Using tuna fish bone waste as mineral sources in feed formulation of tilapia (*Oreochromis niloticus*)

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Abstract. Fish bone is a solid waste that usually found in traditional fish markets of Ternate. The bone fraction contains high minerals such as calcium, phosphorus, and collagen proteins which are important for fish growth. Tuna fish bone waste was processed into dry fish feed and mixed into feed formulation of Tilapia. Four different dose of tuna fish bone powder were applied namely 0%, 1%, 3%, and 5% as mentioned treatment A, B, C, and D. Fish diet were given to Tilapia juvenile for 42 days. Results show that the body weight and specific growth rate are highest in fish which given 3% dose of tuna fish bone than other treatments. It reaches 38.66 g and 9.617% BW/day. The lowest FCR is 1.34 in treatment C and the survival rate is not significantly different among treatments (p>0.05).

1. Introduction

Fish bone has been used as a raw material in feed formulation in aquaculture production for decades. Several advantages of using various types of fish include increasing protein level of diet, as sources of essential amino acid and Polyunsaturated Fatty Acid (PUFA) which are very important in fish growth, and a great source of minerals. However, not all parts of fish’s body can be used since they are competed with human needs. Fishbone fraction, which is still regarded as a waste product, is considered to fulfil mineral requirements of fish. It contains approximately 10-15% of the total body weight [1]. In many countries, large quantities of fish waste and fish by-product are discarded into the environment and converted to an important source of pollution [2]. In recent years, dried fishbone was used as a feed ingredient in diets for fish and other animals, with a positive effect on feed efficiency and growth compared to traditional diets [3]. Fish meal which obtained from whole fish or fish by-products contains approximately 10% minerals, rich of calcium and phosphorus, and also represent a significant source of minerals once mixed into feed [4].

Calcium (Ca) and phosphor (P) are macro minerals that are directly related to the development and maintenance of the skeleton system and participate in various physiological processes of the organism. Ca requirements in fish are influenced by water chemistry, P levels in feed, and species [5]. The addition of Ca in the diet was necessary for the growth of red mullet, Japanese flounder, and scorpionfish [6]. In most fish species, P deficiency results in slow growth, poor feed efficiency, poor bone mineralization, high body lipid content, and low ash content [7]. Calcium and phosphor are minerals that are synergistic with each other [8] and in the form of hydroxyapatite in forming bone [9].

There are limited publications regarding the bioavailability of calcium recovered from fishbone and its potential usability [10]. The effects of fish bone utilization from small fish to human’s Ca content [11], there was a question whether it shifted Ca content in milk or not. A study on the nutritional
composition of yellowfin tuna bone powder showed that tuna bone powder was rich in protein, beneficial fat, calcium, and phosphorus, and can be used in fish feed formulation.

This study aims to determine the levels of Ca and P in fish feed that are suitable for growth and feed efficiency. The benefits that can be obtained from this research are new information for the development of science and technology in the field of nutrition and more knowledge on composition of tilapia feed that is appropriate, accurate and efficient for its growth.

2. Materials and methods
The materials used in this study were bones from yellowfin tuna (Thunnus albacores) obtained from Ternate traditional fish markets. This study was conducted from May to August 2021. The production of tuna bone powder referred to [12]. Bone powder KOH was prepared by boiling tuna bones in an aqueous solution of 0.5% KOH (w/v); quantity of water sufficient enough to completely submerge the bones. Bone powder prepared by boiling tuna bones in potable water was used as control (BP-control). Meat adhering to tuna bones was manually separated. Alkali treated bones were thoroughly washed to remove residual alkali. Washed tuna bones were dried and pulverised to a fine powder. Complete randomized design was used in this study, consisted of four treatments with three replications. The experimental feed formulations were different dose of bone powder which were 0%, 1%, 3%, and 5%.

2.1. Fish rearing and data collecting
The initial weight of Tilapia was 4.60 ± 0.20 g. Fish body weight was measured at the beginning and the end of the study. Before rearing, fish was adapted (acclimated) for 7 days. They were reared in 12 aquaria sized in 60×30×30 cm3 that equipped with a 24-hour aeration system. The stocking densities were 10 fishes per aquarium. The feeding was delivered three times a day, i.e., 8.00, 12.00, and 16.00 using at satiation feeding method.

The growth performance was measured at the end of the study (day 42). The measured parameters were feed consumption, specific growth rate (SGR), and feed conversion ratio (FCR). Feed consumption was calculated using: feed consumption (g/fish) = [Σ consumed diet/ Σ final population]. SGR was calculated using: SGR (%/day) = [Ln Wt – Ln Wo]/T ×100, with Wt is the final average weight, Wo is the initial average weight, and T is the rearing period. FCR was calculated using: FCR = F/[Wt−Wo], Wt is the final fish biomass and Wo is the initial biomass.

