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The effect of protein levels in fermentation feed supplemented *lumbricus* sp. extract as feed additive on growth performance and body chemical composition of milkfish, *Chanos chanos* forskal 1775

Siti Aslamyah, Zainuddin, Badraeni, Muhammad Khairul Umam, Ayu Dewi Lestari, and Haslinda

Aquaculture Study Program Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia

Email: sitiaslamyah1@gmail.com

Abstract Protein is the main nutrient in feed and is sourced from relatively expensive feed ingredients, so it is necessary to optimize use by providing feed additives. This study aims to determine the levels protein of fermented feed supplemented *Lumbricus* sp. extracts as a feed additive that provides the best response to milkfish growth performance and body chemical composition. Milkfish with an initial weight of 8.52 ± 0.29 g head⁻¹ maintained a density of 30 fish per hapa measuring 1 m³ of 24 units. Hapa installed in ponds with a water height of ± 60 cm. The study was designed in a completely randomized design with the treatment of various levels of feed protein, namely 20, 25, 30, and 35%. Before printing into pellets, the feed material is fermented with microorganisms mix for seven days and supplemented with 300 mL kg⁻¹ of feed ingredients *Lumbricus* sp. extracts. During 50 days of rearing fed 5% given of body weight per day three times a day morning, afternoon and evening. The results showed various levels of feed protein gave the same results on milkfish growth performance and body chemical composition. The resulting relative weight growth ranged from 58.47 ± 2.11 - $60.46 \pm 0.40\%$, survival rate 88.89 ± 3.85 - $94.44 \pm 5.09\%$, feed efficiency 40.44 ± 1.98 - $43.06 \pm 1.27\%$, body chemical composition (including protein content 67.52 ± 0.52 - $68.59 \pm 0.67\%$, fat 13.77 ± 0.56 $13.84 \pm 0.22\%$, fiber 1.59 ± 0.03 - $1.66 \pm 0.13\%$, nitrogen-free extract 2.19 ± 0.33 - $2.57 \pm 0.24\%$, and ash 13.41 ± 0.44 - $14.60 \pm 1.61\%$), body energy levels 3545.31 ± 31.24 - 3585.61 ± 14.94 cal kg⁻¹, liver glycogen 5.16 ± 0.71 - 6.34 ± 0.11 - 6.34 ± 0.11 mg g⁻¹ and muscle 4.46 ± 0.30 - 5.11 ± 0.10 mg g⁻¹, and hepatosomatic index 2.28 ± 0.07 - 2.32 ± 0.11 . Thus, 20% protein content in fermented feed supplemented with *Lumbricus* sp. extracts is enough to meet the needs of milkfish.

1. Introduction

The main constituent and energy source for fish is protein, it is considered as the first standard in formulating feed. According to Furuichi (1988) [1], fish protein is more effectively used as a source of energy than carbohydrates. However, the use of feed protein must be optimized because protein sources are generally expensive. Besides, Levit (2010) [2] suggested that the use of high protein as an energy source causes excess nitrogen to be removed in the form of ammonia through the excretion system. Ammonia in an un-ionized form (NH₃) is toxic to fish, even at low levels.



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Various attempts were made to optimize the utilization of protein. One of them is the fermentation method and supplementation of feed additives. Fermentation aims to simplify the complex nutrients contained in all feed ingredients, especially crude fiber, to increase the digestibility and use the value of feed nutrients. Roy et al. (2014) [3] suggested fermented sesame seed flour two strains of phytase-producing bacteria (LF1 and LH1 *Bacillus licheniformis*) which were isolated from the foregut and hindgut areas of *L. rohita* for 15 days at 37±2°C were effective in reducing fiber content crude and anti-nutritional factors such as tannins and phytic acid and enhancing available free amino acids, free fatty acids, and mineral concentration. Sukada et al. (2007) [4] used *Saccharomyces cerevisiae* as a fermenter in pollard, soybean epidermis, and cocoa shells. Aslamyiah et al. (2017) using a mixture of *Bacillus* sp., yeast *Rhizopus* sp., and *Saccharomyces* with a composition of 1mL + 1g + 1g/100 g seaweed flour and proven to increase the percentage of dry matter digestibility and organic matter digestibility, protein content, and nitrogen-free extract (NFE), as well as reducing fiber content rough, fat, and seaweed ash. Fermentation of vegetable waste with 3% rumen fluid for four days produced the highest levels of fat, carbohydrate, and lowest fiber content [5].

