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Analysis of Protein Content and Amino Acid Profile in Fish Feed Made From Maggot Meal and Earthworm Meal

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Abstract. Amino acids were the main components of protein with great potential in feed for fish growth and survival. Some fish feed ingredients sourced from animal protein that can reduce the use of fish meal are maggot and earthworms. The purpose of this study was to determine the protein content and amino acid profile through the use of maggot meal and earthworm meal in fish feed. The use of maggot meal was substituted from 5% to 20%, while earthworm meal was substituted by 4%. Fish feed formulation with ratio of fish meal (FM), maggot meal (MM) and earthworm meal (EM) was tested based on several ratios, namely F1 (30% FM and 5% MM, 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM). The results showed that the protein content produced ranged from 23.65% to 26.66%. Glutamic acid had the greatest amino acid profile across all formulations, followed by aspartic acid, arginine, and lysine. Protein content and amino acid profile in the treatment formulas F3 (15% MM and 4% EM) and F4 (20% MM and 4% EM) always occupy the highest position, while the combination ratio of F1 (5% MM and 4% EM); F2 (10% MM and 4% EM) were generally always in the lower position. This shows that the results of the amino acid profile content of the second combination of this type of natural animal source have increased in line with the increase in the addition of maggot meal.

1. Intruduction

The main source of nutrition for feed, both in terms of quality and quantity, was protein. Protein helps in the growth and performance of living things and was essential for the production of enzymes and other trace elements [1]. Protein will be continuously synthesized and degraded in the fish body. Amino acids and non-specific nitrogen from food sources are required for body maintenance and growth throughout life [2]. Fish protein requirements vary depending on the species, size, meal components, protein quality, and habitat [3].

Protein quality was typically determined by the amino acid profile it contains, whereas protein amount was determined by the nitrogen value produced by proximate analysis measures. Amino acids in animals including fish were in the free form or linked to proteins (linked in peptide chains) [4]. Free amino acids have three primary forms in which feed protein products were hydrolyzed from intestinal absorption, and the final hydrolysis of body proteins [5]. Amino acids can also be used for the synthesis of body proteins or other nitrogenous components (nucleic acids, amines, peptides, hormones, and so on), providing a carbon source for intermediate metabolism or being oxidized to provide energy[6].

Protein quality was related to the amino acid profile it contains. Amino acids were classified according to the body's ability to synthesize and their metabolic needs. This classification was known as essential and non-essential amino acids [7]. The 10 amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan, and valine are necessary for most animals, including fish [3]. Lysine is one of the ten essential amino acids that can be used as a reference amino acid. According to [8] one of the essential amino acids needed by fish was lysine which had the potential for growth and development of fish.

The quality of the source material or material in the feed greatly affects the growth performance of fish. Many artificial feeds were recommended to overcome dependence and problems with feed, but problems then occur because of the need for fish meal raw materials in fish feed formulations [9]. The primary source of protein for commercial fish feed was still imported raw ingredients like fish meal. However, the rapid rise in fish meal prices and demand has severely hampered aquaculture's ability to operate sustainably in recent years. [10]. In addition, using fish meal excessively will burden fishery resources. However, recent years have seriously limited the development of sustainable aquaculture due to sharp increases in prices and demand for fish meal. In addition, there was a fairly high pressure on fish resources due to the excessive use of fish meal. Consequently, a breakthrough in the quest for substitute protein sources was required to ensure sustainable development of the availability of cheaper, environmentally responsible feed.

There were several alternatives to fish meal, such as maggot meal and earthworm, which are an easily available protein source with good nutritional value and environmental benefits. In it was processing, maggot can be processed into meal in fresh or dry form which can be used as a protein source to replace fish feed. Maggot contains protein (approximately 43.23% in dry weight), fat, crude fiber and ash [11]. The use of earthworm meal in artificial fish feed waswidely used in fish aquaculture. Nutrients contained in earthworms such as protein (approximately 54-70% in dry matter), carbohydrates, fats and ash [12]. Earthworms can be obtained either directly from the soil or in an easy and affordable way through propagation. The purpose of this study was to evaluate the protein content and amino acid composition in fish feed containing earthworm and maggot meals.

