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Pet food supplements from the bone waste of canned tuna factories

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ABSTRACT

The aim of this study was to optimize the use of bone waste from tuna canneries. So, bone waste from yellowfin tuna was used to produce dietary supplement tablets for cats. The supplement tablets were made from a combination of 2.5 g bone meal and consumed by two groups of domestic cats. Each group consisted of 3 kittens and the number of feedings was two meals per day including group 1: 1 tablet per meal; group 2: 2 tablets per meal. At the end of the period (21 days), the blood samples of the treated cats were analysed for their calcium, magnesium, and phosphorus contents. The results showed a significant increase in the level of calcium in the blood of the cats. Based on the results obtained in this study and the large amount of waste from fish canneries, it is suggested that bone powder be used to improve the mineral content in the blood of cats.

Keywords: Pets, Bone waste, Tuna, Minerals, Supplementary foods.
Article type: Research Article.

INTRODUCTION

Pet ownership and care are increasing all over the world. According to available reports, the increase in net income in newly developed countries, together with the growth of the culture of urban living and the resulting changes in attitudes, are important factors in the marked elevation in pet ownership, which is also true of developing countries. As a result, the pet food industry is one of the fastest-growing businesses in the world. The development of the pet food industry needs advances in product design, production, education, and implementation of appropriate policies to address the issue of pet food sustainability to reduce malnutrition, food wastage, development of nutrient recycling, and the search for alternative protein sources (Swanson *et al.* 2013; Annooz, & Areaaer 2022; Jaber & Najim 2023). Essential nutrients that animals need include substances that either cannot be produced by the body or are not present in sufficient quantities to keep the animal healthy. One important group of nutrients that animals require are minerals which are very important for bone formation, cartilage, normal organ function, muscle contraction, and transmission of nerve messages (Rivas *et al.* 2013; Kanti Raje & Chaudhary 2018). The most important essential minerals are calcium and phosphorus, which play an important role in structural support of the skeleton and teeth (Flammini *et al.* 2016; Ciosek *et al.* 2021) and are abundant in ready-to-eat and whole foods. Phosphorus is an important mineral for which there is no substitute in animal feed (Ashley *et al.* 2011). The presence and availability of phosphorus in animal sources contribute significantly to the sustainability of this product. Tuna and tuna-like fish are known to be the most important species in the aquatic processing industry. Tuna traded in international markets is mainly used as raw material for the canning industry (fresh, frozen and precooked frozen fillets). Due to the use of the white muscles of tuna in the canning and sashimi industry, a large amount of waste and by-products of this fish is generated, which can represent up to 70% of the raw material. The amount of this waste is estimated to be up to 4.5 million tonnes per year (Nawaz *et al.* 2020). One of the most important wastes from tuna processing is bone tissue, which is a rich source of proteins and minerals. Overall, 60-70% of the components of fish bones are composed of inorganic substances, most of which is hydroxyapatite, the crystallised form of calcium phosphate (Nawaz *et al.* 2020). The tuna bone waste can be used as an inexpensive source of calcium and phosphorus (Flammini *et al.* 2016). In the present study, bone powder was prepared from the bone waste of yellowfin tuna (*Thunnus albacares*) and used to prepare the gelatine supplement tablets fortified with bone powder to evaluate the supply of minerals (calcium, phosphorus, and magnesium) from this product to domestic cats.

MATERIALS AND METHODS

Preparation of tuna bone powder

For the production of tuna bone powder, the bone waste of yellowfin tuna from the tuna cannery was processed. The bone waste was taken to the aquatic processing laboratory in ice containers and kept in the freezer at -18 °C until further steps. The soft tissue on the bone fragments was then cleaned with a wire brush under a stream of cold water. Following the preparation process, the bone fragments were immersed in trypsin enzyme solution (Sigma-Aldrich, Germany) to completely remove flesh, adipose tissue, and connective tissue from the bones and prevent the removal of salts and preserve collagen proteins. Afterward, the bone fragments were placed in a freeze dryer (CHRIST Alpha 1-2 LDplus-Martin Christ, Germany) for 72 h until the samples were completely dried (Malde *et al.* 2010, Namdar Taleshani *et al.* 2021). Thereafter, the bone pieces were crushed and divided into smaller pieces to produce the tuna bone powder and then ground with a grinder until a uniform powder was obtained. Fig. 1 shows the bone powder produced from tuna bone lesions (Fig. 1).

