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Effect of *Spirulina platensis* supplementation on some bone element levels in rats fed with cholesterol and/or hydrogenated vegetable oil

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ABSTRACT

Aim: The aim of study was to evaluate the protective effects of *Spirulina platensis* on some bone element levels in rats fed with cholesterol and/or high hydrogenated vegetable oil. Forty-nine male Sprague-Dawley rats were housed.
Method and materials: The animals were randomly divided into seven equal groups. The trial period was 60 days. *Spirulina platensis* in powder form was administered to experimental animals at a dose of 3g/100g diet. In addition to the basal diet, the experimental groups were fed: Trial 1 (T1), 43% hydrogenated vegetable oil; Trial 2 (T2), 10% cholesterol; Trial 3 (T3), 43% hydrogenated vegetable oil and 10% cholesterol; Trial 4 (T4), 3% *Spirulina platensis*; Trial 5 (T5), 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Trial 6 (T6), 10% cholesterol and 3% *Spirulina platensis*; and Trial 7 (T7), 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*. Ca, Pi, Cr, Ni and Cu levels of tibias were determined using atomic absorption spectrophotometer.
Results: Bone calcium levels did not differ significantly between groups. Phosphorus levels were highest in the group containing 3% *Spirulina platensis*. The lowest was seen in the group to which 10% cholesterol was added. In the 3% *Spirulina platensis* added group, it was significantly higher than that of the 10% cholesterol group and the 43% hydrogenated oil + 10% cholesterol groups. Bone chromium levels were significantly higher in the T1 group containing 43% hydrogenated oil than in the 3% *Spirulina platensis* + cholesterol and 3% Spirulina + cholesterol + hydrogenated oil groups. Nickel levels were significantly higher in the T5 and T6 groups than in the T2 and T3 groups. Copper was found to be significantly higher in the T5, T6 and T7 groups than in the other groups.
Conclusion: It was concluded that feeding 43% hydrogenated vegetable oil and 10% cholesterol reduced bone inorganic phosphorus levels, although it did not affect bone calcium. *Spirulina platensis* supplementation worked to increase bone inorganic phosphorus levels.

Keywords: *Spirulina platensis*, rat, tibia, element, cholesterol

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Introduction

Blue and green algae have been used as an important food source for humans and animals for hundreds of years due to their high nutritional value and high carotenoid content (Abd El-Hady and El-Ghalid, 2018). Spirulina is a filamentous and multicellular cyanobacteria species that belongs to blue algae and grows in alkaline environments (Rubio *et al*., 2021). It is cultivated in temperate waters around the world and is considered a functional food due to its high content of protein, vitamins, minerals, healthy fatty acids and other healing phytonutrients (El Agawany *et al*., 2022). Spirulina, which has a fat content of 6-9%, is rich in unsaturated fatty acids such as linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid and stearidonic acid (Cho *et al*., 2020).

In terms of its composition and nutritional value, Spirulina stands out for its high protein content (55-70% of dry weight) and its richness in both essential and non-essential amino acids compared to other plant protein sources (Rubio *et al*., 2021). Due to its high protein content and abundant essential fatty acids, antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides, commercial Spirulina production has gained popularity for use in pharmaceuticals as well as nutritional supplements for humans and animals (El Agawany *et al*., 2022). It is also rich in vitamins B (B_1, B_2, B_3, B_6, B_9 and B_{12}), C, D and E and contains different photosynthetic pigments such as chlorophyll, xanthophyll and beta-carotene (Rubio *et al*., 2021). The minerals found in it included calcium, magnesium, iron, phosphorus, manganese, potassium, zinc and selenium (El Agawany *et al*., 2022).

Phenolic compounds found in Spirulina are primarily involved in the redox mechanism, function to prevent the formation of reactive oxygen species and eventually inhibit inflammatory responses through anti-oxidative and anti-inflammatory mechanisms, which have protective effects against various diseases (Cho *et al*., 2020). Spirulina has been shown to prevent fatty infiltration of the liver in diabetic rats by inhibiting adipogenesis and lipogenesis (Fournier *et al*., 2016). Additionally, it has been shown to reduce blood cholesterol levels and prevent atherosclerosis associated with a high-cholesterol diet in animal models (Kim *et al*., 2010). It may also increase oral carcinoma regression and antibody production (Ishimi *et al*., 2006). Spirulina has also been shown to improve immune function, reproduction and growth characteristics (Abd El-Hady and El-Ghalid, 2018).

Toxicity from heavy metals is a global concern for humans and the environment (El Agawany *et al*., 2022). Biosorption, which uses dead algae, fungi or bacterial biomass to trap toxic heavy metals, is a well-established method for removing heavy metals from wastewater (Gokhale *et al*., 2009). Microalgae are known to be excellent bioaccumulators of heavy metals (El Agawany *et al*., 2022). Spirulina can be affected by pollution during its development and production in the marine ecosystem, as it can absorb and accumulate toxic pollutants by binding them to its proteins, polysaccharides and amino groups, like other blue algae or cyanobacteria (Rubio *et al*., 2021). *Spirulina platensis* has been reported to protect against metal-induced toxicity in various animal species (Soliman *et al*., 2021). Chan *et al*. (1991) showed that *Spirulina platensis* biomass can be effectively used for nickel and chromium removal from real industrial wastes.