Furthermore, feed additive supplementation can be in the form of flavor agents, antibiotics, antioxidants, enzymes, hormones, vitamins, minerals, immunostimulants, probiotics, and prebiotics to increase animal productivity and quality of production. *Lumbricus* sp. has benefits for human health as additives for animal growth promoters. In fish, flour *Lumbricus* sp. used as a meal replacement for fish meal, as reported by Aslamyiah & Karim (2013) [6] in milkfish feed and Rachmawati et al. (2016) [7] in tiger grouper (*Epinephelus fuscoguttatus*). Utilization of *Lumbricus* sp. as a feed additive due to metabolite compounds contained, such as enzymes lumbrokinase, peroxidase, catalase, ligase, and cellulase and contained arachidonate fatty acids. Besides having high nutrition, as reported by Resnawati (2004) [9] that earthworms contain protein (64-76%), fat (7-10%), calcium (0.55%), phosphorus (1%), and crude fiber (1.08%), as well as essential and nonessential amino acids.

The combination of fermentation and supplementation of feed additives will produce feed with higher digestibility and maximum growth performance. As reported by Aslamyiah et al. (2019) [10] uses 10 mL microorganisms mix/100 g of feed as a fermenter to degrade feed ingredients and supplement *Lumbricus* sp. 300 mL/kg of feed effectively improves the growth performance of milkfish. However, the relation of protein content in feed composition has not been explained. This study aims to determine the level of fermented feed protein supplemented with *Lumbricus* sp. extract as a feed additive that provides the best response to the growth performance and chemical composition of the milkfish body.

2. Research Methods

2.1. Research time and site

The research was conducted from April to July 2019 in the Hasanuddin University education pond in Bojo Village, Mallusetasi District, Barru Regency, South Sulawesi. Some additional activities carried out in the Laboratory of Nutrition and Food Technology, and Productivity and Water Quality Laboratory, Faculty of Marine Sciences and Fisheries, Hasanuddin University.

2.2. Experimental animals and container rearing

The juvenile fish is used as an animal test with a weight size 8:52 ± 0:29 g / tail comes from the nursery Hasanuddin University Education ponds. The fish are stocked in hapa made of waring measuring 1 m³ mounted to the bottom of the pond with a water level of ± 70 cm, every 20 tails per hapa.

2.3. Treatments and experimental design

This study was designed in a Completely Randomized Design (CRD) with four treatments and each with three replications, so there were 12 experimental units. The treatments tested were various protein levels in feed formulations, namely 20, 25, 30, and 35%.

2.4. Experimental feed and fish rearing

The fish are given artificial feed with various levels of protein. In the manufacturing process, feed raw material has been fermented with microorganisms mix and supplemented with 300 mL/kg of feed *Lumbricus* sp. extract. The method of fermentation and supplementation of feed additives refers to Aslamyah et al. (2019) [10]. Acclimatization is carried out for adaptation to culture media and test feed, which is given at satiation for a week. After the acclimation period is complete, the fish fast for 24 hours to eliminate the remaining feed in the body. The fish are kept for 50 days and fed 5% of body weight per day three times a day, at 07.00, 12.00 and 17.00. The weight of the test fish is measured by weighing in each observation period ten days until the end of the experiment. During the experiments, water quality measurements of the maintenance media were carried out, and the observations were within a reasonable range for the survival of milkfish, namely temperature (29-33.4°C), pH (7.1-7.5), salinity (29-30 ppt), dissolved oxygen (4.1-5.6 ppm), and ammonia (0.01-0.12 ppm).

2.5. Research variables and data analysis

2.5.1. The research parameters include the growth performance and chemical composition of the milkfish body. The growth of relative body weight, survival rate, and feed efficiency were calculated by the formula [11].