Fig. 1. Bone waste of yellowfin tuna and bone powder prepared therefrom (Photos taken by the authors).

Chemical analysis of bone powder

Nutrient composition analyses of tuna bone powder were performed in triplicate for moisture, lipid, protein and ash contents. Moisture content was determined gravimetrically after drying in an oven at 105 °C until a constant weight was reached (AOAC 2005). Crude protein was measured using the Dumas method (Müller 2017). Total ash content was measured by burning the samples in an electric oven at 550 °C. Crude fat was analysed by the Soxhlet extraction method using normal hexane as solvent (AOAC 2005). The results were expressed on a dry basis (Malde *et al.* 2010, Namdar Taleshani *et al.* 2021). To determine the content of major mineral elements (calcium, magnesium and phosphorus) in the tuna bone meal, calcium content was determined based on the calibration method of calcium content in the National Standard Feed of Iran-10701- (ISIRI 2009); the magnesium and phosphorus contents, based on the AOAC Animal Feed Method and phosphorus based on the National Standard Feed of Iran -513 (ISIRI 2009).

Production of bone powder supplement tablets

To prepare each supplement tablet enriched with tuna bone powder, 1.5 g of the flavourless and colourless bovine gelatine powder (with an approximate analysis of 85.6% crude protein, 0.1% total fat, 1.3% ash, 13% moisture, 0.06% calcium, 0.02% magnesium and 0.04% phosphorus) was used as starting material. The aforementioned amount of gelatine was added to 5 mL purified water along with the desired amount of bone powder and processed under gentle indirect heat (in the form of a water bath) to form a homogeneous mixture. Then the obtained mixture was poured into the desired moulds and kept at room temperature for 15 min. Afterward, the moulds were placed in a refrigerator at 4 °C until the contents had completely solidified. Fig. 2 illustrates the tablets produced by the abovementioned method (Fig. 2).

Fig. 2. Tablets enriched with the yellowfin tuna bone powder (Photo taken by the authors).

Study design and experiments on domestic cats

In this study, 6 short-haired domestic cats under one year of age were used, which were healthy at the clinical examinations, with a similar physical condition and came from the same mother. The approximate weight of the kittens ranged from 1.5 to 1.8 kg ± 100 g. Three kittens were considered as group 1 and three as group 2. To increase accuracy and reduce errors in the study, competition to avoid food and ensure equal food intake, the cats were kept in individual metal cages (dimensions 90 × 40 cm) during meals. In order to maintain hygiene and follow the rules of animal welfare (AWA), the cat litter boxes were cleaned and replaced three times a day, followed by cleaning the floor of the storage boxes once a day. In addition, large containers with antibacterial soil were used for the cats' sleeping and lying places. In order to acclimate the cats' physiological conditions to the environmental conditions and their habit of eating food daily, the treated cats were given only the basic food for 14 days. The basic diet of the treatments was a combination of chicken breast, potatoes and boiled carrots. The abovementioned foods were fully cooked in approximate ratios of 50, 30 and 20%, respectively, and then mashed. The amount of food prepared for each kitten was 50 g at the beginning of the period and increased proportionally to the need for pet food until it reached 100 g per meal at the end of the period (6 meals per day). Fresh and free water was provided during the treatments. After 14 days and the relative adaptation of the treatments in terms of the basic food consumed and the environmental conditions, as well as complete control of the type of nutrients ingested, the cats were taken to a veterinary clinic where blood samples were taken. In this way, the animals were first immobilised in compliance with all the criteria established by the Ethics and Work on Animals Commission. After restraint, the animal was injected with 11 mg ketamine containing 0.1 mg Acepromazine per kg of body weight into the quadriceps femoris muscle to sedate it and induce analgesia. Then, blood samples were taken from the anterior part of the brachial plexus on the hand using a 1-mL insulin syringe with a 30-gauge needle tip (Fig. 3). The amount of blood collected was 3 mL for each replicate. The collected blood samples were transferred to tubes with EDTA anticoagulant and taken to the laboratory after numbering to profile the main minerals (calcium, magnesium and phosphorus). The main study period began immediately after the end of the preparation and the first sampling and lasted 21 days.