Bone formation is stimulated by decreasing apoptosis and increasing proliferation of osteoblasts. Vitamin D suppresses bone resorption and increases bone mass by promoting bone remodeling (Cho *et al*., 2022). Osteoporosis is a systemic skeletal disease characterized by low bone mass due to microarchitectural deterioration of bone tissue and, consequently, increased susceptibility to bone fragility and fractures. Loss and deterioration of bone tissue structure results from a net imbalance in bone remodeling due to increased activity of osteoclasts or decreased activity of osteoblasts (Devesh *et al*., 2012). Recent data from epidemiological and animal studies strongly support the concept that fat accumulation (obesity) causes deleterious effects on bone mass, which subsequently leads to bone fragility. Fat cells secrete adiponectin and leptin, which are known to affect bone remodeling. It has been suggested that increased loads applied to cortical bone directly stimulate bone formation via leptin, which in turn increases aromatase and estradiol activity, leading to decreased bone resorption and increased bone formation (Cho *et al*., 2022). It has been observed that bone loss is greater in patients with poorly controlled diabetes than in those with well-controlled diabetes (Devesh *et al*., 2012).

Certain marine plants are noted for their ability to improve bone metabolism because they are rich in minerals and growth factors (Devesh *et al*., 2012). İt was noted that some plants, such as seaweed, have a protective role against bone loss (Suzer *et al*., 2020). Rich in minerals and growth factors, Spirulina may be one of the natural sources with potential to improve bone metabolism (Ekeuku *et al*., 2021). The mineral contents of *Spirulina platensis*, especially calcium and phosphorus, have a positive effect on bone calcification and health due to their stimulating effects on mineral absorption in the intestinal microflora. It was also stated that Spirulina's high mineral and protein content may help prevent bone loss by preventing mineral release in kidneys (Suzer *et al*., 2020). Kumar *et al*. (2009) evaluated the protective effect of *Spirulina platensis* against collagen-induced arthritis in rats. A study by Simon *et al*. (2018) showed that Spirulina regulates diabetic pathological pathways through its anti-oxidation and free radical scavenging ability. The aim of this study was to evaluate the protective effects of *Spirulina platensis* on some bone element levels in rats fed with cholesterol and/or high hydrogenated vegetable oil.

Materials and Methods

The study was conducted at Istanbul University and all animal experiments were carried out in accordance with EU Directive 2010/63/EU for animal experimentation. Forty-nine male Sprague-Dawley rats weighing 280-300 g were housed in polypropylene cages under a 12 h dark/12 h light environment. The animals were randomly divided into seven equal groups. The trial period was 60 days. Rats were given food and water ad libitum. 100% pure *Spirulina platensis* in powder form was purchased from Alg BioTek (Feneryolu Mh., Kiziltoprak 34724, Istanbul, Turkey) and administered to experimental animals at a dose of 3g/100g diet. In addition to the basal diet, the experimental groups were fed: Trial 1 (T1), 43% hydrogenated vegetable oil; Trial 2 (T2), 10% cholesterol; Trial 3 (T3), 43% hydrogenated vegetable oil and 10% cholesterol; Trial 4 (T4), 3% *Spirulina platensis*; Trial 5 (T5), 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Trial 6 (T6), 10% cholesterol and 3% *Spirulina platensis*; and Trial 7 (T7), 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*. The composition of the diets is shown in Bilal and Altiner (2020).

At the end of the study, all rats were sacrificed and the tibias were removed. The tibias were cleaned and degreased in ethanol/benzene extraction solution. The dry bones were then ashed at 600˚C overnight. Elemental determination was performed on 200 mg bone ash samples. Ashed bones were digested in nitric acid and diluted in deionized water for elemental determination (Wisser *et al*., 1990). Ca, Pi, Cr, Ni and Cu levels of all samples were determined using atomic absorption spectrophotometer (Sandell, 1959). An Analytik Jena ContrAA 700 High Resolution Continuum Source Atomic Absorption Spectrometer (Analytik Jena, Jena, Germany) equipped with a 300W xenon short-arc lamp (XBO 301, GLE, Berlin, Germany) as a continuum radiation source was used throughout the work. This new equipment presents a compact high-resolution double echelle monochromator and a charge-coupled device array detector with a resolution of about 5 pm per pixel. Measurements were carried out in the spesific wavelength for the each elements. The number of pixels of the array detector used for detection of the line was 5 (central pixel ±2). All measurements were carried out as five replicate.

Data were compared between groups using analysis of variance (ANOVA combined with Tukey's multiple range test). All statistical analyses were performed using the software package program (SPSS for windows, Standard version 10.0, 1999, SPSS Inc., Headquarters, Chicago, IL, USA).

