profile and phosphatase activity of different experimental groups

|                | Protein profile | Phosphatases activity |
|----------------|-----------------|------------------------|
|                | Total protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) | ACP (U/L/mg protein) | ALP (U/L/mg protein) |
| G1 (control)   | 6.76 ± 0.41a | 4.50 ± 0.31a | 2.26 ± 0.17a | 46.12 ± 3.33a | 81.07 ± 5.12e |
| G2 (control 1/20 LD50 Coragen) | 4.10 ± 0.29a | 2.89 ± 0.20c | 1.21 ± 0.10d | 60.11 ± 4.12c | 150.11 ± 7.78a |
| G3 (1/20 LD50 Coragen + 5 g milk) | 5.11 ± 0.32bc | 3.18 ± 0.21bc | 1.93 ± 0.09b | 56.00 ± 3.87bc | 100.21 ± 6.24c |
| G4 (1/20 LD50 Coragen + 5 g yogurt) | 5.20 ± 0.31bc | 3.42 ± 0.19bc | 1.78 ± 0.06bc | 51.11 ± 3.11b | 91.61 ± 6.61d |
| G5 (1/20 LD50 Coragen + 5 g cheese) | 5.13 ± 0.28bc | 3.20 ± 0.22bc | 1.93 ± 0.07b | 55.16 ± 3.22bc | 99.71 ± 7.12c |
| G6 (control 1/40 LD50 Coragen) | 4.68 ± 0.31 | 3.10 ± 0.18c | 1.58 ± 0.08c | 55.51 ± 4.00c | 141.11 ± 8.82b |
| G7 (1/40 LD50 Coragen + 5 g milk) | 5.41 ± 0.32bc | 3.61 ± 0.18b | 1.80 ± 0.08b | 52.14 ± 3.27bc | 94.71 ± 5.41cd |
| G8 (1/40 LD50 Coragen + 5 g yogurt) | 5.62 ± 0.40b | 3.72 ± 0.27b | 1.90 ± 0.10b | 50.11 ± 3.27b | 90.00 ± 5.55d |
| G9 (1/40 LD50 Coragen + 5 g cheese) | 5.44 ± 0.36b | 3.66 ± 0.26b | 1.78 ± 0.11bc | 53.00 ± 4.00bc | 95.00 ± 5.61d |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

### Table 7: The antioxidant capacity and activity of different experimental groups

|                | Antioxidant |
|----------------|-------------|
|                | Total antioxidant capacity (mM/L) | GSH (mM/mL) | GST activity (mM/mL) | GPx activity (U/L) | SOD activity (U/L) |
| G1 (control)   | 1.75 ± 0.09a | 0.52 ± 0.031a | 53.27 ± 3.71a | 920.516 ± 50.11a | 351 ± 20.1a |
| G2 (control 1/20 LD50 Coragen) | 1.50 ± 0.10b | 0.38 ± 0.020c | 46.72 ± 2.94b | 785.11 ± 41.11b | 262 ± 18.3c |
| G3 (1/20 LD50 Coragen + 5 g milk) | 1.60 ± 0.07ab | 0.42 ± 0.023bc | 48.66 ± 3.00ab | 812.03 ± 51.03ab | 298 ± 19.4b |
| G4 (1/20 LD50 Coragen + 5 g yogurt) | 1.67 ± 0.09a | 0.44 ± 0.030b | 50.97 ± 2.94a | 851.21 ± 43.94a | 300 ± 20.2b |
| G5 (1/20 LD50 Coragen + 5 g cheese) | 1.62 ± 0.08a | 0.42 ± 0.026bc | 48.71 ± 2.48ab | 801.11 ± 52.22ab | 295 ± 18.9b |
| G6 (control 1/40 LD50 Coragen) | 1.54 ± 0.08b | 0.40 ± 0.028bc | 49.88 ± 3.01ab | 800.21 ± 50.31ab | 284 ± 18.8bc |
| G7 (1/40 LD50 Coragen + 5 g milk) | 1.68 ± 0.09ab | 0.46 ± 0.029b | 50.27 ± 3.11ab | 842.00 ± 42.48ab | 317 ± 20.4ab |
| G8 (1/40 LD50 Coragen + 5 g yogurt) | 1.70 ± 0.07ab | 0.48 ± 0.031ab | 51.34 ± 3.12ab | 867.77 ± 49.99a | 321 ± 21.0ab |
| G9 (1/40 LD50 Coragen + 5 g cheese) | 1.67 ± 0.07ab | 0.44 ± 0.030b | 50.32 ± 3.23ab | 851.21 ± 54.21ab | 311 ± 22.10ab |

