Testing of artificial feed by using raw material from *Euthynnus affinis* waste flour for gourami (*Osphronemus gourami*)

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Abstract. Utilization of fish based-waste as feed is a very efficient alternative in reducing pollution and environmental damage, and meeting the nutritional needs of fish growth. This study aims to test the quality of fish feed made from waste flour *Euthynnus affinis* based on physical, chemical and biological tests for gourami. This research was conducted in 2024. The study used a completely randomized design (CRD) with 4 replications and 5 treatments. The addition of fish waste flour includes: P1 (0%) control, P2 (35%), P3 (40%), P4 (45%) and P5 (50%). The results showed that the ANOVA test on the physical test, the addition of waste flour in artificial feed for gourami significantly different ($P < 0.05$) on the breaking speed and sinking speed with the highest value in treatment P5 (50%) which is 14.51 minutes and 0.05 seconds. However, there was no significant effect ($P > 0.05$) on the dispersion of solids, level of hardness, level of homogenesis and feed attractiveness. The results of chemical tests (proximate) showed that feed without the addition of waste flour and feed with the addition of waste flour provided protein content values of 20.89% and 30-32%. In biological studies, the addition of waste flour to feed had no significant impact ($P > 0.05$) on gourami survival, but it had an important effect ($P < 0.05$) on weight. The highest growth value was found in the P4 treatment (45%) which amounted to 2.46 g with survival ranging from 90% ± 0.00. Therefore, from each treatment it can be concluded that the addition of raw materials of waste flour in feed provides a good nutritional value for the growth of gourami.

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1 Introduction

Fish feed is the most significant component of the aquaculture sector, which accounts for 60–70% of overall production cycle expenses [1]. In order to reduce production costs, it is necessary to have independent feed sources with alternative ingredients. These alternative should provide adequate nutritional value for fish growth, be cost-effective, widely available, sustainable, and not compete with human food sources [2]. According to [3, 4], the fisheries industry generates a large number of byproducts in the form of waste that can be turned into usable outputs employing technology. Growing amounts of organic waste will pose a new threat to the environment and the well-being of the local population around the disposal site. Waste from byproducts is a significant issue that needs to be handled carefully. Furthermore, appropriate waste management can help achieve SDG 14, or Life Below Water. Therefore, appropriate waste management is necessary to prevent issues with environmental quality and aquatic animal sustainability.

Gourami fish is one of the leading types of freshwater fish commodities native to Indonesia which has important economic value and high nutritional content [5]. However, gourami fish has a relatively slow growth rate [6]. To increase its growth, gourami fish needs a protein intake of around 38-40% in their feed [7]. Utilizing the waste to produce ingredients for fish feed is one technique to lessen the issue of fish processing waste and fish sustainability.

Tuna (*Euthynnus affinisI*) is one of the widely consumed marine fish and is one type of fish processing commodity. Tuna fish has a protein content of 67.47% and Omega 3 [8] According to [9], tuna waste includes the head (12.0%), bones (11.7%), fins (3.4%), skin (4.0%), spines (2.0%), and offal (4.8%). The average protein content of fish offal reached 16.72% [10], entrails 15.48% [11], and fish bones 9.45% [12], so that the waste produced if processed properly can be used as flour as a composition in the process of making fish feed. Previous studies have highlighted the potential of fish head and bone waste as a viable fishmeal substitute [13]. Additionally, fish offal can serve as an alternative raw material in making fish feed [14]. [15], waste of tamban fish (*Sardinella lemuru*) heads and bones are quite potential to be used as fishmeal and as an alternative raw material for making cheap feed for marine fish farming. In addition, the substitution of fishmeal with earthworm flour at different levels produces the same feed quality and is within the range of fish needs [16]. Feed quality could be measured by conducting physical, chemical and biological tests [17]. By utilizing tuna waste, it is expected to reduce production costs for fish farmers and be able to meet the nutritional needs of fish for growth.

The aim of this study is to assess the quality of feed made from tuna waste flour through physical, chemical, and biological tests for gourami fish.

2 Materials and methods

2.1 Materials

The materials used in this research are as follows: 1) gourami fish obtained from local fish shop in Aceh Besar; 2) Tuna fish waste; 3) Fish meal; 4) Soybean meal; 5) Corn flour; 6) Fine bran; 7) Tapioca flour; 8) Minerals; 9) Fish oil; 10) Vitamins; and 11) Cr_2O_3 (chromium).

