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The Natural Calcium and Phosporus Content in Two Fishbone of Commercial Species from Enggano Waters

The Natural Calcium and Phosporus Content in Two Fishbone of Commercial Species from Enggano Waters

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Abstract. Calcium and phosphorus are important elements needed for the growth and development of the body both in humans and other living things. The bones of Tiger Grouper Fish (*Epinephelus fuscoguttatus*) and Lencam Fish (*Lethrinus lentjan*) have a fairly high content of calcium and phosphorus. The purpose of this study was to determine the calcium and phosphorus content in the bones of Tiger Grouper Fish and Lencam Fish. This research was carried out in September-November 2023 with direct sampling of collectors in Banjarsari Village, Enggano Island. The calcium content test was performed using an Atomic Absorption Spectrophotometer (AAS) and the phosphorus content test was performed using a UV-Vis spectrophotometer. The results of this study showed that the calcium content in the bones of Tiger Grouper was 8.84% and for Lencam Fish was 7.97%, and the phosphorus content produced in both samples was 1.4% for Tiger Grouper Fish of 1.4% and 1.43% for Lencam Fish. Based on the results of the study, the calcium content in Tiger Grouper is greater than that of Lencam, while the phosphorus content in Tiger Grouper is smaller than Lencam Fish.

1. Introduction

The production of catches produced on Enggano island can reach 27.89% of the total production of capture fisheries in North Bengkulu [1,2]. Snapper, Grouper and Pomfret are the three types of marine capture fisheries that produce the highest production from sea waters compared to coastal areas [3]. The high development of the industry in the field of processing fishery products will have the potential to increase organic waste in the area [4–6]. According to [7], The most common types of fishery waste are fish bones, fish heads, fish guts, and even shells, while this waste cannot be recycled due to the many limitations of its advanced processing [8]. Actually, it is estimated to be a material that can be used again, especially the use of fish bones which have natural calcium and phosphorus content.

Calcium and phosphorus are very important for bone and tooth growth and calcium intake must be considered early to meet the needs of bone growth in humans [9]. Several health problems can arise due to calcium and phosphorus deficiency, namely the risk of osteoporosis. In Indonesia, osteoporosis is caused by the low average calcium intake of the Indonesian population which is only 254 mg per day.

The average daily calcium requirement is 300-400 mg for infants, 500 mg for children, 600-700 mg for adolescents, and 500-800 mg for adults [10].

Fish bones are a type of fishery waste that contains a number of important benefits for humans. The main components of fish bones are calcium, phosphorus, and carbonate, so that fish bones themselves have the highest calcium content compared to other parts of the fish's body [11]. Calcium derived from fish bones is a waste product and is currently not used on a large scale for human needs. Therefore, further research is needed on the benefits of fish bone waste to be used as processed products that are beneficial for fisheries and marine problems [12]. One of the uses of fish bones is to process them into fish bone meal.

According to [13], calcium and phosphorus are macrominerals that are directly related to the development and maintenance of the skeleton system and participate in various physiological processes of the organism's body. The nutritional content of fish bones in 100 g of fish bone meal is calcium 735 mg, protein 9.2 g, fat 44 mg, phosphorus 345 mg, iron 78 mg, ash 24.5 g, and carbohydrates 0.1 mg [14]. Calcium has a role as an important micronutrient in addition to phosphorus for the growth and development of children and calcium and phosphorus are very important in the bone mineralization process [15]. Calcium plays an important role in physiological processes, such as cell metabolism, muscle contraction, and bone growth [16]. Phosphorus has metabolic functions to build bones, scales, adenosine triphosphate, cell membranes, and nucleic acids [4]. Chemical analysis revealed that fish bones are a valuable source of calcium phosphate as an economical source for synthetic hydrocytopathy. Bone is a biological composition consisting of an inorganic phase (calcium phosphate with a structure like carbonated hydroxyapati) [17].

The fisheries from the waters of Enggano that are consumed are Tiger Grouper Fish and Lencam Fish, is one type of fish waste that has the potential to be researched because it is included in the target fish species and is also the most dominant economic fish found [18]. Morphologically and physiologically the two samples are different, but the living habits or habitats of the two samples are almost the same, so this test was carried out to determine the difference in calcium and phosphorus content of Tiger Grouper and Lencam fish.