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.
controls the release of calcium from intracellular stores in insects [8]. The flow of calcium is regulated by ryanodine receptors, which mediate several physiological cellular processes such as skeletal muscle excitation-contraction coupling process, neurotransmission, neurohormones release, and cardiac contraction [48]. In our previous study, Coragen with different doses reduced serum calcium in rats [13]. There is no report available regarding the possibility of bone toxicity and osteoporosis after prolonged exposure to Coragen in rats. Therefore, this study evaluated the effect of Coragen at two different doses and assessed the potential ameliorative effect of milk and milk products.

Bone is the main component of the skeletal system and consists of 50–70% of minerals, 20–40% of organic matter, and 5–10% of water. The bone functions are locomotion, bone marrow protection, and storage of calcium and phosphate. Calcium and phosphate are key components for hydroxylapatite which is an essential mineral compound in normal bone and responsible for the rigidity of bones [17]. When the calcium circulation level decreases after calcium elimination from the body through urination, parathyroid hormone is activated causing increased bone turnover [49]. Blood calcium deficiency is associated with the risk of osteoporosis [46]. Thus, calcium and phosphorus intake is important for healthy bones and normal BMD. The high dietary ratio of Ca/P has a positive effect on bone mass [50]. Dairy products are considered the best dietary source of calcium due to their high calcium content and high absorption rate [51]. Cow milk and its products (yogurt and soft cheese) have higher calcium content than camel and buffalo milk and considerable amounts of phosphorus and vitamin A and D more than camel and buffalo milk [52]. Numerous clinical studies on dairy products and calcium supplementation in children reported that dairy products and calcium have a beneficial effect on bone mineral mass during growth [53,54]. Bone mineral density and bone strength were increased after treatment with cheese fortified with calcium in rats [55]. Bovine milk provided a positive effect on bone strength, bone length, and bone mineralization in rats [56]. Dried yogurt supplemented with chicory increased the strength of bones and bone calcium concentration in calcium-deficient rats [57].

In the current study, the reduction of calcium and phosphorus levels in blood and bones by Coragen was reversed by treatment with milk products. Calcium content reduction was associated with a reduction in breaking force, total, proximal, and distal BMA, BMC, and BMD in Coragen intoxicated control groups. Treatment with milk, yogurt, and soft cheese exhibited a positive effect on bone characters (BMA, BMC and BMD) and breaking force.

Yogurt was the best treatment to protect against bone loss, which may be due to its richness in probiotics. Probiotics produce short-chain fatty acids, which decrease the pH of the intestinal tract, consequently improving intestinal calcium absorption and may prevent or decrease bone loss and restore the decreased levels of plasma Ca [58]. Studies reported that some strains of probiotics (Lactobacillus casei, Lactobacillus plantarum, Lactobacillus paracasei, and Bifidobacterium longum) had a positive influence on osteoporosis [58–60]. Osteoblasts and osteoclasts cells are responsible for bone formation and bone resorption, respectively, and both influence bone density. When osteoplastic bone resorption rate becomes higher than osteoplastic bone formation rate, bone mass reduces and osteoporosis occurs [61]. Probiotics affect osteoblasts and osteoclasts cells during the process of bone remodeling [58]. Lactobacillus casei 393 from fermented milk improved BMD reduction in ovarie- tomized rats and increased bone strength [59]. Moreover, probiotics synthesize vitamins such as vitamin K, D, C, and folate which are essential for bone formation and growth [58]. There is some evidence suggesting that Lactobacillus plantarum has degradation potential toward organophosphate pesticides [27,62,63].