2.2 Research methods

This study was carried out from May to June 2024 at the Laboratory of Fish Rearing and Hatchery, Faculty of Marine and Fisheries, Universitas Syiah Kuala. Meanwhile, sample

testing at the Laboratory of the Industrial Services Standardisation and Policy Centre (BARISTAND), Banda Aceh. This study used the Completely Randomised Design (CRD) method with 5 treatments and 4 repetition. The treatments (T) tested in thisstudy are as follows:
Treatment 1: Without the addition of tuna waste flour (0%)
Treatment 2: Addition of 35% tuna waste flour
Treatment 3: Addition of 40 % tuna fish waste flour
Treatment 4: Addition of 45% tuna waste flour
Treatment 5: Addition of 50 % tuna fish waste flour

2.3 Research procedure

The research was conducted in several stage, including: a) Preparation of tuna waste flour including head, fish offal, and bone; b) formulated of test feed; c) Proximate test; d) preparation of container of gourami fish; and e) Feed manufacturing.

2.4 Data analysis

The data were analyzed using Analysis of Variance (ANOVA) in SPSS 27 software to evaluate the quality of feed based on physical, chemical, and biological tests for gourami fish. If significant results were found, further analysis was conducted using the least significant difference (LSD) test.

3 Results and discussion

3.1 Feed physical test

According to the ANOVA test results in Table 1, the addition of tuna waste flour significantly ($P < 0.05$) affects the parameters of the breakage speed test and feed sinking speed test, but it has no effect ($P > 0.05$) on the parameters of the solids dispersion test, attractiveness, hardness level, and homogenesis level.

Table 1. The results of physical test parameters on feed.

Test Parameters	Treatments				
	T1 (0%)	**T2 (35%)**	**T3 (40%)**	**T4 (45%)**	**T5 (50%)**
Breakage speed (minutes)	3,59±1,02^{a}	4,83±0,97^{a}	5,48±0,50^{a}	8,60±2,06^{b}	14,51±1,73^{c}
Solids dispersion (%)	0,39±0,22^{a}	0,19±0,13^{a}	0,51±0,19^{a}	0,39±0,08^{a}	0,49±0,12^{a}
Hardness level (g)	0,42±0,30^{a}	0,59±0,31^{a}	0,64±0,34^{a}	0,51±0,32^{a}	0,46±0,31^{a}
Homogenesis level (g)	0,59±0,13^{a}	0,64±0,20^{a}	0,61±0,10^{a}	0,66±0,26^{a}	0,61±0,06^{a}
Sinking speed (seconds)	0,01±0,05^{a}	0,05±0,00^{b}	0,05±0,01^{c}	0,03±0.01^{c}	0,05±0,00^{c}

Feed lure (seconds)	0,21±0,20[a]	0,15±0,04[a]	0,21±0,07[a]	0,21±0,09[a]	0.15±0,08[a]

The findings of the ANOVA test in Table 1 indicate that the addition of tuna waste flour as a raw material for artificial feed has a significant effect (P <0.05) on the physical test parameters of the speed of breaking the feed. Treatment T5 (50%) is the highest value of 14.51 minutes and the lowest value in treatment P1 (0%) control is 3.59 minutes. These results are higher than the results of the research of [18] which amounted to 2.60 minutes in all treatments. The high value of the breaking speed is influenced by the level of dryness of the pellets. [18] stated that the rapid rupture of artificial feed in water is caused by the level of dryness. The results of physical parameters also show that artificial feed with the addition of cob waste flour breaks faster than commercial feed and can be given to gourami.

The ANOVA test results in Table 1 shows that the addition of cob waste flour as a raw material for artificial feed is not significantly different (P>0.05) to the physical test parameters of solid dispersion in feed. The highest value results were found in the T3 treatment (40%) which amounted to 0.59% and the lowest value results were found in the T2 treatment (35%) which amounted to 0.19%. The results of the dispersion value of solids are much lower than the results of research by [19] on the treatment of the addition of seaweed *E. denticulatum* which amounted to 14.36%. According to earlier studies, less refined raw materials used to make feed may have contributed to the low solid dispersion value. A number of parameters, including the fineness of feed raw materials and the method of mixing ingredients during the feed-making process, influence the stability of feed in water, according to [20]. [16] claimed that because artificial feed has poor water stability, it is rapidly damaged and spread, making it impossible for test animals to consume. [21] Because it has a significant impact on the amount and quality of nutrients in the feed, the degree of solids dispersion should not exceed 10%.

Based on the ANOVA test results in Table 1, it shows that the addition of tuna waste flour is not significantly different (P>0.05) to the level of hardness. The highest value was found in the T3 (40%) treatment which was 0.64% and the lowest value was found in the T1 (0%) control treatment which was 0.42%. Compared to the results of research conducted by [16] with an average value of 84% of all treatments. The results of the highest value in this study were influenced when making feed using raw materials that were less refined. According to [22] artificial feeds with higher hardness are made from relatively finer raw materials. Thus, although the results of the physical test of hardness were not significantly different and less good than the results of previous studies, artificial feed with the addition of cob waste flour can be used as a substitute for commercial feed for gourami.