Fig. 3. Taking blood from groups of the kitten in the veterinary clinic (Photo taken by the authors).

During this period, the basic meals were administered 6 times per day as usual. The mineral preparation prepared with the diet was given to the experimental animals in two portions of six meals each, 12 h apart. Throughout the feeding period, the process of food intake was studied and the complete absorption of the food by the experimental animals was ensured. During this period, the cats were divided into two treatment groups. For group 1, an amount of 5 g per day (2.5 g per serving equals one tablet per serving) and for group 2, an amount of 10 g per day (5 g per serving equals 2 tablets per serving) was fed orally. At the end of the 21-day experimental period, the treated animals were taken to a veterinary clinic where blood samples were taken from them. The blood samples were taken to the laboratory under the abovementioned conditions, where the changes in the essential mineral elements (calcium, magnesium and phosphorus) in the blood of the animals were studied.

Statistical analysis

To analyse the obtained results and compare the mean values of blood indices between the two groups after treatment, the T-test for independent samples was used. The condition for the application of this test is the equality of variances of the test groups. Leven's test was used to check the equality of variances. SPSS version 25 software was used for the statistical analysis of the data and the significance of the changes during the test period was also evaluated at a 95% level. Excel 2010 software was used to create all the graphs.

RESULTS AND DISCUSSION

Results of initial analysis and mineral content (calcium, magnesium, and phosphorus) of bone powder

Results of the initial analysis and the number of essential minerals (calcium, magnesium, and phosphorus) in the yellowfin tuna bone meal were presented in Table 1.

Table 1. Results of approximate analysis and analysis of mineral elements of yellowfin tuna bone powder (n = 3)

Analysis	Unit	Tuna bone meal
Crude protein	%	31.03 ± 0.08
Fat	%	4.84 ± 0.92
Ash	%	49.53 ± 0.41
Moisture	%	2.55 ± 0.19
Calcium	%	20.87 ± 0.02
Magnesium	%	0.34 ± 0.01
Phosphorus	%	7.62 ± 0.06

Results of trace essential minerals (calcium, magnesium, and phosphorus) in the blood of domestic cats

Table 2 shows the results of analyses and tracking essential minerals (calcium, magnesium and phosphorus) in the blood of each treatment separately, before and after taking supplements with tuna bone powder. As shown in Table 2, the amount of calcium in the blood of both groups of cats that received supplement tablets containing 5 and 10 g bone powder per day increased compared to the time before supplement feeding ($p < 0.05$). The amount of phosphorus in the blood of the first group was not increased ($p > 0.05$), while in the second group (who received supplemental food tablets containing 10 g bone powder per day) was elevated ($p < 0.05$). The amount of magnesium in the both groups was not significantly upraised.

Table. 2. Blood mineral content results of domestic cats fed with supplements containing 5 and 10 g of bone powder per day.