2. Methodology

2.1 Material

The raw materials for making feed include fish meal, earthworm meal, maggot meal, corn meal, rice bran, copra meal, tapioca meal, fish oil, and premix. Maggot and earthworms were obtained from local maggot and earthworm farms, Tegal agrofarm Indonesia. The manufacture of maggot meal refers to [13] a slight modification, starting with cleaning the maggot with tap water, then spreading it on an aluminum container. Then dry the maggot while using a hot air oven with a temperature of 60 ºC for 36 hours, then the dried maggot is mashed using a grinder and placed in a plastic bag.

The production of earthworm meal refers to [14] with a slight modification, first the earthworms were washed with running water, then steamed at 70 ºC for 10 minutes with an autoclave and then grinded with a grinder until the particles are fine. After that, dry for 10 to 12 hours in a 60 ºC oven. The dried samples were broken up and put through a sieve with a mesh size of around 50.

2.2 Feed Formula Design

Feed formula with four treatments (F1, F2, F3, and F4) based on different percentages of fish meal (FM), maggot meal (MM), and earthworm meal (EM). F1 = 30% fish meal (FM) + 5% maggot meal (MM) + 4% earthworm meal (EM), F2 = 25% fish meal (FM) + 10% maggot meal (TM) + 4%

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earthworm meal (EM), F3 = 20% fish meal (FM + 15% maggot meal (MM) + 4% earthworm meal (EM), and F4 = 15% fish meal (FM) + 20% maggot meal (MM) + 4 % earthworm meal (EM). Other additives include, tapioca flour (10%), bran flour (18%), copra flour (18%), corn flour (10%), premix (1%), and fish oil (2%). all ingredients were mixed homogeneously according to the formula and then put into the extruder feed molding machine.

Table 1. Composition (% dry weight) of raw materials in each formula

Materials	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Fish meal	30	25	20	15
Maggot meal	5	10	15	20
earthworm meal	4	4	4	4
Tapioca meal	10	10	10	10
Bran meal	18	18	18	18
Copra meal	10	10	10	10
Corn meal	20	20	20	20
Premix	1	1	1	1
Fish oil	2	2	2	2
Total	100	100	100	100

2.3 Nutrition analysis

Nutrient analysis (protein, fat, water, ash, and fiber) was carried out according to the standard method of the AOAC [15]. Protein content analysis was carried out using the Kjeldahl method [16]. Entering as much as 2 grams of sample, crushed then put in a Kjeldhal flask and then added 3 grams of the digestion mixture (1 part $CuSO_4$ and 9 parts K_2SO_4 and 20 ml concentrated H_2SO_4). The sample solution from the destruction was put in a steam distillation device and added 3 drops of phenolphthalen indicator. The distillation was ended. When the dripping distillate reacts neutrally to red litmus and the color of the reservoir solution becomes green. Do the titration with 0.1 N HCl solution in the container solution marked by the color of the solution turning pink (pink). The experiment's direct results for protein content are presented in percentages (%) and soxhletation extraction was used to analyze fat.

2.4 Amino acid analysis

Amino acid analysis refers to the method carried out by [17] with slight modifications. A total of 30 mg of protein hydrolyzate was then added with 4 ml of 6 N HCl heated for 24 hours at a temperature of 110 °C. Cool to room temperature, then neutralized (pH = 7) with 6 N NaOH then the sample is added with distilled water to a volume of 10 ml, and filtered with Whatman filter paper 0.2 m. Take 50 µl of sample plus 300 µl of OPA (Orthophalaldehyde) solution, stir for 5 minutes and then add 10 µl of HPLC injector.

2.5 Data Analysis

Analysis of variance was used in statistical data analysis to evaluate the level of significance. Tukey's further test was used to see if there was a change between treatments.

3. Results and Discusion

3.1 Analysis of protein content

The results of the proximate analysis to determine the nutritional value of the treatment feed showed that the protein and fat content (%) from treatment F1 to F4 were respectively; 23.65 and 5.14; 24.74 and 5.64; 25.32 and 6.60; 26.66 and 7.66 (Table 3). This was because maggot meal had a higher protein content (44.13%) in dry matter than fish meal 40.43% (Table 2). The combination of earthworm meal and maggot meal at F1 (30% FM and 5% MM, 4% EM) showed the lowest protein level.

