Potential Protein Source From Black Soldier Fly (Hermetia Illucens) Larvae As A Substitution For Fish Meal In Feed Formulation Of Common Carp (Cyprinus Carpio)

Ayu Azkiyah Azizah, Arning Wilujeng Ekawati, Happy Nursyam

Abstract: The purpose of this research was to determine the effect of maggot meal substitution on fish meal as the main source of protein in carp feed formulations. Maggot are cultivated on pollard meal media, then check the nutritional content and amino acids. Substitution of maggot meal with fish meal, with treatments A (0%), B (25%), C (50%), D (75%) and E (100%). The main parameters observed included survival rate, specific growth rate, feed conversion ratio, protein efficiency ratio. The results biomass weight is 263.16 g, with a nutrient content of 54.34% protein, 16.02% fat, 10.00% ash, and crude fiber 10.83%. And the results use of maggot meal substitution in feed did not have an effect on the survival of carp, but had a very significant effect on the specific growth rate of 45%; 2.16 BB / day; 44 % for feed conversion ratio 1.94, 52% for protein efficiency ratio 1.65.

Index Terms: Hermetia illucens larvae, Substitution, Fish meal, Protein source, Common carp.

1. INTRODUCTION

During this time, the source of protein for feed products for fish is very dependent on fish meal while on the other hand the problem of high production costs as a result of the high price of feed manufacturers is not comparable with the prices prevailing in the market, so economically the level of efficiency is still quite low [1]. Cultivating maggot as a source of animal feed is now familiar. Maggot or larvae of the fly black soldier fly (Hermetia illicens) is one alternative feed that meets the requirements as a source of protein, with a high protein and fat content of 54% and 49%, these results can be obtained based on the substrate where it grows and in the cultivation process [2] [3]. Nutrient content of these larvae can be used as natural feed raw materials considering that until now Indonesia still imports fish feed, especially fish meal. Black soldier fly larvae can be used as feed for several species of fish, one of which is carp [4]. Maggot is one of the alternative feeds chosen by farmers. The term maggot is the name shown for black soldier fly larvae. Black soldier flies or Hermatia illucens are a common type of fly family Stratiomydae and can be widely found in grasses and leaves, maggot works to convert organic waste into simpler biomass. Black soldier flies or Hermatia illucens are a common type of fly family Stratiomydae and can be widely found in grasses and leaves, maggot works to convert organic waste into simpler biomass. The black soldier fly larva can be used as a good source of nutrition because it contains proteins, lipids and minerals.

2 MATERIAL AND METHOD

Cultivating and Harvesting Larvae

Maggot eggs are obtained from maggot cultivators in Jakarta. Maggot eggs weighed 0.10 g and then placed on pollard meal media at 500 g. Maggot is maintained for approximately 25 days and then can be harvested. The collected prepupae were washed with tapwater and frozen overnight. While freeze-drying mayresult in less complete moisture removal compared to oven drying, this diff erence is minimal and freeze-drying guarantees a better preservation of nutrients.

Proximate Analysis

Proximate analysis of Black soldier fly larvae consists of Protein (%), Fat (%), Ash (%), crude fiber (%) tested in Proximate Analysis Results of Fisheries Product Engineer, Faculty of Fisheries and Marine Science, Brawijaya University.

Amino Acid Analysis (UPLC)

Testing of amino acids using the Ultra Performance Liquid Chromatography (UPLC) method Analysis of amino acids using the UPLC consists of several stages, namely. The sample was weighed as much as 0.1 g was crushed and put into a closed test tube. The sample solution was added with 6 N HCl as much as 5-10 mL, hydrolyzed in an oven at 110 ° C for 22 hours, then cooled at room temperature and transferred to a 500 mL measuring flask. then added aquabides to the boundary and filtered with a 0.45 µL filter and piped 10 µL, add 70 µL AccQ Fluoric Borate and divorce. Then 20 µL of the Flour Adan reagent was added to be cooked and left to stand for 1 minute and added for 10 minutes at 55 ° C. then injected into the UPLC as much as 1 µL with chromotographic conditions using ACCQ-Tag Ultra C18 column, temperature 49 ° C, phase of system motion PDA composition gradient detectors, flow rate 0.7 µL / minute and wavelength 260 nm.