Results and Discussion

Group means, standard errors of the means, and p values were recorded (Table 1). Bone calcium levels did not differ significantly between groups. Phosphorus levels were highest in the group containing 3% *Spirulina platensis*. The lowest was seen in the group to which 10% cholesterol was added. In the 3% *Spirulina platensis* added group, it was significantly higher than that of the 10% cholesterol group and the 43% hydrogenated oil + 10% cholesterol groups. Inorganic phosphorus levels were significantly higher in all groups containing Spirulina than in all groups containing cholesterol but not Spirulina. Bone chromium levels were significantly higher in the T1 group containing 43% hydrogenated oil than in the 3% *Spirulina platensis* + cholesterol and 3% Spirulina + cholesterol + hydrogenated oil groups. The highest was detected in the T1 group and the lowest in the T7 group.

Tibia nickel levels were lowest in the T4 group (3% *Spirulina platensis* group) and highest in the T6 group (3% Spirulina + cholesterol group). Nickel levels were significantly higher in the T5 (Spirulina + hydrogenated oil group) and T6 (Spirulina + cholesterol group) groups than in the T2 (cholesterol group) and T3 (hydrogenated oil + cholesterol group) groups. Copper was found to be significantly higher in the T5 (Spirulina + oil group), T6 (Spirulina + cholesterol group) and T7 (Spirulina + oil + cholesterol) groups than in the other groups. Tibia copper levels were lowest in the T1 group (hydrogenated oil group) and highest in the T6 group (Spirulina + cholesterol group).

Protein deficiency in human diet is a major concern for most developing countries. Therefore, there is a need to develop new and unconventional protein sources (El Agawany *et al*., 2022). Algae have long been recognized as a valuable source of nutrients and minerals. Algae provide numerous bioactive substances such as vitamins, minerals, polyunsaturated fatty acids, polysaccharides and pigments (Siddiqui *et al*., 2024). Spirulina is a complete food both qualitatively and quantitatively when consumed in certain doses, as it is rich in micro and macro nutrients (Moreira *et al*., 2013).

Table 1. Mean tibial element levels of the groups and their statistical comparisons.

Element	Group	Mean	SEM	IQR					P value
				Min	25	Med.	75	Max	
Ca (mg/g)	T1	2.30	0.11	1.97	2.05	2.27	2.47	2.89	0.08
	T2	2.75	0.25	2.16	2.25	2.42	3.25	4.02	
	T3	2.87	0.23	1.66	2.49	3.03	3.2	3.84	
	T4	2.40	0.05	2.16	2.29	2.43	2.53	2.55	
	T5	2.42	0.11	2.10	2.17	2.32	2.74	2.84	
	T6	2.42	0.11	2.10	2.17	2.32	2.74	2.84	
	T7	2.07	0.31	0.001	2.04	2.26	2.52	2.93	
P_i (mg/g)	T1	0.82^{abc}	0.21	0.001	0.28	0.92	1.29	1.57	0.001
	T2	0.43^{a}	0.08	0.10	0.25	0.44	0.61	0.76	
	T3	0.69^{ab}	0.21	0.001	0.04	0.75	1.23	1.45	
	T4	1.31^{c}	0.07	0.98	1.12	1.40	1.46	1.53	
	T5	1.13^{bc}	0.06	0.92	1.01	1.10	1.19	1.47	
	T6	1.13^{bc}	0.06	0.92	1.01	1.10	1.19	1.47	
	T7	0.81^{abc}	0.05	0.59	0.74	0.79	0.87	1.10	
Cr (mg/kg)	T1	2.74^{b}	0.35	1.26	2.21	2.75	2.98	4.77	0.004
	T2	2.07^{ab}	0.52	0.86	1.37	1.59	2.11	5.54	
	T3	2.29^{ab}	0.73	0.93	1.17	1.69	2.19	7.27	
	T4	1.86^{ab}	0.30	0.58	1.25	2.00	2.19	3.43	
	T5	2.09^{ab}	0.23	1.34	1.60	1.97	2.50	3.26	
	T6	0.74^{a}	0.32	0.001	0.23	0.40	0.98	2.74	
	T7	0.55^{a}	0.25	0.001	0.22	0.33	0.55	2.21	
Ni (mg/kg)	T1	2.84^{c}	0.52	1.02	1.83	2.80	3.30	5.81	0.001
	T2	1.07^{ab}	0.27	0.001	0.48	1.00	1.73	2.15	
	T3	0.68^{ab}	0.15	0.001	0.44	0.66	1.04	1.18	
	T4	0.53^{a}	0.24	0.001	0.001	0.27	0.89	1.95	
	T5	2.87^{c}	0.43	0.83	2.17	2.86	3.71	4.64	
	T6	3.08^{c}	0.57	1.47	2.01	2.58	3.76	6.47	
	T7	2.06^{bc}	0.06	1.97	1.98	2.01	2.05	2.45	
Cu (mg/kg)	T1	3.37^{a}	0.21	2.72	2.97	3.24	3.70	4.47	0.001
	T2	3.70^{a}	0.20	2.88	3.41	3.64	3.91	4.82	
	T3	3.84^{a}	0.32	2.45	3.29	3.59	4.71	5.06	
	T4	3.51^{a}	0.20	2.73	3.16	3.43	3.92	4.34	
	T5	5.57^{bc}	0.15	4.89	5.37	5.57	5.72	6.37	
	T6	6.34^{c}	0.47	5.02	5.18	6.04	7.30	8.71	
	T7	5.20^{b}	0.23	4.10	4.87	5.23	5.54	6.24	