One of the indicators of the existence of Tiger Grouper fish is the presence of coral reefs in a water area to be able to shelter and as a place to forage [19]. Adult Tiger Grouper Fish can reach depths of more than 2 m. This type of fish often lives in clear waters with many coral reefs and depths of up to 60 m. The habitat of this fish is in the shallow waters of coral reefs, rocky seabeds, lagoon tops, coral channels, and the outermost part of steep coral reefs. This Grouper also likes waters with a sandy, rocky bottom and seaweed growing at the bottom [20]. In general, groupers are solitary, but when they want to spawn the fish in groups together. In general, this fish lives at a depth of 5-20 m in all types of corals in good condition. Most Tiger Grouper fish use burrows/cavities/holes in coral reefs as shelter and usually move around.

Lencam fish usually live in coastal areas, coral waters and belong to the group of underwater fish. The habitat of the Lencam fish is coral reefs, seagrass meadows, mangrove areas and shallow coastal waters with a sandy bottom at a depth of 30 m [21]. Lencam fish consists of 29 species spread around the world and about 81% of them are found in Indonesian waters. This fish continues to be threatened both directly and indirectly due to exploitation caused by various human activities [22].

2. Materials and Methods

2.1 Study area

This research was carried out in September – December 2023 with sampling obtained from fish collectors in Banjarsari Village, Enggano Island, Enggano District, North Bengkulu Regency, Bengkulu Province (Figure 1). Further sample analysis was carried out at the Marine Bioecology Laboratory and the Oceanography and Marine Instrumentation Laboratory at Sriwijaya University.

Figure 1. Sampling location of tiger grouper and lencam fish in Enggano Waters

2.2 Sample Identification and Preparation

The samples used were taken from fish collectors in Banjarsari Village, Enggano Island. The fish samples amounted to 2 Tiger Grouper Fish and 2 Lencam Fish. The fish was still fresh, seen from its red color and not slimy. The sample preparation process was carried out at the Marine Bioecology Laboratory, starting with dissecting the fish using a knife. Then the dissected sample was washed and then boiled using a hot plate with a temperature of 60-80 °C for 60 min. After boiling, the fish was cleaned again using a brush to remove the meat that was still attached to the fish bones, then NaOH alkaline extraction was carried out which aimed to remove fat and blood levels in the sample. Samples that have been extracted for 60 min were cleaned again using aquades and then baked for 48 h at a temperature of 60°C. After the sample dried, it was crushed with mortal until it became fish bone meal powder which will later be used to make calcium and phosphorus test solutions. Fish bone meal powder was taken as much as 1 g (calcium) and 2 g (phosphorus) weighed using an analytical scale. Each sample taken was put into the erlenmeyer to make a test solution. The preparation of this test solution was carried out in the fume hood with the addition of NaOH 1.5 N, HNO_3, HCI, H_2O_2, $NaHCO_3$, $(NH_4)_6Mo_7O_{24}.4H_2O$, $H_2O(SbO)C_4H_4O_6$ 0.5K, H_2SO_4 and PO_4. After the fish bone sample becomes powder, a destructive process is carried out to determine the calcium and phosphorus content. The calcium content test itself was carried out using an Atomic Absorption Spectrophotometer (AAS) with a wavelength of 420 nm, while the phosphorus content testing was carried out using a tool called the Uv-Vis Spectrophotometer with a wavelength of 889 nm.

2.3 Sample Destruction

Common destructions to determine the mineral components present in foodstuffs were known to be of two types, namely dry and wet [23]. Sample destruction in this study will use the wet destruction method referring to the study [24]. Their results showed that wet destruction gave better results than dry destruction because the temperature used could not exceed the boiling point of the solution and in general the carbon was destroyed faster than using dry ashing.

2.4 Calcium level testing

The calcium content was determined by measuring the sample by wet destruction using an Atomic Absorption Spectrophotometer (AAS) with a wavelength of 420 nm, and the calcium content was checked using a modified method [25]. To analyze the calcium content, it can be done by weighing as much as 0.1 g of fine samples and transferring them to a 100 mL measuring flask. The destruction process was carried out by adding 15 mL of hydrochloric acid (HCl) to digest the sample. The solution was decomposed until it was clear and then cooled. Filtration volumes up to 100 mL can be measured with atomic absorption spectrophotometer (AAS) [26].