Treatments / Parameters	Group 1 (5 g of bone powder per day)		Group 2 (10 g of bone powder per day)	
	Before Fed with supplements containing bone powder	After Fed with supplements containing 5 g of bone powder per day (21 days)	Before Fed with supplements containing bone powder	After Fed with supplements containing 10 g of bone powder per day (21 days)
Calcium (mg/dl)	9.60 ± 0.30^{b}	10.63 ± 0.47^{a}	9.23 ± 0.25^{b}	10.77 ± 0.40^{a}
Phosphorus (mg/dl)	8.67 ± 0.45^{a}	9.53 ± 0.81^{a}	5.50 ± 0.92^{b}	6.89 ± 0.55^{a}
Magnesium (mg/dl)	2.06 ± 0.32^{a}	2.66 ± 0.06^{a}	2.33 ± 0.06^{a}	2.78 ± 0.06^{a}

*Different letters in each row and each treatment separately show a statistically significant difference ($p < 0.05$).

*The results are reported in percentage and based on the average ± standard deviation. (n=3).

DISCUSSION

Bone wastes from tuna contain an average of 30% organic compounds (Herpandi *et al.* 2011), most including collagen amino acids such as glycine, leucine, proline, hydroxyproline, and lysine, in addition to meeting some of the animals' needs for protein sources to improve the digestion and absorption of bone minerals and increase their bioavailability (Suntornsaratoon *et al.* 2014, Thammayon *et al.* 2017). In addition, the wastes contain monounsaturated fatty acids (MUFA) and also EPA and DHA, although lower than in the filet of this fish, albeit high percentage among the other fatty acids. Tuna bones consist of an average of 50 to 70% minerals, most of them contain elements such as calcium and phosphorus. The tuna bones used in this study exhibited a calcium-phosphorus ratio of 2.5 to 2.7:1, consistent with the results of Nemati *et al.* (2016). Because of the abundance and importance of the three elements, i.e., calcium, phosphorus, and magnesium in the bodies of mammals, including dogs and cats, these elements play a vital role in intracellular and extracellular function and in building structural strength. Animals require these three elements especially during pregnancy, lactation and adolescence. Therefore, we decided to use the tuna bone powder in order to provide mineral preparations with a gelatinous structure consumed by domestic cats in two amounts of 5 g and 10 g per day. Numerous factors are involved in the digestion, absorption, and alterations in mineral content of the blood and various tissues in monogastric animals such as cats. Given the importance and major role of parathyroid hormone (PTH) in the control and absorption of calcium and phosphorus, the most important factor stimulating the absorption and excretion of calcium from the intestine can be attributed to the role of this hormone in the production of 1 and 25 dihydroxyvitamin D. This vitamin is the most important factor in precisely controlling the absorption of calcium from the intestine in the presence of imbalanced conditions of this element in the blood. Continuous decrease of this element in blood serum of cats (hypocalcemia) stimulates secretion of parathyroid hormone and the elevated absorption of calcium in bone tissues, absorption of calcium from consumed food through intestine and the decreased excretion of this mineral provides ionization (iCa) necessary for the blood (Coady *et al.* 2019). For this reason, insufficient dietary calcium intake over a long period of time leads to a decrease in calcium density in bone tissue, resulting in osteoporosis and pathological fractures (Zafalon *et al.* 2020). The problem is much more serious in lactating animals, which must supply calcium to the milk they produce, and in growing young animals. As mentioned earlier, the ratio of calcium and phosphorus in the diet plays a much more important role in the absorption of these two elements from the gastrointestinal tract than the amount of these two elements in the diet. The lowest recommended ratio of calcium to phosphorus in cats is 1 to 1, while the highest is 2 to 1(Stockman et al. 2021), which is generally not observed in meat-containing foods and home-cooked meals. For the bone powder prepared in this study, this ratio was slightly higher than the recommended value (2.5 to 2.75 to 1). This is reflected in the low phosphorus intake, which is due to the high ratio of calcium to phosphorus in the bone meal, since the absorption of phosphorus in any form is influenced by other minerals, particularly calcium (Summers *et al.* 2020). In cats, many factors such as parathyroid hormone, 1 and 25-dihydroxyvitamin D, fibroblast growth factor 23, thyroid hormone, glucocorticoids, estrogens, and metabolic acidosis are just some of the endogenous metabolic factors that can be incorporated into phosphorus, directly or indirectly alter or increase or decrease renal excretion of this element (Lederer 2014). The most important factors in phosphorus absorption in most animal species include intestinal pH, the phosphorus requirement of the animal, the source of phosphorus supply, interaction with other nutrients such as calcium, magnesium, phytate (a form of phosphorus stored in cereals), while the ratio of calcium to phosphorus is the most important factor (Laflamme *et al.* 2020). When there is a magnesium deficiency in the body, absorption usually occurs through the active transfer process. However, if there is a concentration gradient in the gastrointestinal tract, inactive transfer from the lumen of the gastrointestinal tract to the extracellular space may also occur. Magnesium deficiency, like phosphorus, is rare in the body and occurs only in the pure magnesium-deficiency diets. Stockman and Villaverde (2021) found in a study that the negative effects of dietary magnesium deficiency are exacerbated by an elevation in dietary phosphorus. So, it is also particularly important to pay attention to a balanced phosphorus and magnesium content in the diet of dogs and cats. Elevating dietary calcium intake also affects dietary magnesium intake in cats. This effect may be due to the formation of soluble calcium-magnesium-phosphorus complexes that reduce the bioavailability of magnesium (Akter *et al.* 2018). To determine the standard levels of calcium, phosphorus, and magnesium in the blood of subjects before and after taking bone supplements, the number of minerals in the blood of subjects at the above time points was compared with the standard range given by Fielder (Fielder *et al.* 2019). According to the researchers, the standard levels of calcium, phosphorus and magnesium in the blood of cats are 8-11, 3.8-10.9 and 1.3-2.5 mg dL^{-1}, respectively.