Alkaline phosphatase and acid phosphatase are markers for bone formation and bone resorption, respectively [64]. ALP and ACP activity were significantly increased in Coragen-induced rats when compared with normal control rats. Dutta et al. [12] reported that Coragen increased the level of alkaline phosphatase and that induction was reduced by Pterocarpus santalinus treatment in rats. The elevated ALP and ACP activity could contribute to a high bone turnover rate, through an elevation in bone formation and resorption, with bone resorption usually higher than bone formation which may cause bone loss [65]. The positive role of milk and milk products supplemented diets was observed in the present study through the improvement in bone metabolic markers ALP and ACP activity. Hypoproteinemia is associated with hypocalcemia [66]. There is a positive correlation between albumin/globulin ratio and bone mineral density [67]. Al-Aqaby et al. [68] reported that total protein, albumin, and globulin levels increased after treatment with milk supplemented with probiotics. In the present study, protein profile (total, albumin, and globulin) was decreased by Coragen. Treatment with milk, yogurt, and soft cheese reversed that reduction. The alteration of serum total protein and protein fractions level (albumin and globulin) resulted in parallel changes in serum calcium level in the present study.

One of many possible underling mechanisms of pesticide toxicity is oxidative stress production. Coragen
administration has been found to cause oxidative stress and alteration of the antioxidant defense system [12–14]. Oxidative stress occurs by an imbalance between the antioxidant defense system and the production of free radicals and that can lead to tissue damage and numerous pathological conditions. The antioxidant defense system (enzymatic and non-enzymatic) is scavenging various reactive oxygen species and free radicals by different mechanisms [69]. SOD, catalase, GPX, and GST enzymes are considered the first line of defense during the reactive oxygen species scavenging process and maintain the balance between the antioxidant defense system and the production of free radicals [69]. SOD catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide; hydrogen peroxide, in turn, is converted by catalase to oxygen and water. GPX is an antioxidant enzyme that plays a vital role in the reduction of hydrogen peroxide by holding the status of a redox system (GSH/GSSG) in the nonenzymatic antioxidant GSH system. Glutathione transferase has several biological roles including cell protection against xenobiotics and oxidative stress [69]. In the present study, the significant reduction in total antioxidant capacity, GSH level, and the antioxidant enzyme activity of SOD, GPX, and GST due to exposure to Coragen (1/20 LD50 and 1/40 LD50 doses) for a prolonged time suggests the onset of Coragen-induced oxidative stress and free radical production in rats. Yogurt treatment was superior in increasing the antioxidant defense system in rats induced by Coragen at a dose of 1/20 LD50. Yogurt antioxidant efficacy may be attributed to its probiotic and prebiotic content which is usually more than milk or soft cheese. In vitro and in vivo studies reported that lactic acid bacteria and yogurt supplementation modulate free radical production by reducing the oxidative stress marker level and increasing antioxidant enzyme activity [70–72]. Lactobacillus acidophilus increased the total antioxidant capacity in pesticides-induced rats [29]. Some studies showed that dried plums rich in antioxidant agents had a positive effect on the whole body and spine BMD, and the trabecular bone [73].

5 Conclusion

Coragen ingestion had negative effects on calcium and bone characters leading to osteoporosis as a result of BMD reduction. Moreover, Coragen ingestion showed bone osteoclasts activity higher than bone osteoblasts activity because of ALP and ACP activity alteration. Treatment with milk, yogurt, and soft cheese attenuated the disturbing effects of Coragen toxicity. These desirable influences of cow milk and its products varied with the different products. Yogurt treatment resulted in the highest improvement for the studied parameters of intoxicated animals. Several essential nutrients and different components are provided by milk and functional dairy products. Yogurt was superior to milk and soft cheese treatments, which may be due to the high prebiotic and probiotic content. Adding milk and milk products to the diet may protect against the toxicological effects of Coragen.

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References

[1] Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. Sci World J. 2016;6:295–310. doi: 10.1100/tsw.2006.38.
[2] Tudi M, Daniel Ruan H, Wang L, Luu Y, Sadler R, Connell D, et al. Agriculture development, pesticide application and its impact on the environment. Int J Env Res Public Health. 2021;18(3):1112. doi: 10.3390/ijerph18031112.
[3] Compston JE, Vedi S, Stephen AB, Bord S, Lyons AR, Hodges SJ, et al. Reduced bone formation after exposure to organophosphates. Lancet. 1999;354(9192):1791–2. doi: 10.1016/s0140-6736(99)04466-9.
[4] Hodgson S, Thomas L, Fattore E, Lind PM, Alfenen T, Hellström L, et al. Bone mineral density changes in relation to environmental PCB exposure. Env Health Perspect. 2008;116(9):1162–6. doi: 10.1289/ehp.11107.
[5] Rignell-Hydbom A, Skerfving S, Lundh T, Lindh CH, Elmstähl S, Bjellerup P, et al. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. Env Res. 2009;109(8):991–6. doi: 10.1016/j.envres.2009.08.008.
[6] Ali SJ, Ellur G, Patel K, Sharan K. Chlorpyrifos exposure induces parkinsonian symptoms and associated bone loss in adult Swiss Albino mice. Neurotox Res. 2019;36(4):700–11. doi: org/10.1007/s12640-019-00092-0.
[7] Ali SJ. Monocrotophos, an organophosphorus insecticide, induces cortical and trabecular bone loss in Swiss Albino mice. Chem Biol Interact. 2020;25(39):109112. doi: 10.1016/j.cbi.2020.109112.