Based on the ANOVA test results in Table 1, it shows that the addition of raw materials of tuna waste flour as a substitute for fish flour is not significantly different (P>0.05) to the homogeneity level parameter. The highest value of each treatment was found in the T3 (40%) treatment with a value of 0.66% and the lowest value was found in the T1 (0%) control treatment which was 0.59%. The high value of homogeneity in the P3 (40%) treatment with a value of 0.66% is very much different compared to the results of research conducted by [23] by getting the best value in treatment (2.5%) which is 2.67%. The low results of the study were caused by the use of less refined and unevenly distributed raw materials during the feed-making process, which resulted in uneven dough during printing. [24] recommended that the mixing stage be carried out as effectively as feasible in order to obtain homogenous raw materials in feed. To put it simply, mixing can be done by hand, but for larger volumes, a mixing equipment (mixer) that employs electrical energy can be used to ensure that the mixing process is completed as efficiently as possible [19]. Thus, good fish feed has a compact texture and particle size of raw materials that are smooth and uniform [22].

The ANOVA test results in Table 1, it shows that the addition of tuna waste flour in gourami feed is significantly different (P <0.05) to the sinking speed of the feed. The results of the BNT further test showed that the feed with the addition of cob waste flour had the highest value in the P3 (40%) treatment with a value of 0.05 seconds and the lowest value was in the P1 (0%) control treatment with a value of 0.01 seconds. The results of this research value are very much different from Research conducted by [23] with the best value found in treatments (3%) and (3.5%) with the same value of 1.27 seconds for the addition of lamtaro leaf flour. The results of the research value are caused by differences in treatment in the process of mixing feed raw materials and water stability in feed. As we know during the process of making feed with the treatment of adding different raw materials of cob fish waste flour and mixing simply, when making dough using hands, so the possibility of all raw materials is not perfectly distributed which causes the feed dough not to mix evenly well. [19] stated that feed with the addition of tuna waste flour showed a slower sinking speed compared to the control feed, due to differences in the process of mixing feed raw materials and commercial feed in the mixing process using modern equipment, so that the feed ingredients are evenly mixed. [25] also stated that the higher the specific gravity with a smaller particle size, the better the stability in water but the faster the sinking speed. Thus, the addition of tuna waste flour to the physical test on the parameter of sinking speed is not good because the results of the value are very much different from the results of previous research values.

Based on the ANOVA test results in Table 1, it shows that the addition of tuna waste flour is not significantly different (P>0.05) to the attractiveness of artificial feed. The highest value was found in treatment T1 (0%) with a value of 0.21 cm/second and the lowest value was found in treatment T4 (45%) with a value of 0.12 cm/second. The results of this research value are very much different from the results of research conducted by [18] with the results of the best value in treatment (3.5%) which is 3.00 minutes on feed containing M. pruriens flour. The lack of research time is due to the percentage of raw materials for feed formulations that are used such as fish oil and tuna waste flour which contain smell against gourami stimuli, the more delicious the aroma of feed raw materials, the faster the stimulation of fish to the pellets given. [19] stated that in their research, the attractiveness of feed can be influenced by the quality and quantity of feed aroma so that it is more quickly detected by receptor organs. Feed that is too long in the water in addition to reducing the quality and quantity of nutrients will also reduce the value of its attractiveness or the delicious power of pellets [23]. Thus, artificial feed with the addition of raw materials of cob waste flour is better than the artificial feed from previous studies and is good for gourami.

3.2 Feed chemical test

Proximate analysis data on gourami (*Osphronemus gouramy*) test feed with the addition of tuna waste flour (*Euthynnus affinis*), can be seen in the table below.

Table 2. The proximate test results of gourami feed.

Test Parameters	Treatmets				
	T1 (0%)	**T2 35%)**	**T3 (40%)**	**T4 (45%)**	**T5 (50%)**
Moist (%)	8,22	7,74	7,38	7,02	7,67
Ash (%)	13,49	12,57	12,34	12,48	11,98
Protein (%)	20,89	30,55	30,47	30,45	31,76
Fat (%)	3,08	6,71	8,09	10,37	10,85

Carbohydrate (%)	28,75	24,94	23,96	20,55	18,03
Crude fibre (%)	6,77	5,76	5,53	3,97	3,10
Cromium (%)	1,17	0,88	0,72	0,72	1,26

Based on the chemical test results in Table 2, the proximate feed made by gourami to the addition of tuna waste flour shows that the best proximate feed test results are found in the T4 treatment (45%) with a protein content value of 30.44% and the lowest value is in the control treatment P1 (0%) with a protein c o n t e n t value of 20.89%. [26] Ahmad *et al*. (2017) suggested that good feed is usually feed with a higher protein content than carbohydrates because protein is the main source of energy for fish. The chemical test shows that the nutrients in pellets made from tuna waste flour are very good for gourami feed formulation because they meet the requirements for gourami feed. Good nutritional requirements for gourami are moisture content of 7.79%, protein 28.01%, fat 7.05%, ash content 11.03% and crude fibre 3.92% [27] (Indonesian National Standard, 2009). The composition of good feed for gourami fish is 30-32% protein and 20-30% carbohydrates in this composition, it can be seen that the protein content is a more dominant amount than carbohydrates [26] (Ahmad *et al.*, 2017). Thus, it can be concluded that the nutritional content of artificial feed with the addition of cob waste flour shows sufficient nutrition for the needs of gourami and is very well used as an artificial feed formulation for gourami.