Since the normal blood calcium, phosphorus, and magnesium levels of cats tested in this study before and after taking bone powder supplements fall within the range between the minimum and maximum levels of these elements in the blood of cats, it can be concluded that in the complete cat health, factors other than consumption of calcium-rich foods play a role in maintaining calcium balance in the cat's blood. (Mack *et al.* 2015). According to the findings of (Mack *et al.* 2015), dietary calcium concentration has nothing to do with the actual digestibility of calcium; however, the ratio of dietary calcium to phosphorus seems to have little effect on the actual digestibility of calcium in cats. They found that the factors affecting the actual digestibility of calcium in dogs and cats exhibits different influences on the actual digestibility of phosphorus in these animals. According to the results (Fig. 2), the elevated consumption of tablets containing bone powder in the diets of cats displayed no significant effect on the serum magnesium levels in the treatments. Since too much calcium is absorbed through the intestines of cats, this causes the kidneys to reabsorb the magnesium and reduce it in the urine. Increasing supplementation in the diets of the cat groups did not have a large effect on intestinal absorption of magnesium, which is consistent with the results of the present study and its relationship to the findings of other researchers (Pastoor *et al.* 1994, Stockman *et al.* 2021).

CONCLUSION

Given the increasing importance of domestic animals as one of the largest consumers of protein resources, it is of particular importance to utilize waste protein sources consumed by humans, which have a high potential to meet the nutritional needs of these animals. The tuna bone waste, which constitutes a large portion of the waste from the tuna canning and fileting industry, is a rich source of organic and inorganic nutrients and has a very high bioavailability that can meet the mineral requirements of humans and animals. In the present study, the bone waste of tuna used in the canning industry was processed with the best method and the least damage to the nutrients and used to produce bone supplement tablets for feeding domestic cats and the possibility of providing elements. The mineral requirements (calcium, phosphorus and magnesium) of these animals were studied. The results of this study showed that tuna bones were rich in minerals. Due to the control of the animal feed during the test period, no significant difference was found in the content of some minerals in the blood of the treatments, especially magnesium. Based on the obtained results (increase in the content of calcium and phosphorus in the blood of cats) and given the fact that a large amount of bone waste is produced in factories for water treatment, it is proposed to use it for the production of food supplements for pets. By this method it is possible to produce a food supplement with the required calcium and phosphorus at a low cost. On the other hand, the reuse of the waste contributes to the protection of the environment.

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