2.2. Data analysis
The survival rate (SR), specific growth rate (SGR), and FCR were analysed using one-way ANOVA with a 95% confidence level and were further tested using Duncan’s test. Statistical analysis was conducted using Excel 2019 software.

3. Result and discussion
Study results showed that mixing 3% of fish bone into diet formulation gave highest body weight and specific growth rate which were 36.66 g and 9.617%BW/day, respectively. It was followed by other treatments (D, B, and A) which had 38.36 g; 32.57 g; and 24.74 g of body weight. While the specific growth rate was 9.525%BW/day; 8.695%BW/day; and 7.486%BW/day, respectively. Those results indicated that treatment C and D were almost similar, means that mixing 3% to 5% fish bone powder into diet formulation would gain similar weigh to Tilapia (Figure 1, 2 and 3). Even though 3% fish bone in diet formulation was not completely consisted of pure Calcium, it could be assumed that most of bone parts were calcium content. According to [13], African Catfish which given 3 g Ca per kg feed would increase 25.2 g BW and SGR 1.70% day.
Figure 1. Mean body weight of Tilapia (*Oreochromis niloticus*) in different dose of tuna fish bone powder in feed formulation for six weeks.

Figure 2. Final weight of Tilapia given different dose of Tuna bone powder during research.

The largest amount of calcium source comes from fish environment whether in marine or freshwater [14]. It varies in regarding calcium absorption and typically depends on hypo- and hyper-calcaemic hormones, species specific, and calcium absorption capacity of species in which marine fish can absorb more Ca than freshwater fish. Based on [15], the highest specific growth rate in juvenile grouper was reached when added 0 g of Ca source into diet and combined 6 g of P in 1 kg diet. It can be seen that fish can absorb more Ca from ocean which provide minerals. Other study [16] revealed that Goldfish and brook trout absorbed more calcium from diet fed when those fish reared in low calcium water than in high calcium water.
Figure 3. Specific growth rate (%BW/day) in each treatment during research

FCR was determined by dividing the total feed used in a production unit or entire aquaculture facility by the net production of culture species. Varieties of FCR depended on several factors, including species, feed type and quality, production system, feeding technique, and water quality conditions. Warmwater fish and shrimp farms typically achieved FCR values of 1.5 to 2.5, and for general purposes, FCR often was assumed to be 2.0 [17].

Figure 4. The amount of feed conversion ratio (FCR) each treatment during research

The lowest FCR was found on treatment C with a value of 1.34, followed by treatment D (1.40), B (1.43), and the highest one in treatment A (1.75). Tilapia converted feed efficiently become weight gain during rearing time. The less diet used for certain period of time in aquaculture production and the more fish body weight was achieved, the better feed formulation was chosen for fish. It can be a proper choice when driving an aquaculture activity both indoor and outdoor. It can be also concluded that all types of diet were lower FCR implying that all the diets contained sufficient protein content which is very useful for body building. As [18] stated that high FCR was related to higher fibre
content and lower protein used for body gained. Furthermore, reduction of those components was crucial to increase fish absorption in feed efficiently.

Figure 5. Survival rate of Tilapia fed by different dose of tuna fish bone powder for six weeks

Figure 5 above shows the results of percentage survival and mortality of fish after 42 days of feeding trial. The result obtained revealed that percentage of fish that survived was non-significantly different among treatments. Treatment C and D were 100% survive, followed by treatment A 96.66%, and treatment B 93.33%, respectively. The result of the study showed that all experimental diets were accepted by juvenile and had lower risk for their survival rate.

Water quality parameters were measured at the beginning and at the end of rearing period. Table 1 shows that temperature, pH, and dissolved oxygen were in proper condition for supporting juvenile growth.

| Treatment | Water quality parameters |
|-----------|--------------------------|
|           | Temperature (°C) | pH | DO (mg/l) |
| A (0%)    | 23.77 - 24.84   | 7.4 – 7.6 | 4.9 – 6.5 |
| B (1%)    | 24.11 - 24.77   | 7.3 – 7.6 | 4.3 – 5.2 |
| C (3%)    | 24.62 - 24.88   | 7.4 – 7.7 | 3.7 – 4.5 |
| D (5%)    | 24.75 – 25.20   | 7.7 – 7.9 | 4.3 – 4.7 |

There was a close relationship between water quality condition in aquaculture system and the health performance of fish. It is important to remember that the failure or successful of aquaculture business plan is connected to water quality controlling during fish rearing time [19].

4. Conclusion

Studies on using tuna fish bone waste as mineral sources in diet formulation remain challenging. Though 3% dose of fish bone mixed in feed formulation gave the highest result on growth and specific rate, and also had the lowest FCR, the exact calcium requirements for supporting fish growth still needs further study. At the same time, the proportion of Ca and P was also important to study further.

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