2.5 Phosphorus level testing

The determination of phosphorus content in the solution of fish bone samples aimed to obtain the concentration of UV-Vis spectrophotometer (ppm) readings. The determination of the maximum wavelength P was carried out by analyzing the sample of phosphorus sample using a UV-Vis spectrophotometer at a wavelength of 889 nm. With this determination of wavelength, the maximum result of the P content in the fishbone sample [27]. The dissolved concentration obtained from measurements using a UV-Vis spectrophotometer was then recorded so that a graph of the wavelength and absorbance relationship in each fishbone sample used in testing the content of each sample was obtained.

2.6 Data analysis

2.6.1 Moisture content analysis

%Moisture content was calculated based on SNI-19-1705-2000 and AOAC (1995).

$$\%Moisture\ content\ = \frac{Wo-Wt}{Wt}\ x\ 100\%\ \ fk = \frac{100}{100-\%\ Moisture\ content}$$

Information:

Wt : Final weight of fish bone meal (g)
Wo : Initial weight of the fish bone (g)
Fk : 100 / (100 - % of air)

2.6.2 Calcium concentration data analysis

The concentration obtained from the curve was a concentration in mg/L. While the sample used in this study can be in the form of a solid so that it will be converted into mg/kg. To calculate the level of calcium in fish bone samples in ppm by sample weight, which was formulated as follows:

$$\text{Mineral concentration (mg/kg)} = \frac{\text{Sample concentartion } \left(\frac{\text{mg}}{\text{L}}\right) \text{x sample volume (L)}}{\text{Sample weight (kg)}} \text{ x } D_f$$

Information :

D_f : Dilution factor

2.6.3 Phosphorus concentration data analysis

The processing of phosphorus concentration data that was previously in the form of ppm units is converted into % which is formulated as follows:

P_2O_5 levels available (ppm) = ppm curve x ml extract/1,000 mL x 1,000 g (g example) x D_f x 142/190 x M_f

Information :

ppm curve	:	the sample level obtained from the relationship curve between the standard series and its reading after blank correction
D_f	:	dilution factor (if any)
142/190	:	PO_4 to P_2O form conversion factor
M_f	:	moisture content correction factor

3. Result and Discussion

3.1 Description of Tiger Grouper Fish

Based on the identification [28,29] the fish obtained were classified as *Epinephelus fuscoguttatus species*. Observations of the morphology were size, body shape of the fish, the position of the mouth, the type of fins and also the type of scales.

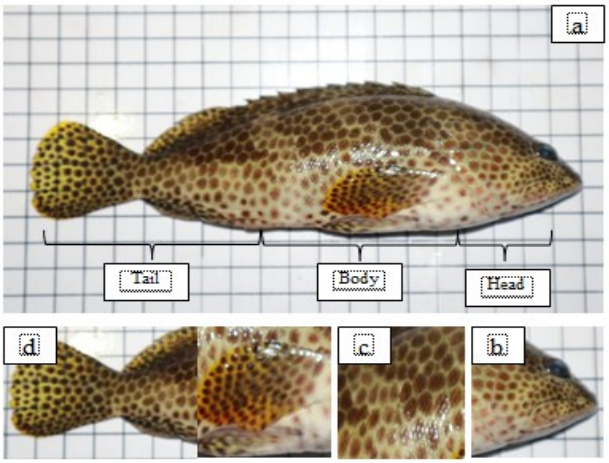

Figure 2. (a) Fish body parts, (b) Mouth type, (c) Scale type, (d) Fin type

Observation of the morphology of the Tiger Grouper fish consists of parts of the fish's body consisting of the head, body, fins and tail. The body shape of this fish in figure 3 (a) is compressed. In Figure (b) the mouth type is a terminal type whose upper and lower jaws can be seen to be the same length (parallel), and is often used to eat prey floating in front of it. Figure (c) body type covered by scales with a brownish body color and filled with reddish spots. While Figure (d) is a rounded fin type, meaning that the dorsal fin is elongated and totals 8-10 pieces and the caudal fin totals 15-17 pieces.

3.2 Description of Lencam Fish

The determination of the morphological characteristics of Lencam fish includes body shape, mouth position, caudal fin shape, and scale type.