[8] Bassi A, Rison JL, Wiles JA. Chlorantraniliprole (DPX-E2Y45, Rynaxypyr®; Coragen®), a new diame insecticide for control of codling moth (Cydia pomonella), Colorado potato beetle (Leptinotarsa decemlineata) and European grapevine moth (Lobesia botrana). In: Bassi A, Rison JL, Wiles JA, editors. Proceedings 9th Slovenian Conference on Plant Protection with International Participation. Nova Gorica, Slovenia: Plant Protection Society of Slovenia; 2009 March 4–5. p. 39–45.

[9] EFSA. Conclusion on the peer review of the pesticide risk assessment of the active substance chlorantraniliprole. EFSA J. 2013;11(6):3143. doi: 10.2903/j.efsa.2013.3143.

[10] Kadala A, Charretton M, Charnet P, Collet C. Honey bees long-lasting locomotor deficits after exposure to the diame chlorantraniliprole are accompanied by brain and muscular calcium channels alterations. Sci Rep. 2019;9:2153. doi: org/10.1038/s41598-019-39193-3.

[11] Bantu N, Vakita VR. Acute toxicity of chlorantraniliprole to freshwater fish Channa punctatus (Bloch). Adv Zool Botany. 2013;1(4):78–82. doi: 10.13189/azb.2013.010402.

[12] Dutta K, Ali M, Najam A, Kumar R, Kumar A. Ameliorative effect of seed extract of Pterocarpus santalinus on Coragen induced haematological alterations and serum biochemical changes in Charles Foster rats. J Toxicol Env Health Sci. 2014;6:194–202. doi: 10.5897/JTEHS2014.0324.

[13] Abdel-Mobdy YE, Moustafa MAM, Nahas AHA, Abdel-Rahman HR. Sub-acute and sub-chronic effect of chlorantra-niliprole (coragen®20% sc) on albino rat. J Plant Prot Path, Mansoura Univ. 2017;8(6):297–303. doi: 10.21608/jppp.2017.64308.

[14] Meligi NM, Hassanand HF, Honyda SM. Coragen induced toxicity and the ameliorative effect of an Origanum majorana L, in male albino Rats. J Am Sci. 2019;15(9):33–44. doi: 10.7537/marsjas150919.05.

[15] Hassan HF, Mohammed HS, Meligi NM. Potential impact of marjoram on coragen-induced physiological and histological alteration in male albino rats. Egypt J Zool. 2021;75:25–38. doi: 10.12816/EJZ.2020.49316.1044.

[16] Mishra AK, Chandraseharan VK, Jose N, Sudarsanam TD. Chlorantraniliprole: an unusual insecticide poisoning in humans. Indian J Crit Care Med. 2016;20(12):742–4. doi: 10.4103/0972-5229.195718.

[17] Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. J Clin Pathol. 2008;61(5):577–87. doi: 10.1136/jcp.2007.048868.

[18] Blair HC, Schlesinger PH, Huang CLH, Zaidi M. Calcium signaling and calcium transport in bone disease. Subcell Biochem. 2007;45:539–62. doi: 10.1007/978-1-4020-6191-2_21.

[19] Bhat ZF, Bhat H. Milk and dairy products as functional foods: a review. Int J Dairy Sci. 2011;6:1–12. doi: 10.3923/ijds.2011.1.12.

[20] Savaiano DA, Hutkins RW. Yogurt, cultured fermented milk, and health: a systematic review. Nutr Rev. 2021;79(5):599–614. doi: 10.1093/nutrit/nuaa013.

[21] Marteau PR, Vrese MD, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr. 2003;72(2):430S–6S. doi: 10.1093/ajcn/73.2.430s.