Abbreviations: IQR: Interquartile Range, SEM: Standard error of the mean. Min: Minimum, Max: Maximum, 25: 25 Percentile, 75: 75 Percentile, Med: Median, T1: 43% hydrogenated vegetable oil group, T2: 10% cholesterol group, T3: 43% hydrogenated vegetable oil + 10% cholesterol group, T4: 3% Spirulina group, T5: 3% Spirulina + 43% hydrogenated vegetable oil group, T6: 3% Spirulina + 10% cholesterol group, T7: 3% Spirulina + 43% hydrogenated vegetable oil + 10% cholesterol group, a,b,c: There are significant differences between the means indicated by different letters for each element.

Spirulina is labeled as a powerful food rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols, and some vital elements such as calcium, chromium, iron, zinc, magnesium, manganese, and selenium. Spirulina is also a natural source of the full spectrum of vitamin B_{12}, vitamin E, ascorbic acid, tocopherols and natural mixed carotene and xanthophyll phytopigments (Devesh *et al*., 2012). Spirulina, one of the most concentrated natural nutrient sources, contains all the essential amino acids and is also rich in beta-carotene and natural phytochemicals (Ishimi *et al*., 2006). It has been claimed that chlorogenic acid, synaptic acid, salicylic acid, trans-cinnamic acid and caffeic acid are commonly found in Spirulina (Cho *et al*., 2020). Spirulina also contains phycocyanin (14%), chlorophyll (1%) and carotenoids (0.37%) (James *et al*., 2009). The high protein concentration of various microalgae species, especially *Spirulina platensis*, makes them an excellent source of this nutrient (El Agawany *et al*., 2022). Another beneficial component of Spirulina is γ-linolenic acid. It has been discovered that the amounts of γ-linolenic acid vary between 0.16 g/100 g and 1.24 g/100 g and constitute an average of 14% of the total polyunsaturated fatty acids in Spirulina (Cho *et al*., 2020).

Environmental factors such as pH and sea currents affect the levels of trace elements and toxic metals in water and algae growing in water (Rubio *et al*., 2021). *Spirulina platensis* biomass can be used for metal removal from wastewater with different pH values through bioaccumulation and biosorption processes (Zinicovscaia *et al*., 2019). Additionally, *Spirulina platensis* may protect against the toxicity of copper sulfate and copper oxide nanoparticles through its ability to absorb metal ions (Soliman *et al*., 2021). Metal bioaccumulation is a complex process involving intracellular oxidation or reduction reactions of metal ions and extracellular metabolic transformations (Zinicovscaia *et al*., 2019). In addition to calcium, algae also contain minerals such as magnesium, phosphorus and potassium, which are essential for bone health (Siddiqui *et al*., 2024). Spirulina contains 0.66% magnesium and 1% phosphorus in addition to 0.35% calcium. Daily consumption of Spirulina food supplements should be considered as an additional dietary source of these metals (Rubio *et al*., 2021). Venkataraman *et al*. (1994) discovered that vitamin-mineral premixes commonly added to chicken feed rations could be improved when Spirulina was added due to its nutrient-rich composition.

In addition to protecting bone health, Spirulina also has other health-promoting effects, such as anti-inflammatory and antioxidant properties (Siddiqui *et al*., 2024). It preserves the structural integrity of cells and repairs degenerated tissues through its bioactive components (Soliman *et al*., 2021). The prophylactic effect of Spirulina may be attributed to its ability to quench free radicals, especially reactive oxygen species that cause oxidative stress (Wu *et al*. 2003). Antioxidant compounds such as phycobilins and phycocyanins in Spirulina inhibit the activities of catalytic enzymes such as lipoxygenase and cyclooxygenase or increase the activity of enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. Polyphenols in Spirulina have been reported to have anti-inflammatory, antiviral, antioxidant, antithrombotic, vasodilator, antidiabetic, neuroprotective, hepatoprotective and anticarcinogenic properties (Cho *et al*., 2020). Spirulina's chlorophyll acts as a cleansing and detoxifying phytonutrient against toxic substances. Its β-carotene keeps the mucous membrane tight and thus prevents toxic elements from entering the body (James *et al*., 2009). Nakagawa *et al*. (2000) suggested that dietary supplementation of Spirulina may increase vitamin C metabolism in young sea breams.