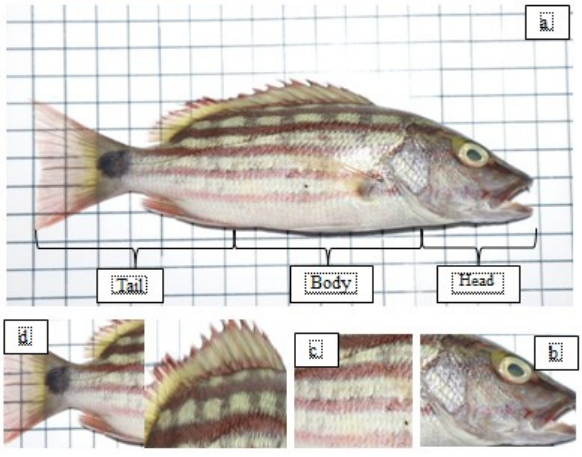

Figure 3. (a) Fish body parts, (b) Mouth type, (c) Scale type, (d) Fin type

Lencam fish are characterized by a flattened body that is covered with many scales, smooth cheeks, at the base of the ventral fin there are large scales (axillary scale) that are well developed. The mouth is located at the front end of the fish's head (terminal type) with a relatively thick lip, the front of the jaw has strong canines, on the side of the jaw there are conical teeth and large molars, there are no teeth at the top of the oral cavity. The first dorsal fin is fused with the second fin, consisting of 9 hard fingers and 9 or 10 soft fingers, the anal fin with 3 hard fingers, 8-10 soft fingers. (a) is a part of the

body of a fish consisting of 3 parts, namely the head, body and tail, in Figure (b) the mouth type of the Lencam fish is a terminal mouth type, meaning it has the same length of upper and lower jaws, and often eats prey floating in front of it. Figure (c) the shape of the body is rather tall and flattened, and the body is covered by scales. While Figure (d) is the type of *Homocercal fin,* the shape of the pinna caudalis is curved or not and supported by the spokes of the tail fin. In this type, the length between the two ends of the tail is the same length and also forms like the letter V.

3.3 Yield Value of Tiger Grouper Fish and Lencam Fish

The yield value in this study is the result of a comparison between the initial weight of the fish bone and the amount produced from the final weight of the fish bone powder. According to [30], the level of yield value produced shows that the number of products produced is also high, indicating that the process used is more effective.

Table 1. Yield Value

Types of fish	Bone weight (g)	Fish bone powder (g)	Yield value (%)
Tiger Grouper Fish	17.98	6.7	2.6836
Lencam Fish	13.04	4.05	3.2198

The yield value obtained in this study can be an indicator of the quality of bone powder in both types of fish samples. It can be assumed that a high yield value indicates good flour quality. The magnitude of the yield obtained is influenced by the effectiveness in the extraction process. According to [31], a high yield will not necessarily produce the highest calcium levels, but it is also determined by other factors such as the low protein content in the material. The low yield is also suspected to be due to the influence of drying, where drying is the process of removing or disposing of liquid materials from a material.

3.4 Calcium Content of Tiger Grouper Fish and Lencam Fish Bones

The analysis of calcium content in this study was obtained from measurements or readings using an atomic absorption spectrophotometer (AAS).

Table 2. *Calcium Content*

Types of Fish Bones	Conc (ppm)	Ca (%)	Quality Standards (%)
Tiger Grouper Fish	8.479	8.48	20-30
Lencam Fish	7.967	7.97	

The results of the measurement of calcium content in the bone samples of Tiger Grouper and Lencam Fish are known that the calcium content in Tiger Grouper is 8.84%, while in Lencam Fish it is 7.97%.