[22] Vasiljevic T, Shah NB. Probiotics – from Metchnikoff to bioactives. Int Dairy J. 2008;18(5):714–28. doi: 10.1016/j.idairyj.2008.03.004.

[23] Martins N, Oliveira MBPP, Ferreira ICFR. Development of functional dairy foods. In: Mérillon JM, Ramawat K, editors. Bioactive molecules in food. Reference series in phytochemistry. Cham: Springer; 2018. p. 1–14. doi: 10.1007/978-3-319-54528-8_35.

[24] Tanabe R, Haraiwama K, Sogabe N, Sugimoto A, Kawamura Y, Takasugi S, et al. Retention of bone strength by feeding of milk and dairy products in ovariectomized rats: involvement of changes in serum levels of 1alpha, 25(OH)2D3 and FGF23. J Nutr Biochem. 2013;24(6):1000–7. doi: 10.1016/j.jnutbio.2012.07.004.

[25] Đorđević TM, Siler-Marinković SS, Durović-Pejević RD, Dimitrijević-Branković SI, Gajić Umljeniđić JS. Dissipation of pirimiphos-methyl during wheat fermentation by Lactobacillus plantarum. Lett Appl Microbiol. 2013;57(5):412–9. doi: 10.1111/lam.12128.

[26] Zhang YH, Xu D, Liu JQ, Zhao XH. Enhanced degradation of five organophosphorus pesticides in skimmed milk by lactic acid bacteria and its potential relationship with phosphatase production. Food Chem. 2014;164:173–8. doi: 10.1016/j.foodchem.2014.05.059.

[27] Daisley BA, Trinder M, McDowell TW, Collins SL, Sumarah MW, Reid G. Microbiota-mediated modulation of organophosphate insecticide toxicity by species-dependent interactions with Lactobacilli in a Drosophila melanogaster insect model. Appl Env Microbiol. 2018;84:e02820-17. doi: 10.1128/AEM.02820-17.

[28] George F, Daniel C, Thomas M, Singer E, Guilbaud A, Tessier F, et al. Occurrence and dynamism of lactic acid bacteria in distinct ecological niches: a multifaceted functional health perspective. Front Microbiol. 2018;9:2899. doi: 10.3389/fmicb.2018.02899.

[29] Bahr HI, Hamad R, Ismail SAA. The impact of Lactobacillus acidophilus on hepatic and colonic fibrosis induced by ethephon in a rat model. Iran J Basic Med Sci. 2019;22(8):956–62. doi: 10.22038/ijbms.2019.32936.7866.

[30] Shallan MA, Abdel-Mobdy YE, Hamdi E, Abdel-Rahim EA. Coragen (Chlorantraniliprole) insecticide effects on male albino rats. Res J Pharm Biol Chem Sci. 2016;7(6):1536–45.

[31] Panesar PS. Fermented dairy products: starter cultures and potential nutritional benefits. Food Nutr Sci. 2011;2(1):47–51. doi: 10.4236/fns.2011.21006.

[32] Fahmi AH, Sharara HA. Studies on Egyptian Domiati cheese. J Dairy Res. 1950;17(3):312–28. doi: org/10.1017/5002202990005860.

[33] AOAC. Official methods of analysis. In: Helerich K, editor. Vol. I. 15th ed. Arlington, VA and Washington DC, USA: Association of Official Analytical Chemists Inc.; 1990. p. 200–10.

[34] Murthy GK, Rhea U. Determination of major cations in milk by atomic absorption spectrophotometry. J Dairy Sci. 1967;50(3):313–7. doi: 10.3168/jds.50022-0302(67)87416-2.

[35] Lane-Petter W, Pearson AE. Dietary requirements. In: Lane-Petter W, Pearson AE, editors. The laboratory animal-principles and practice. London: Academic Press; 1971. p. xi+293.

[36] Gregor A, Kostzrezewska E, Godorowska W. Determination of serum proteins in the presence of dextran by means of the biuret reaction. Infusionsther Klin Ernahr. 1977;4(1):48–50. doi: 10.1159/000219790.
[37] Bergmeyer HU, Bergmeyer J, Grassl M. Samples, reagents, assessment of results. In: Bergmeyer HU, editor. Methods of enzymatic analysis. Vol. XXVI. Weinheim – Deerfield Beach – Basel: Verlag Chemie. 1983. p. 605.

[38] Kind PR, King EL. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol. 1954;7(4):322–6. doi: 10.1136/jcp.7.4.322.