The morphological and material properties of bones affect all properties of the bone. As a natural composite material, bone contains a substantially hard mineral phase (mainly hydroxyapatite crystals) and a softer collagen matrix (Suzer *et al*., 2020). Additionally, there are several types of cells in bone, such as osteoblasts, osteoclasts, and osteocytes, which work together to ensure the bone's metabolic functions and its ability to adapt and repair under stress (Siddiqui *et al*., 2024). In humans, bones develop through endochondral and intramembranous ossification, respectively, and are formed by osteoblasts and osteoclasts (Carnovali *et al*., 2019). Osteoblasts are the cells responsible for creating bone tissue. They are active on the bone surface and help bone grow, repair, and maintain bone density by depositing bone matrix (the structural framework of bone) and minerals. Osteoblasts synthesize collagen and other proteins that store minerals such as calcium and phosphorus (Yang *et al*., 2024). Osteocalcin, produced abundantly by osteoblasts, plays an important role in bone mineralization (Cho *et al*., 2022). It has been reported that 25-OH-vitamin D leads to an increase in the level of osteogenesis in vivo, which indicates increased osteoblastic activity (Ekeuku *et al*., 2021).

Bone is a connective tissue that plays important roles in both metabolism and mechanical functions. The basic component of bone is mineralized collagen fibrils, which have a highly organized and hierarchical structure (Siddiqui *et al*., 2024). It has been reported that physiological concentrations of circulating vitamin C are necessary for the maintenance of an optimum collagen network, which is associated with maximum load, stiffness, fracture, and post-yield displacement (Jepsen *et al*., 2015). 90% of the collagen matrix of bone consists of type I collagen. Vitamin C stimulates type I collagen deposits. Collagen increases bone hardness. A decrease in cross-link concentration is associated with decreased bone stiffness and energy absorption capacity (Suzer *et al*., 2020). Bone health is a critical aspect of overall health and well-being, as bones provide structural support and protection to our bodies. As we age, bone loss increases, leading to conditions such as osteoporosis and fractures. Bone fracture is a major global health problem, especially among older people and more specifically among postmenopausal women. Osteoporosis is a medical condition characterized by microarchitectural deterioration that leads to low bone mass, low bone mineral content, brittle bones, and an increased risk of fractures (Siddiqui *et al*., 2024).

Bone tissue has a lifelong cycle of construction and destruction. The most important factor driving the cycles is calcium. A harder bone contains more inorganic components. Increased mineralization results in an increase in hardness and as a result the bone can withstand greater loads. Plant foods containing rich minerals and growth factors can improve bone metabolism (Suzer *et al*., 2020). The presence of inorganic minerals in the diet is essential for healthy bones. Other cationic minerals such as boron, copper, iron, magnesium, manganese, potassium, selenium, silicon, strontium and zinc are believed to play a vital role in the formation and maintenance of strong and healthy bones (Siddiqui *et al*., 2024). Although their incorporation into mineralized bone is critical, some trace elements are required for the formation and mineralization of organic bone matrix, as well as the regulation of osteoblast and osteoclast metabolism (Zofková *et al*., 2013).

Treatment with natural herbs causes fewer side effects than currently used synthetic oral preparations such as glitazones, which have negative effects on bones (Sumeet *et al*., 2010). Certain marine plants have recently gained attention for their ability to improve bone metabolism because they are rich in minerals and growth factors (Ishimi *et al*., 2006). It has been suggested that algae supplements improve bone microarchitecture as well as increase bone mineral density. Seaweed is full of nutrients that aid in bone development, strength and metabolism, such as vitamins, minerals, chlorophyll, amino acids and growth factors (Wu *et al*., 2003). There are various types of seaweed, such as red, green and brown seaweed, that have been found to be beneficial for bone health and contain various minerals such as calcium, magnesium and phosphorus, which are essential for the development and maintenance of healthy bones. In addition to these minerals, seaweed also contains bioactive compounds such as polysaccharides, polyphenols, and carotenoids, which have antioxidant and anti-inflammatory properties that may support bone health. Studies suggest that consumption of seaweed or seaweed-derived supplements may increase bone density and reduce the risk of osteoporosis in both animals and humans. Trace minerals from marine red algae can be used to prevent progressive bone mineral loss in conjunction with calcium and may be beneficial as part of an osteoporosis prevention strategy. Algae extracts have shown an overall positive effect on human and animal bones by maintaining and improving bone density and sustainably producing components important for bone metabolism. Some studies have found that polysaccharides such as fucoidan from brown seaweed and carrageenan from red seaweed are rich sources of several vitamins, such as vitamin K and vitamin D, which may have potential benefits for bone health by increasing bone mineral density and improving bone strength (Siddiqui *et al*., 2024). Another study in rats found that supplementing with the brown seaweed *Sargassum horneri* increased bone mineral density and improved bone strength (Chen *et al*., 2020).

Spirulina is considered a superfood as it contains a wide range of vitamins, minerals and functional components that are vital for bone formation and maintenance (maximum non-toxic intake 1-3 gm/day) (Andrade *et al*., 2018). In a study, bone surface strength and integrity were improved by Spirulina (Cho *et al*., 2022). Another study in rats found that Spirulina supplementation improved bone mineral density and prevented bone loss (Carson and Clarke, 2018). Spirulina contains high concentrations of functional bioactive nutrients that stimulate osteoblast differentiation and thus improve skeletal integrity (Cho *et al*., 2022). Another study showed increased numbers of osteocytes and osteoblasts in groups treated with Spirulina (Ekeuku *et al*., 2021). Spirulina is a rich source of vitamin B_{12}, which improves osteoblast maturation and thereby reduces osteoclastogenesis. Tartrate-resistant acid phosphatase type 5b is a marker reflecting the number and activity of osteoclasts and was found to be reduced by Spirulina. This means that there is a decrease in osteoclast activity and subsequent bone resorption (Cho *et al*., 2022). In another study, Spirulina supplementation did not affect osteoclast activity (Ekeuku *et al*., 2021).