Table 2. Nutrient composition (% dry weight) on fish meal, maggot meal, and earthworm meal

Ingredient	Protein	Moisture	Ash	fat	fiber
Fish meal	40.43^{c}±0.09	8.67^{a}±0.01	16.48^{a}±0.27	8.41^{b}±0.01	4.40^{c}±0.12
Maggot meal	44.63^{b}±0.02	6.34^{c}±0.06	12.53^{b}±0.03	11.50^{a}±0.11	10.19^{a}±0.04
Earthworm meal	54.46^{a}±0.09	8.18^{b}±0.02	5.61^{c}±0.03	7.43^{c}±0.02	6.39^{b}±0.14

Note: The number after ± was the standard error value, unequal superscript letters on the same line indicate significantly unequal treatment effects (P <0.05).

Table 3. Nutrient composition (% dry weight) and total energy (kcal.kg^{-1})

Formulation	Protein	Moisture	Ash	fat	Energy
F1	23.65^{d}±0.02	9.87^{a}±0.02	12.76^{a}±0.03	$5,14^{e}$±0.01	3818.2
F2	24.74^{c}±0.02	9.75^{b}±0.01	12.24^{b}±0.02	5.64^{c}±0.01	3853.7
F3	25.32^{b}±0.03	9.36^{c}±0.01	11.63^{c}±0.25	6.60^{b}±0.02	3899.3
F4	26.66^{a}±0.03	9.25^{d}±0.02	10.88^{d}±0.02	7.66^{a}±0.01	3936.5

Note: the number after ± was the standard error value; unequal superscript letters on the same line indicate significantly unequal treatment effects (P <0.05). F1 (30% FM and 5% MM, 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM).

Through a research approach that revealed that small fish require higher protein levels than larger fish due to their higher metabolism and growth rate, the existence of different protein level treatments was used to find the ideal protein level. According to the claim [18] regarding several factors that affect feed protein requirements such as fish size, water that this study also considered other aspects that affect feed protein requirements, including fish size, water temperature, feeding, the amount of non-protein energy in the feed, protein quality, natural feed availability, and feed management practices [19].

The main approach to protein in this case was amino acids because protein was used for growth and maintenance of the body so naturally all the energy used by fish comes from protein. Although there are several sources of protein for feed, the quantity and quality will vary. Amino acids in animals, including fish, were either free or linked to proteins. Free amino acids have three primary forms in which feed protein products were hydrolyzed from intestinal absorption and the final hydrolysis of body proteins [20]. Amino acids could be used for the synthesis of body proteins or other nitrogenous components (nucleic acids, amines, peptides, hormones, and so on), providing a carbon source for intermediate metabolism or being oxidized to provide energy [21].

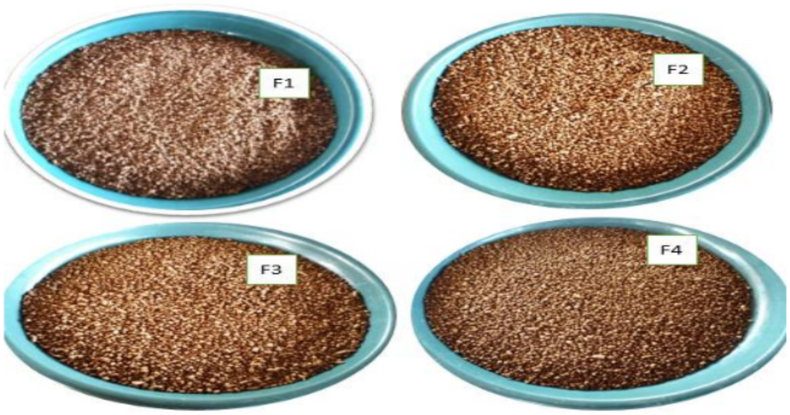

Figure 1. Fish feed products containing maggot meal and earthworm meal

3.2 Amino acid profile

Protein quality was typically determined by the amino acid profile it contains, whereas protein amount was determined by the nitrogen value produced by proximate analysis measures. Amino acids in animals including fish are in the free form or linked to proteins (linked in peptide chains) [22]. Free amino acids have three primary forms in which feed protein products are hydrolyzed from intestinal absorption, and the final hydrolysis of body proteins. Amino acids can also be used for the synthesis of body proteins or other nitrogenous components (nucleic acids, amines, peptides, hormones, and so on), providing a carbon source for intermediate metabolism or being oxidized to provide energy [23] .