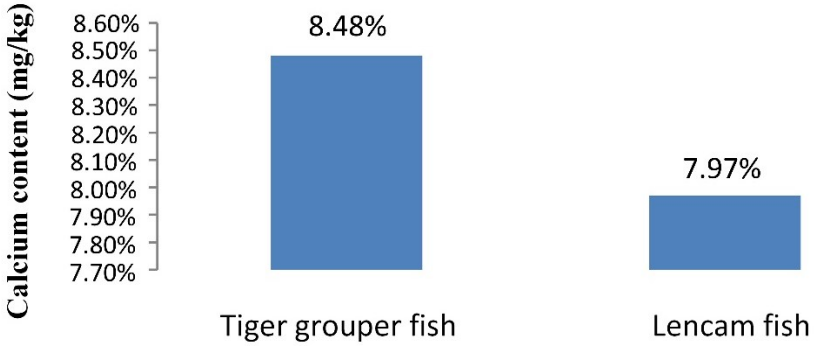

Figure 4. Measurement of calcium content

The difference in calcium content in the two samples can be influenced by the difference in the samples used, because basically different types of fish affect the value of the calcium levels produced. The results of measuring the calcium content of both Tiger Grouper and Lencam fish bones with different sizes are assumed that the sample size affects the mineral content in the fish's body, one of which is the bones. This is in line with the opinion [32,33], that minerals play an important role in the maintenance of every body function at the level of cells, tissues, organs, and overall body functions, and the presence of macro minerals in the body of fish is influenced by several factors, such as the size of the fish.

3.5 Phosphorus Content of Tiger Grouper Fish and Lencam Fish Bones

The analysis of phosphorus content in this study was obtained by reading the results of the UV-Vis Spectrophotometer. The phosphorus content obtained in the bones of Tiger Grouper Fish and Lencam Fish with the same weight category in this study is seen in Table 3.

Table 3. Phosphorus Content of Fish Bones

Types of Fish Bones	Conc (ppm)	P (%)	Quality Standards (%)
Tiger Grouper Fish	1,401.49	1.40	20-30 %
Lencam Fish	1,432.58	1.43	

Results of testing phosphorus content in the bones of Tiger Grouper and Lencam fish in the same weight category in mg/kg and percentage (%). Based on the quality standards of fish bone meal according to SNI 01-3158-1992, it is understood that the phosphorus content in the two samples does not meet the quality requirements. Furthermore, the results that have been obtained are visualized in the form of a graph to show a comparison of the results between the two fish, which can be seen in the following graph.

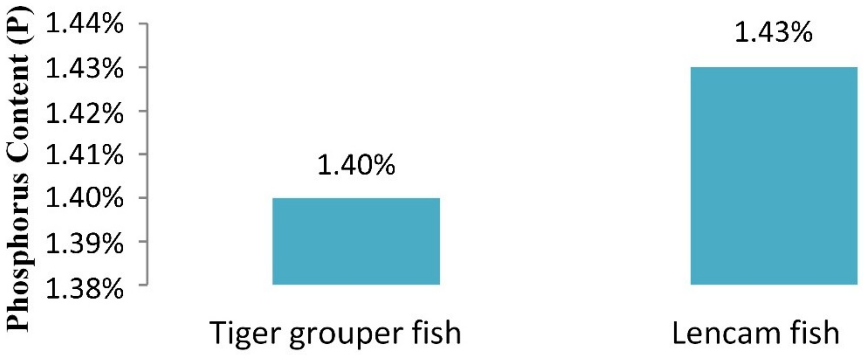

Figure 5. Measurement of phosphorus content

The phosphorus content in Lencam fish bone samples was higher than the phosphorus content in Tiger Grouper fish bone samples. The phosphorus content in Tiger Grouper can be seen at 1.4%, while in Lencam fish the content is slightly higher at 1.43%. The comparison of these two samples is around 0.03%. The type of sample, the material and the procedure used to measure the calcium and phosphorus content can also affect the results obtained**.** Different types of fish used in the study can affect the calcium and phosphorus content obtained, because the mineral content in each fish is different. Another factor that can cause this difference is also due to the sample size and the process in handling the sample.

The type of sample, materials and procedures in the study used to measure calcium and phosphorus levels can also affect the results obtained. This was revealed by [12] in the results of the study, the analysis of various types of frequencies in the boiling process affected the calcium content produced in the bones of Belida fish (*Chitala lopis*) ranging from 28.25% and the phosphorus content varied between 3.98% - 4.04%. The difference in NaOH concentration during the extraction process

affects the calcium and phosphorus content obtained, namely the concentration of 1.5 N provides the best content compared to the concentration of 1 N and 2 N. The selection of the test method in measuring the calcium and phosphorus content can affect the results obtained in each sample used.

3.6 *Calcium and phosphorus content in several types of fish bones*

Based on Table 4, it is the result of a comparison of calcium and phosphorus content from various sources and also from various other types of marine fish. The different calcium and phosphorus content in the table below is caused by various factors, especially from the type of fish, the size of the fish and also influenced by the sample handling process before testing the content level in each sample. The method used in each source is also different, so it can be stated that the content is different because it is also due to the method used, because each method uses a different solution and also the processing process before being made into a solution is also different.