Spirulina can increase bone growth, bone strength, bone mineral content and antioxidant activities by regulating growth hormone, IGF-1, osteocalcin and parathyroid hormone under normal nutritional conditions, and the higher the Spirulina content, the greater the positive effect. The results showed that 50% or 70% Spirulina protein substitution may provide more favorable effects on osteogenesis compared to control and low-concentration Spirulina substitution. Spirulina treatment significantly increased osteocalcin levels over time (Cho *et al*., 2020). It was concluded that Spirulina treatment would be quite beneficial in reducing the risk of bone fractures in diabetic rats treated with Pioglitazone and dexamethasone (Devesh *et al*., 2012). Sixabela *et al*. (2011) reported that dietary supplementation of *Spirulina platensis* significantly increased tibial length in rats.

Vitamin E found in Spirulina can protect cells from oxidative damage by scavenging free radicals that can attack osteoblasts and damage cells important in bone metabolism. Vitamin D promotes transcription of osteocalcin and has bidirectional effects on gene transcription of type I collagen and alkaline phosphatase. Spirulina is a rich source of vitamin D. It was observed that serum 25-OH-vitamin D level was significantly higher in the Spirulina supplementation group (Cho *et al*., 2020). Spirulina supports bone formation, as evidenced by increased osteoblast numbers and osteocalcin expression, and the hypoglycemic properties of Spirulina and its ability to elevate serum 25-OH-vitamin D may explain its bone-protective properties (Ekeuku *et al*., 2021).

The rich mineral and protein content of *Spirulina platensis* also stimulates bone development by affecting the intestinal microflora and stimulating mineral absorption. Therefore, *Spirulina platensis* may cause an increase in bone mineralization (Suzer *et al*., 2020). Gutierrez-Salmean *et al*. (2015) reported that the improvement of mechanical and material properties of bone is the result of *Spirulina platensis*' rich protein (60-70%), vitamin C and minerals such as iron, calcium and phosphorus. Ishimi *et al*. (2006) also observed that Spirulina can improve the collagen structure and elastic properties of bone due to its protein content. Nakagawa *et al*. (2000) suggested that Spirulina preserves and improves the content of vitamin C, a co-factor for collagen synthesis in muscle and bone against degradation. Suzer *et al*. (2020) reported that bones were slightly harder in both the low and high dose Spirulina groups than in the control group, but there was no significant difference between the groups. Siddiqui *et al*. (2024) stated that *Spirulina platensis* may have positive effects on bone growth and biomechanical bone properties and can be used as a food additive to support bone health in growing animals. Gunes *et al*. (2017) also reported that *Spirulina platensis* led to a significant increase in type I collagen in human skin cell cultures.

Spirulina may act as an anti-obesity drug (Cho *et al*., 2022). It can inhibit fat accumulation without interfering with normal body and organ growth (Cho *et al*., 2020). In a study, Spirulina reduced triglyceride accumulation and inhibited adipogenesis by reducing the protein expression of adipogenic regulators (DiNicolantonio *et al*., 2020). In a clinical study, individuals with obesity experienced beneficial effects in regulating body weight after taking Spirulina supplements (Yousefi *et al*., 2018). It has been reported that feeding with Spirulina improves dietary hyperlipidemia caused by a high fructose diet through an increase in lipoprotein lipase activity (Ishimi *et al*., 2006). Algae may have cholesterol-lowering effects, potentially reducing the risk of cardiovascular disease (Siddiqui *et al*., 2024). Spirulina supplementation may exert hypocholesterolemic properties by reducing cholesterol absorption and/or synthesis in the gastrointestinal tract and increasing Lactobacillus population (Abd El-Hady and El-Ghalid, 2018). Spirulina contains high levels of γ-linolenic acid, an essential polyunsaturated fatty acid that is 170 times more powerful than linoleic acid and has the capacity to lower cholesterol levels. γ-Linolenic acid in Spirulina can be used to effectively lower cholesterol and treat atopic eczema, breast cancer and premenstrual disorders (Cho *et al*., 2020). Increases in unsaturated lipid levels and acyl chain lengths may alter membrane fluidity and function, leading to increased intestinal mineral absorption. Mineral bioavailability may be affected by the lipid composition of a high-fat diet (Cho *et al*., 2022). Serum HDL, alcium and inorganic phosphorus concentrations of chickens fed with 3% and 6% Spirulina were significantly increased compared to the control group (Abd El-Hady and El-Ghalid, 2018).

It is widely known that obesity reduces bone strength. Obesity may decrease osteoblastogenesis and increase adipogenesis. This is because osteoblasts and adipocytes are derived from the same multipotential mesenchymal stem cells. Obesity may increase bone resorption through upregulation of proinflammatory cytokines such as TNF-α, which stimulate osteoclastic differentiation (Cao, 2011). In one study, a decrease in bone formation and mineralization occurred in the obese control group due to the high fat diet-high fat emulsion used to induce obesity (Cho *et al*., 2022).