3.3 Biological test

The results of the 20-days study showed that feeding gourami with the addition of tuna waste flour (*Euthynnus affinis*) had a significant effect ($P<0.05$) on weight and length parameters absolute, but has no significant effect ($P>0.05$) on survival parameter. the data can be seen in the table below.

Table 3. The biological test parameters of gourami.

Test Parameters	**Treatments**				
	T1 (0%)	**T2 (35%)**	**T3 (40%)**	**T4 (45%)**	**T5 (50%)**
Absolute weigh (g)	$0,54\pm0,41^{a}$	$1,11\pm0,20^{ab}$	$1,67\pm0,72^{ac}$	$2,46\pm0,72^{bc}$	$2,25\pm0,59^{c}$
Survival (%)	$77,5\pm9,57^{a}$	$85,0\pm5,77^{a}$	$85,0\pm5,77^{a}$	$90,0\pm0,00^{a}$	$86,5\pm5,77^{a}$

Based on the results of the ANOVA test in Table 3, it shows that the addition of tuna waste flour as a raw material in artificial feed is significantly different ($P <0.05$) on growth parameters, the absolute weight of gourami. From these results it can be seen that the T4 (45%) treatment is the treatment with the highest value, which is 2.46 g and the lowest value is in the T1 (0%) treatment or control, which is 0.54 g. Compared to the results of research conducted by [28] based on the absolute weight growth of gourami showed that the highest absolute weight growth was obtained in treatment D with 20% artificial feed dosing with a weight of 2.44 g and the lowest absolute weight growth in treatment A with 5% artificial feed dosing with a weight of 2.29 g.

The high growth of absolute weight in the T4 treatment (45%) is due to differences in the composition and percentage of feed, namely cob waste flour and sufficient nutrients of protein levels of 30.45% and carbohydrate levels of 20.55% contained in artificial feed for the growth of gourami fish seeds. [26] added that good feed for gourami is 30-32% protein

and 20-30% carbohydrates in the composition it can be seen that the protein content is a more dominant amount than carbohydrates. The low value of absolute weight growth of feed in the control treatment or T1 (0%) (without the addition of cob waste flour) is due to the lack of nutrients in feed raw materials and the slow growth of fish seeds. [29] stated that based on the analysis of variance, the provision of different protein levels had a significant effect on the weight growth of gourami. Thus, it can be concluded that the addition of cob waste flour as a raw material for artificial feed on absolute weight parameters is very good to be given to gourami fish seeds as an alternative feed to replace commercial feed.

Based on the results of the ANOVA test in Table 3, the addition of raw materials for fish cob waste flour shows that the survival parameters of gourami fish seeds are not significantly different (P>0.05). From the data values in table 4.3, it can be seen that the highest value is in the P4 treatment (45%) which is 90.0% and the lowest value is in the control treatment. or T1 (0%) which is 77.5%. The results of the highest or best value in this study are the same as the results of research conducted by [30-32] with the highest survival value of 90.00%. The high survival rate indicates the quality and quantity of feed given is sufficient to meet the main needs of the body, and can even increase growth. The low survival value results were stated by [33] that environmental factors also determine the survival rate of fish where stressed fish and low body resistance cause a large number of deaths. The low survival rate is also considered less successful, because based on SNI (2000) the good survival rate for gourami fish maintenance is 80 - 95%. With such results in the survival parameter of gourami fish fry is very good even though it is not significantly different (P>0.05) to the ANOVA test.

4 Conclusion

The results of the physical test showed that the addition of tuna waste flour in gourami feed had a significant effect (P <0.05) on the breaking speed with the highest value found in the P5 (50%) treatment, which was 14.51 minutes and the sinking speed with the highest value found in the P5 (50%) treatment, which w a s 0.05 seconds but had no significant effect (P>0.05) on the dispersion of solids, the level of hardness, the level of homogenesis and the allure of the feed. The biological test results of gourami growth and absolute weight showed a significant effect (P <0.05) with the highest and best value. The results of the chemical test (proximate) feed showed that the best value for the growth of gourami fish seeds was in the T4 treatment (45%) with the addition of tuna waste flour showing the value of protein content of 30.45% and was carried out descriptively.

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