[39] Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54(5):356–61. doi: 10.1136/jcp.54.5.356.

[40] Belfield A, Goldberg DM. Hydrolysis of adenosine monophosphates by acid phosphatases as measured by a continuous spectrophotometric assay. Biochem Med. 1970;4(2):135–48. doi: 10.1016/0006-2944(70)90090-6.

[41] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium, biochemical role as a component of glutathione peroxidase. Science. 1973;179(4107):588–90. doi: 10.1126/science.179.4073.588.

[42] Grant DF, Matsumura F. Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant Aedes aegypti. Pest Biochem Physiol. 1989;33(2):132–43. doi: 10.1016/0487-5357(89)90004-7.

[43] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(1):130–2. PMID: 6490072.

[44] Iwamoto J, Shimamura C, Takeda T, Abe H, Ichimura S, Sato Y, et al. Effects of treadmill exercise on bone mass, bone metabolism, and calcitropic hormones in young growing rats. J Bone Min Metab. 2004;22(1):26–31. doi: 10.1007/s00774-003-0443-5.

[45] Tamaki H, Man SL, Ohta Y, Katsuyama N, Chinen I. Inhibition of osteoporosis in rats fed with sugar cane wax. Biosci Biotechnol Biochem. 2003;67:423–5. doi: 10.1271/bbb.67.423.

[46] Costa AL, da Silva MA, Brito LM, Nascimento AC, do Carmo Lacerda Barbosa M, Batista JE, et al. Osteoporosis in primary care, an opportunity to approach risk factors. Rev Bras Reumatol Engl Ed. 2016;56(2):111–6. doi: 10.1016/j.jbree.2015.07.016.

[47] Eilonheiro H, Lange R, Tolenon H, Kolossa-Gehring M. Environmental substances associated with osteoporosis—a scoping review. Int J Env Res Public Health. 2021;18(7):738. doi: 10.3390/ijerph18070738.

[48] Copping LG, Duke SO. Natural products that have been used commercially as crop protection agents. Pest Manag Sci. 2007;63(6):524–54. doi: 10.1002/ps.1378.

[49] Ilic K, Obradovic N, Vujasinovic-Stupar N. The relationship among hypertension, antihypertensive medications, and osteoporosis, a narrative review. Calcif Tissue Int. 2013;92(3):217–27. doi: 10.1007/s00223-012-9671-9.

[50] Ito S, Ishida H, Uenishi K, Murakami K, Sasaki S. The relationship between habitual dietary phosphorus and calcium intake, and bone mineral density in young Japanese women, a cross-sectional study. Asia Pac J Clin Nutr. 2011;20:411–7. PMID: 21859660.

[51] Sunyecz JA. The use of calcium and vitamin D in the management of osteoporosis. Ther Clin Risk Manag. 2008;4(4):827–36. doi: 10.2147/tcrm.s3552.

[52] Hamad EM, Abdel-Rahim EA, Remeih EA. Beneficial effect of camel milk on liver and kidneys function in diabetic Sprague–Dawley rats. Int J Dairy Sci. 2015;6(3):190–7. doi: 10.3923/ijdls.2015.190.197.

[53] Huncharek M, Muscat J, Kupelnick B. Impact of dairy products and dietary calcium on bone-mineral content in children, results of a meta-analysis. Bone. 2008;43(2):312–21. doi: 10.1016/j.bone.2008.02.022.

[54] Nguyen VH. School based nutrition intervention can improve bone health in children and adolescents. Osteoporos Sarcopenia. 2021;7(1):1–5. doi: org/10.1016/j.j-afos.2021.03.004.

[55] Kato K, Takada Y, Matsuyama H, Kawasaki Y, Aoe S, Yano H, et al. Milk calcium taken with cheese increases bone mineral density and bone strength in growing rats. Biosci Biotechnol Biochem. 2002;66(11):2342–6. doi: 10.1271/bbb.66.2342.

[56] Cakebread JA, Wallace OAM, Kruger MC, Vickers MH, Hodgkinson AJ. Supplementation with bovine milk or soy beverages recovers bone mineralization in young growing rats fed an insufficient diet, in contrast to an almond beverage. Curr Dev Nutr. 2019;3(11):nzz115. doi: 10.1093/cdn/nzz115.