Amino acid profiles can be identified using the high-performance liquid chromatography method, as was done by [6], [24]. Amino acid values in the (FM)/(MM)/(EM) ratio treatments (15:20:4)% in the F4 and (FM)/(MM)/(EM) ratio (20:15:4)% in the F3 treatment always occupies the highest position while the combination (FM)/(MM)/(EM) ratio (30:5:4)% in the treatment F1 was generally always at it was lowest (Table 4). This indicates that the amino acid profile of the addition of natural feed sources from maggot meal which was increased from 5% to 20% and earthworm meal (4%) can increase the value of the amino acids it contains. This indicates that the addition of maggot meal and earthworm meal can also increase the number of amino acid values in the feed formula.

In Figure 1 it can be seen that there were nine profiles of essential amino acids out of ten required to be present in the four types of feed, namely arginine, histidine, isoleucine, leucine, lysine, threonine, valine, methionine, and phenylalanine. The highest amino acid profiles were glutamic acid (5.78%), aspartic acid (3.22%), arginine (2.72%), and lysine (2.52%) respectively, the highest of the fifteen amino acids detected in the diet. There were several reasons to choose lysine as the reference amino acid. First, since it was requirements were unaffected by other metabolic functions, lysine's primary job in the animal body was the deposition of protein tissues. [25]. Second, depending on the fish species and the type of feedstock, lysine usually has a major role in limiting amino acids because it was known that the need for lysine was much greater than for other amino acids [26].

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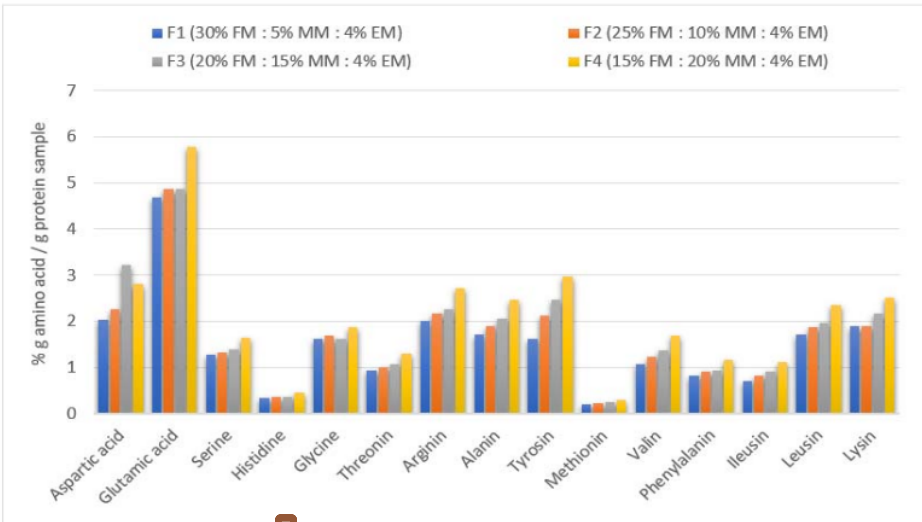

Figure 2. Profile of essential and non-essential amino acids in each feed treatment

The amino acid content of lysine in formulas F1 to F4 showed an increase, namely F1 (1.90%), F2 (1.90%), F3 (2.18%), and F4 (2.52%). This shows that the content of the amino acid lysine value was also influenced by the addition of maggot meal. Figure 2 shows the presence of an amino acid with the greatest value detected in the five types of feed, glutamic acid. Glutamic acid serves as an energy source for the brain and was found in serum, muscle, and cerebrospinal fluid [27]. One of the 20 amino acids included in the standard genetic code was glutamate, which had an amide side chain created by switching the glutamic acid's hydroxyl side chain for an amine functional group [28]. According to [22] suggested that the amino acid requirement for fish was essential and should be linked or even regulated by the pattern of amino acid present in muscle tissue. Imbalance of the amino acid profile in the diet can reduce food intake and reduce the efficiency of utilization of essential amino acids.

Conclusion

The highest protein content and amino acid profile in the treatment formulas F3 (15% MM and 4% EM) and F4 (20% MM and 4% EM) always occupy the highest position, while the combination ratio of F1 (5% MM and 4% EM); F2 (10% MM and 4% EM) were generally always in the lower position. This shows that the protein content and amino acid profile of the combination of the two types of natural animal sources produced has increased significantly in line with the constant increase in maggot meal and earthworm meal.

Acknowledgments

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