Relative weight growth is calculated according to the formula :

$$RWG = \frac{W_t - W_o}{W_o} \times 100$$

RWG = Relative Weight Growth (%)
 W_o = Average weight of The fish at the beginning of the study (g)
 W_t = Average weight of the test fish at time t (g)

Survival Rate is calculated according to the formula :

$$SR = \frac{N_t}{N_o} \times 100$$

SR = Survival Rate (%)
 N_t = final number of the fish
 N_o = the initial number of the fish.

Feed efficiency is calculated according to the formula:

$$FE = \frac{(W_t + W_d) - W_o}{W_p}$$

FE = Feed Efficiency (%)
 W_t = Initial total weight (g)
 W_d = Dead weight during the study (g)
 W_p = Amount of feed eaten (g)
 W_o = Final total weight (g)

2.5.2. *The hepatosomatic index.* The hepatosomatic index is calculated by comparing total fish weight with liver weight. First of all, the fish are weighed. After that, they are dissected on the surface of the ice. Surgery is done carefully and as quickly as possible. Then the heart is weighed. The hepatosomatic index value is calculated based on the following Kindom & Alisson (2010) [12] equation:

$$IH = HP/W \times 100$$

IH = Hepatosomatic index
 HP = Hepatosomatic weight (g)
 W = Fish weight (g)

2.5.3. *The glycogen levels.* The glycogen levels in the liver and muscle of the test fish were measured at the end of the experiment. Muscles are taken from the dorsal part. The procedure for analyzing glycogen levels follows the method of Wedemeyer & Yasutake (1977) [13].

2.5.4. *The chemical composition of the fish's body.* The content of protein, fat, crude fiber, nitrogen-free extract (NFE), and fish test ash at the beginning and end of the study, were analyzed using the proximate method [14]. Ash content by the dry grinding method, water content by the oven method, fat content by the Soxhlet method, protein content by the Kjeldahl method, and carbohydrates by different methods, and energy by the bomb calorimeter.

2.5.5. *Data analysis.* Data obtained in this study were analyzed using variance with the help of the SPSS 12.0 program.

3. Results

The growth performance of milkfish which was treated with protein levels of fermented feed supplemented with *Lumbricus* sp. extract includes the relative weight growth, survival rate, feed efficiency, index hepatosomatic, levels of liver glycogen and muscle are presented in Table 1, while the body chemical composition, including the levels of protein, fat, ash, crude fiber, and NFE, as well as the levels of body energy, are presented in Table 2.

Table 1. The growth performance of milkfish by treating protein levels of fermented feed supplemented *Lumbricus* sp. extract.

| Parameter | Treatment (Protein Level %) | | | |
|-------------------------------|-----------------------------|--------------|--------------|--------------|
| | 20 | 25 | 30 | 35 |
| Initial Weight (g) | 8.57 ± 0.31 | 8.57 ± 0.31 | 8.43 ± 0.42 | 8.50 ± 0.35 |
| Final Weight (g) | 20.64 ± 0.53 | 21.30 ± 0.45 | 21.22 ± 0.21 | 21.50 ± 0.90 |
| Relative Weight Growth (%) | 58.47 ± 2.11 | 59.79 ± 0.60 | 60.24 ± 2.33 | 60.46 ± 0.40 |
| Survival Rate (%) | 88.89 ± 3.85 | 95.56 ± 5.01 | 91.11 ± 5.09 | 94.44 ± 5.09 |
| Feed Efficiency (%) | 40.44 ± 1.98 | 42.58 ± 1.19 | 43.27 ± 2.60 | 43.06 ± 1.27 |
| Index Hepatosomatic | 2.28 ± 0.07 | 2.29 ± 0.07 | 2.32 ± 0.01 | 2.32 ± 0.11 |
| Liver Glycogen Levels (mg/g) | 5.16 ± 0.71 | 6.33 ± 0.09 | 6.26 ± 0.16 | 6.34 ± 0.11 |
| Muscle Glycogen Levels (mg/g) | 4.46 ± 0.30 | 5.08 ± 0.23 | 5.09 ± 0.55 | 5.11 ± 0.10 |