In a study by Elefteriou *et al*. (2004) it was found that leptin secreted by adipocytes has effects on bone mass in an obese rat model. Overproduction of leptin levels resulted in decreased osteoblastic activity and subsequent low bone mass. Spirulina may act as an anti-obesity drug while protecting bones from fractures. Spirulina helped increase the mineral density of the femur (Cho *et al*., 2022). A study by Cho *et al*. (2020) showed that Spirulina can increase bone strength in rats not on a high-fat diet in a 7-week treatment. Spirulina supplementation provided beneficial protection against bone fragility in diet-induced obesity through enhancement of gene expression for bone formation genes (ALP and osteocalcin) and suppression of bone resorption genes (osteoprotegerin, nuclear factor kappa-B ligand, tartrate-resistant acid phosphatase type 5b and peroxisome proliferator-activated receptor gamma) (Cho *et al*., 2022).

Calcium is an essential mineral that performs various biological functions. Studies have shown a relationship between low calcium intake and chronic diseases such as osteoporosis, colon cancer, hypertension and obesity (Moreira *et al*., 2013). Calcium is critical for bone structure and is part of the hydroxyapatite in the collagen matrix of bones (Cho *et al*., 2022). 99% of the body's calcium is stored in the bones, and the recommended daily intake varies with age. Studies have suggested a positive correlation between calcium intake and bone health and have shown that calcium plays a vital role in maintaining healthy bones, but calcium alone is not enough because vitamin D is important in increasing calcium uptake by the gut. Inadequate calcium intake leads to a decrease in serum calcium levels, triggering mobilization of bones and release of their contents into the blood. This leads to decreased bone mineral density and increased risk of osteoporosis (Siddiqui *et al*., 2024). Increased osteoblastic activity is associated with the movement of calcium and phosphate from the blood to the bone for the bone formation process, leading to decreased blood calcium and phosphorus levels. On the other hand, with increased resorption of bone tissue, calcium and phosphate are released from the bone into the blood, leading to increased serum calcium and phosphate levels (Ekeuku *et al*., 2021). It has been reported that the calcium-phosphorus ratio is important to measure the risk of decreased decalcification capacity (Suzer *et al*., 2020).

Consuming the recommended amount of phosphorus (700 mg per day) is not detrimental to bone health (Siddiqui *et al*., 2024). Increases in phosphorus trigger the release of parathyroid hormone. This will lead to a high bone turnover where calcium is drawn from the bones (Cho *et al*., 2022). However, excessive phosphorus intake can be harmful, especially if associated with low calcium intake. Adequate phosphorus intake is crucial for bone formation during growth periods because low serum phosphate levels can limit bone formation and mineralization. Low serum phosphorus levels may indicate malnutrition, which is a risk factor for osteoporosis and fractures. When phosphorus uptake is insufficient or negative, osteoblast performance decreases, while osteoclast activity and bone turnover increase (Siddiqui *et al*., 2024). One study showed that the obese control group had higher bone phosphate and calcium levels than the normal group. Ekeuku *et al*. (2021) found low levels of 25-OH-vitamin D in the diabetic control group, indicating reduced osteogenesis and osteoblastic activity evidenced by increased blood calcium and phosphorus levels.

Calcium, an essential mineral that provides structural support for bones and teeth, is one of the primary bioactive compounds in algae. Algae, especially certain types of seaweed such as Lithothamnium sp. and Laminaria sp., are rich sources of calcium, with some species containing up to 10 times more calcium by weight than milk (Siddiqui *et al*., 2024). A study by Devesh *et al*. (2012) proved that Spirulina is rich in minerals such as calcium and phosphate. Ramírez-Moreno and Olvera-Ramírez (2006) reported that the nutritional composition of Spirulina species for human consumption should contain 0.1–0.4% calcium. Moorhead *et al*. (2012) explained that Spirulina contains approximately 26 times more calcium than milk, which supports bone and tooth development, and also contains phosphorus, which affects the remineralization of teeth. Craig and Mangels (2009) stated that calcium in Spirulina and its high absorption are associated with bone health balance.

Due to the presence of calcium and phosphorus minerals, Spirulina supplementation improved the structure and composition of the bone surface (Cho *et al*., 2022). A study in rats has shown that the bioavailability of calcium in *Spirulina platensis* can preserve bone integrity (Suzer *et al*., 2020). Data showed that calcium and inorganic phosphorus concentrations of tibia ash were significantly ($P<0.05$) increased in chickens fed *Spirulina platensis* algae powder compared to the control group. It was concluded that the addition of *Spirulina platensis* algae powder to the diet could improve calcium-phosphorus metabolism in the blood and tibia bone. Similarly, in our study, bone inorganic phosphorus levels were found to be significantly higher in the T4 group fed with *Spirulina platensis*. In our study, feeding hydrogenated vegetable oil and cholesterol along with Spirulina reduced the inorganic phosphorus levels of the bone. In another study, serum phosphate and calcium levels were found to be significantly lower in the Spirulina group compared to obese control groups (Cho *et al*., 2022). Similar to our findings, serum calcium and phosphorus levels were reduced and bone strength and stiffness were improved with Spirulina (Ekeuku *et al*., 2021). Both adequate blood and bone calcium concentration are necessary for bone hardness and rigidity (Suzer *et al*., 2020). Metformin, Spirulina, and metformin + Spirulina treated groups showed a significant increase in 25-OH-vitamin D levels and a significant decrease in phosphate levels compared to the diabetic control. A significant decrease in calcium levels was found in the Spirulina-treated group, which was not seen in the metformin and metformin + Spirulina-treated groups (Ekeuku *et al*., 2021).