Table 4. Calcium and Phosphorus Content in Several Types of Fish Bones

No	Types of Fish Bones	Calcium %	Phosphorus %	References
1	*Epinephelus sp*	8.48	1.40	This results
2	*Lethrinus sp*	7.97	1.43	This results
3	*Chitala lopis*	28.25-31.31	3.98- 4.06	[12]
4	*P, albilabris*	48.10	35.40	[34]
5	*Thunnus sp.*	39.24	13.66	[35]
6	*Trichiurus savala*	19.33	7.21	[36]
7	*Sardinella longiceps*	26.39	16.57	[36]
8	*Scomber scombrus*	14.3	8.6	[37]
9	*Salmo trutta*	14.7	8.7	[37]
10	*Salmo salar*	13.5	8.1	[37]
11	*Micromesistius poutassou*	17.0	8.7	[37]
12	*Clupea harengus*	16.1	9.4	[37]

Based on the comparison table of calcium content in the table above, it can be stated that the calcium content in fish bones is lower than the calcium content in crab shell shells. This was also explained by [38,39], it was explained that the calcium carbonate content reached 53.70%-78.40% contained in the shell of the shell. As for the phosphorus content in fish bones from various sources presented in the table above, it is known that the phosphorus content is higher than the phosphorus content in seaweed. According to [40], his research explained that the phosphorus content in seaweed is >0.05, it can also be concluded that the phosphorus content is indeed higher than the content of other species. The difference in calcium content is also caused by the difference in the species analyzed, because every difference in a living creature can cause the results of the analysis related to the nutrient content in that type or species to be different. The difference between this study and the previous study lies in the category of fish sample size used and the chosen destruction method. The sample size was made in 3 categories to see the difference between them, while the selection of the method for phosphorus testing with P Bray I where the phosphorus read was only in the form of $H_2PO_4^-$ and PO_4^{3-}. In line with the results of a study [41], which tested soil samples with two different methods.

Calcium is an important mineral that can be relied on in the human body in a variety of several important processes [42]. Most of the calcium in the body is in the bones. Your circulatory system also has a certain amount of calcium which is essential for blood clotting as well as the function of blood vessels, nerves, muscles and heart. Calcium and phosphorus are one of the substances that have an important role in various bodily functions, such as bone mineralization, functioning as cofactors in many enzyme systems, maintaining muscle and nerve excitation, and in the case of iron, maintaining oxygen transport and blood capacity [43]. Many female athletes consume less calcium than the recommended nutritional adequacy rate. It is also a concern due to the need to reach peak bone mass during adolescence and the possible relationship of poor calcium intake with stress-induced fractures. Calcium and

phosphorus are available in various supplement forms, both can be taken together as individual supplements, and there are calcium phosphate supplements that are a combination of the two minerals. The role of phosphorus in the human body functions in the growth and development of energy in the body. The high content of calcium and phosphorus in fish bones makes fish bones can be processed into natural ingredients to replace bones or hydroxyapathic materials. According to [26], hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic material that is biocompatible, biodegradable, and non-toxic. Hydroxyapatite can be synthesized from natural materials that contain relatively high levels of calcium which will later be used as calcium precursors because hydrosapathic compounds can be obtained by mixing calcium precursors with phosphate precursors. The functions of calcium in the body, especially in fish, include as the main component of the formation of bones, teeth, skin and scales, as well as other functions can maintain the strength of the body's skeleton, thicken the blood, become an intracellular regulator which can also help regulate the activity of skeletal muscles, heart and other tissues, muscle contraction and relaxation, help the absorption of vitamin B12, maintain osmotic balance [9][44]. This is supported by research [13], which states that calcium plays an important role in skeletal mineralization and bone formation, not only that calcium is also needed for bone growth and strength.

Conclusion

Grouper Fish Bones have a higher calcium content of 8.48% compared to Lencam Fish bones of 7.97%. Meanwhile, Lencam fish bones have a phosphorus content 1.43% higher than Tiger Grouper 1.4%. Based on the quality requirements of fish bone meal according to SNI 01-3158-1992, the calcium and phosphorus content of the bones of Tiger Grouper Fish and Lencam Fish do not meet the quality requirements.

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