Table 2. The body chemical composition of the milkfish by treating protein levels of fermented feed supplemented *Lumbricus* sp. extract.

| Body Chemical Composition | Beginning | Treatment (Protein Level %) | | | |
|---------------------------|-----------|-----------------------------|------------|------------|------------|
| | | 20 | 25 | 30 | 35 |
| Crude protein (% dw) | 58.75 | 67.52±0.52 | 67.85±1.99 | 68.59±0.67 | 68.57±0.86 |
| Crude fat (% dw) | 13.98 | 13.80±0.16 | 13.77±0.57 | 13.74±1.02 | 13.84±0.22 |
| Ash (% dw) | 21.60 | 14.52±0.74 | 14.60±1.61 | 13.74±1.97 | 13.41±0.44 |
| Crude fiber (%dw) | 2.95 | 1.59±0.03 | 1.60±0.04 | 1.66±0.13 | 1.60±0.02 |

| | | | | | |
|-------------------|-----------|-----------|-----------|-----------|-----------|
| NFE (% dw) | 2.72 | 2.57±0.12 | 2.19±0.33 | 2.27±0.42 | 2.57±0.24 |
| Body Energy Level | 3,256.711 | 3,545.31 | 3,544.69 | 3,570.80 | 3,585.61 |
| cal/kg) | | ±31.24 | ±61.01 | ±113.89 | ±14.94 |

The results of the analysis of variance showed that protein levels of fermented feed supplemented of *Lumbricus* sp. extract maintenance for 50 days did not affect ($P > 0.05$) on growth performance and body chemical composition of fish. However, an increase in growth and body protein levels compared with the beginning of maintenance.

4. Discussion

Protein is one of the essential macromolecules in feed, which is needed as a bodybuilding agent to replace and maintain damaged cells. Therefore, the fulfillment of protein requirements in milkfish cultivation is vital. In this study, the difference in protein levels in feed did not affect the growth performance and body chemical composition of the milkfish. This is related to the effective use of protein as growth material that occurs after fermentation of feed raw materials and supplementation of *Lumbricus* sp. extract as a feed additive.

Fermented feed results in the availability of simpler nutrients so that the digestive process and absorption of feed nutrients become more effective. Roy et al. (2014) [3] suggested *Labeo rohita* growth and feed utilization efficiencies of diets containing 30% fermented oilseed meal were superior to diets containing raw meals. Mumi et al. (2019) [15] the substitution of tofu with 66.67% rumen fluid fermented vegetable waste in feed can increase the activity of digestive enzymes, total digestibility, nutrient digestibility, growth, and survival of juvenile vannamei shrimp. Phulia et al. (2018) [16] reported that rohu seed could efficiently utilize fermented *Jatropha* kernel meal in diets without adverse effects on growth performance, nutrient utilization, and metabolic response.

Lumbricus sp. extract supplementation as a feed additive can also increase feed digestibility. As reported by Samatra et al. (2017) [17], *Lumbricus* sp. extracts containing serine protease. Serine protease is an enzyme that functions to break peptide bonds in proteins so that it can increase protein digestibility and encourage the absorption of protein in feed. The maximum utilization of protein also has an impact on feed efficiency.

At the level of the body's metabolism, extracts of *Lumbricus* sp. can maximize growth. As reported by Aslamyah et al. (2019) [10] that supplementation dose 300 mL/kg of *Lumbricus* sp. in fermented feed can increase absolute growth ($16.94 \pm 4.0g$), relative growth ($48.71 \pm 5.77\%$), feed efficiency ($40.74 \pm 37\%$), and hepatosomatic index ($1, 50 \pm 0.17$) milkfish. This is, *Lumbricus* sp. extract also contains an insulin-like growth factor [17]. IGF-I is a mediator for the stimulation of growth hormone [18]. The growth hormone stimulates the production of IGF-I in the liver, which plays an essential role in regulating several physiological processes such as growth, metabolism, development [19,20].