Copper can be found in biological tissues in the form of organic complexes such as metal proteins with enzymatic activity (Moreira *et al*., 2013). It serves an important function as a cofactor in several enzymes but is toxic to cells when present in high concentrations (Soliman *et al*., 2021). Oxygen use during cellular respiration, energy utilization, and synthesis of essential compounds are examples of metabolic reactions mediated by enzymes that require the presence of copper to have catalytic activity. Therefore, copper is essential for human metabolism, although it is required in low concentrations (Moreira *et al*., 2013). It has been reported that adequate copper supplements increase immunity, connective tissue synthesis and bone development (Asnayanti *et al*., 2024). Severe copper deficiency can lead to skeletal problems. The role of copper in bone metabolism can be attributed to lysyl oxidase, an enzyme required for the formation of chemical bonds in collagen and elastin derived from lysine.

Ramírez-Moreno and Olvera-Ramírez (2006) reported that the nutritional composition of Spirulina species for human consumption should contain 0.0012% copper. In a study, Spirulina played a protective role against copper toxicity in *Cirrhinus mrigala*. This indicates that Spirulina has the ability to eliminate and detoxify accumulated copper, and was demonstrated by the improvement of feeding and growth parameters in sublethal exposure of *Cirrhinus mrigala* fed Spirulina-supplemented diets. It is possible that dietary Spirulina may also reduce the metal level in tissues and protect *Cirrhinus mrigala* from copper toxicity. Spirulina reduces copper accumulation in the tissues of fish, increases the excretion of the accumulated metal through feces, and reduces the metal load and toxicity in fish. As dietary levels of Spirulina in the diet increased, fecal excretion of accumulated copper increased. A positive correlation coefficient was obtained for the relationship between dietary Spirulina supplementation and fecal copper excretion and was found to be statistically significant. Excretion of copper via feces reduced the copper burden in the body, which directly improved food utilization, phosphatase activities and hematological parameters. Alkaline phosphatase activities were improved in copper-exposed fish fed Spirulina-supplemented diets (James *et al*., 2009). In our study, no significant difference was found in terms of copper accumulation in the bone between the groups fed with Spirulina or hydrogenated vegetable oil and/or cholesterol supplements, that is, the first 4 groups. Spirulina plus fatty feeding significantly increased bone copper accumulation.

Some metals, such as chromium, are essential in the human diet but become harmful when intake levels are exceeded (El Agawany *et al*., 2022). Chromium is an essential trace element that is necessary for normal carbohydrate, protein, nucleic acid and lipid metabolism, can activate certain enzymes, stabilize proteins and nucleic acids, improve insulin sensitivity and protect against glucose intolerance by participating in the glucose tolerance factor (Li *et al*., 2006). Chromium is important in maintaining glucose metabolism due to the potentiation of insulin action at the cell membrane level (Moreira *et al*., 2013). Chromium binds to a peptide known as Apo LMWCr, which binds to the insulin receptor and increases activity (Sumeet *et al*., 2010). It also functions in corticosteroid metabolism and maintenance of bone density (Li *et al*., 2006). The presence of chromium in Spirulina makes it a very useful adjuvant treatment (Devesh *et al*., 2012). The chromium content in natural Spirulina is very low (e.g. 5.41 mg/kg) (Li *et al*., 2006). The plasma glucose lowering effect observed in the Spirulina and metformin treated groups may be attributable to the chromium naturally present in Spirulina (Ekeuku *et al*., 2021). In our study, Spirulina feeding tended to reduce chromium levels in bone.

In living cells, nickel ions can be transported into the cell via the energy-dependent magnesium transport system or microprecipitated on the cell surface in the form of nickel hydroxide (Zinicovscaia *et al*., 2019). High nickel intake can cause toxicity in humans and the tolerable daily intake has been determined as 2.8 µg Ni/kg body weight/day. People with hypersensitivity to nickel or kidney problems are susceptible to damage from high intake of this element (Rubio *et al*., 2021). In this study, the lowest tibia nickel levels were found in the group given only Spirulina. It was found to be significantly higher in the Spirulina and oil fed groups.

Conclusion

It was concluded that feeding 43% hydrogenated vegetable oil and 10% cholesterol reduced bone inorganic phosphorus levels, although it did not affect bone calcium. *Spirulina platensis* supplementation worked to increase bone inorganic phosphorus levels.

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