[57] Herminiati A, Rimbawan R, Setiawan B, Astuti DA, Udin LZ, Pudjiastuti S. The application and effectiveness of Diflucure Anhydride III to increase absorption of calcium in calcium-deficient rats. Funct Foods Health Dis. 2020;10(4):168–79.

[58] Collins FL, Rios-Arce ND, Schepper JD, Parmeswaran N, Mccabe LR. The potential of probiotics as a therapy for osteoporosis. Microbiol Spectr. 2017;5(4):1–29. doi: 10.1128/microbiolspec.BAD-0015-2016.

[59] Kim JG, Lee E, Kim SH, Whang KY, Oh S, Imm JY. Effects of a Lactobacillus casei 393 fermented milk product on bone metabolism in ovariectomised rats. Int Dairy J. 2009;19(11):690–5. doi: 10.1016/j.idairyj.2009.06.009.

[60] Rodrigues FC, Castro AS, Rodrigues VC, Fernandes SA, Fontes EA, de Oliveira TT, et al. Yacon (Smallanthus sonchifolius) for the treatment of osteoporosis: a randomized clinical trial. Biomed Res Int. 2014;2014:115. doi: 10.1155/2014/734275.

[61] Kim T, Ha H, Kim N, Park E, Rho J, Kim E, et al. ATP6v0d2 deficiency increases bone mass, but does not influence ovariectomy-induced bone loss. Biochem Biophys Res Commun. 2010;403(1):73–8. doi: 10.1016/j.bbrc.2010.10.117.

[62] Li C, Ma Y, Mi Z, Hao R, Zhou T, Hai H, et al. Screening for Lactobacillus plantarum strains that possess organophosphorous pesticide-degrading activity and metabolomic analysis of phorate degradation. Front Microbiol. 2018;9:2048. doi: 10.3389/fmicb.2018.02048.

[63] Kumral A, Kumral NA, Gurbuz O. Chlorpyrifos and deltamethrin degradation potentials of two Lactobacillus plantarum (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains. Turk J Entomol. 2020;44:165–76. doi: 10.16970/entoted.625156.

[64] Bull H, Murray PG, Thomas D, Fraser AM, Nelson PN. Acid phosphatase. Mol Pathol. 2002;55(2):65–72. doi: 10.1136/mp.55.2.65.

[65] Elwafik AM, Hassan HA, Gharib NS. Osteoprotective effect of Soybean and sesame oils in ovariectomized rats via estrogen-like mechanism. Cytotechnology. 2014;66(2):335–43. doi: 10.1007/s10616-013-9580-4.
[66] Gutman AB, Gutman EB. Relation of serum calcium to serum albumin and globulins. J Clin Invest. 1937;16(6):903–19. doi: 10.1172/JCI100917.

[67] Furukawa K, Zenke Y, Menuki K, Yamanaka Y, Sakai A. Correlation of albumin/globulin ratio with forearm bone mineral density in women above 50 years of age. HAND 2016;11(1 Suppl):49S. doi: 10.1177/155894471666055Scd.

[68] Al-Aqaby ARA, Glaskovich AA, Kapitonova EA, Losev E. Study the effect of using probiotic (Vetlactoflorum) on some of biochemical and immunological parameters of broiler chickens. Basra J Vet Res. 2014;3(1):166–79. doi: 10.33762/bvtr.2014.88137.

[69] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9–19. doi: 10.1097/WOX.0b013e3182439613.

[70] Gao D, Zhu G, Gao Z, Liu Z, Wang L, Guo W. Antioxidative and hypolipidemic effects of lactic acid bacteria from pickled Chinese cabbage. J Med Plants Res. 2011;5:1439–46. doi: 10.5897/JMPR.9000235.

[71] Chen XL, Gong LZ, Xu JX. Antioxidative activity and protective effect of probiotics against high-fat diet-induced sperm damage in rats. Animal. 2013;7:287–92. doi: 10.1017/S1751731112001528.

[72] Lasker S, Rahman M, Parvez F, Zamila M, Miah P, Nahar K, et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Sci Rep. 2019;9(1):20026. doi: 10.1038/s41598-019-56538-0.

[73] Rendina E, Hembree KD, Davis MR, Marlow D, Clarke SL, Halloran BP, et al. Dried plum’s unique capacity to reverse bone loss and alter bone metabolism in postmenopausal osteoporosis model. PLoS One. 2013;8(3):e60569. doi: 10.1371/journal.pone.0060569.