This research shows what is called the protein-sparing action for growth. Protein is only used for growth and repair of damaged cells, not as an energy source so that at the level of 20% is satisfactory growth performance. According to Lim et al. (1979) [21], although carbohydrates are not an excellent source of energy for fish over proteins and fats, carbohydrates digested from food can show what is called protein-sparing action for growth. In the treatment of higher protein shows the same growth performance, it is suspected that the excess protein is converted into energy. Daudpota et al. (2014) [22] revealed that excess protein in fish feed could be wasted and converted into energy.

The survival rate of fish fed with protein levels supplemented *Lumbricus* sp. extracts did not have a significant effect on various treatments. This illustrates that low protein feed can be maximized. The maximum utilization of protein in feed increases fish body immunity. Cooper & Hirabayashi (2013) [23] revealed that *Lumbricus* sp. contains lubricin, which can inhibit the growth of positive bacteria and negative bacteria, so that fish immunity becomes good and suppresses mortality. The maximum utilization of protein in feed can increase tissue growth in the body so that it can improve the immunity of the fish body.

Feed efficiency is the wet weight of fish meat obtained per unit dry weight of feed was given [11]. The efficiency of feed obtained increases with increasing protein levels in feed. However, it did not show any

real effect between treatments. Growth is closely related to the amount of feed consumed, protein content, and the ability of fish to utilize feed. The higher the feed efficiency, the better the growth rate. This is presumably due to *Lumbricus* sp., which is used as a feed additive in feed composition, also has the potential to maximize feed efficiency even with low protein content.

Different feed efficiency results were obtained for 30% and 35% protein feed treatments. This is also suspected because the protein levels have exceeded the protein requirements in milkfish so that the fish's body cannot utilize the entire fermented feed protein and has been supplemented with *Lumbricus* sp. Extract. If the protein in the feed is lacking, the fish growth becomes slow, so that the protein in the body's tissues will be used to maintain more important body tissue functions, but if the protein in the excess feed will be excreted as nitrogen in the form of ammonia.

Measurements of growth material, including hepatosomatic index, liver, and muscle glycogen levels, and the body's chemical composition show patterns that coincide with growth and feed efficiency. Protein levels of 20-35% in the diet indicate hepatosomatic indexes, liver and muscle glycogen levels, and the same body chemical composition. However, it increased compared to the beginning of the experiment. The effective process of digestion and absorption of feed nutrients causes an increase in the efficiency of the use of feed protein for growth and replacement of damaged cells, which in turn results in an increase in protein deposition in the body. The study of Kumar et al. (2006) [24] on α -amylase supplementation in *Labeo rohita* diets can reduce the use of feed protein. The hepatosomatic index (HSI), liver and muscle glycogen, and intestinal amylase activity are significantly increased.

The liver functions as a very dynamic place for glycogen storage is sensitive to the level of feed consumption over a short period and can show the level of nutritional conditions in the waters. Ghaffari et al. (2011) [25] state that the liver is a metabolic organ in fish so that the IHS value can also be used as a biomarker in detecting the environmental conditions of test animals. Quantitatively stated in IHS is also called the liver somatic index (LSI), which is a comparison between the weight of the liver and the total weight of fish.

One of the organs that functions to store fat and glycogen is the liver—the liver functions primarily for fatty acid synthesis, detoxification, and nutrient storage [26]. Fish do not rapidly monopolize glycogen reserves stored in their hearts when they are starving. The decrease in the hepatosomatic index in milkfish is influenced by the juvenile phase so that in its development, the fish need a lot of energy to support growth. The average fish used in this study was 8.52 ± 0.29 g, which was still in the juvenile phase. Young fishes will have relatively fast growth, and this indicates having a lot of energy needs. Other than for metabolic processes, energy in young fish will be used as growth and development of reproductive organs. Yandes et al. (2003) [27] explain energy conversion apart from the body also uses energy reserves in the liver. The increase in IHS values indicates an increase in the number of nutrients absorbed and then causes the number of nutrients to accumulate in the liver to increase.

5. Conclusion

Based on the results of research that has been conducted, it is concluded that protein levels of feed provide the same effect on the growth performance and body chemical composition of the milkfish. Therefore, milkfish can be fed with fermented feed supplemented with *Lumbricus* sp. extract with a protein content of 20%.

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