Parameter and Data AnalysisThe main parameters observed included survival rate, specific growth rate, feed conversion ratio, and protein efficiency ratio. Data analysis was performed by analysis of variance ANOVA .

3. RESULT

Biomass and Proximate Analysis

Based on the results of maggot cultivation on pollard meal media obtained the number of biomass and proximate results

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are biomass weight is 263.16 g, with a nutrient content of 54.34% protein, 16.02% fat, 10.00% ash, and crude fiber 10.83%. Nutritional content of pollard meal media is dry matter 88, 40%, protein 79,45%, fat 5,1%, ash 24%, mineral 11,60%, crude fiber 8,8%, and BETN 45%.

Table 1. *Composition of Feed Inggridients*

Type of Inggridients	Dry matter (%)	Water content (%)	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)	BETN **	Energy kkal/g ***
Fish meal*	89,15	10,85	58,22	5,73	22,75	3,50	9,80	2,75
Maggot meal*	93,64	6,36	54,34	16,02	10,00	10,83	8,82	3,42
Soy meal*	92,83	7,17	33,63	22,41	5,30	8,41	30,25	3,75
Bran meal*	89,68	10,32	10,01	11,01	7,17	8,89	62,92	2,82
Tapioca*	91,59	8,41	0,14	0,04	0,05	0,80	98,96	2,48

Note :

* : Proximate Analysis Results of Fisheries Product Engineer, Faculty of Fisheries and Marine Science, Brawijaya University.

** : BETN = 100-Protein-Fat-Ash-Crude fiber

*** : Energy = (3,5 x %Protein) + (8,1 x %Fat) + (2,5 x %BETN)

Table 2*. Common carp Feed Formulation*

Type of Inggridients (%)	Treatments A (0:100)	B (25:75)	C (50:50)	D (75:25)	E (100:0)
Fish meal	37,41	28,06	18,71	9,35	0,00
Maggot meal	0,00	10,02	20,04	30,06	40,08
Soy meal	30,03	30,03	30,03	30,03	30,03
Bran meal	11,21	11,21	11,21	11,21	11,21
Tapioca	16,48	13,03	9,57	6,12	2,66
Cr_2O_3	0,50	0,50	0,50	0,50	0,50
Vit and Minerals	2,00	2,00	2,00	2,00	2,00
CMC	2,37	5,16	7,95	10,73	13,52
Total (%)	100,00	100,00	100,00	100,00	100,00
Protein (%)	33,00	33,00	33,00	33,00	33,00
Energy (kkal/g)	287,78	287,78	287,78	287,78	287,78

Amino Acids Analysis

Based on the results of amino acid testing using the UPLC method (Ultra Performance Liquid Chromatography) found 15 amino acids in maggot meal, which consists of 8 types of essential amino acids (Figure 1) and 7 (Figure 2) types of non essential amino acids.

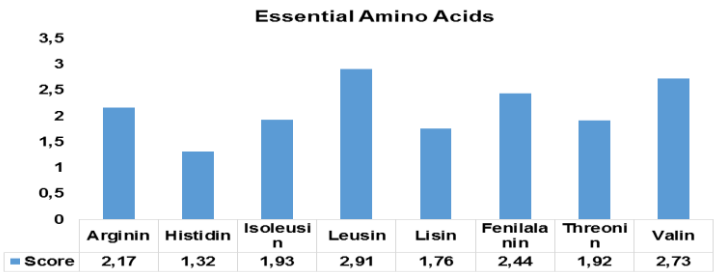

Figure 1. *Essential Amino Acids of Maggot Meal*

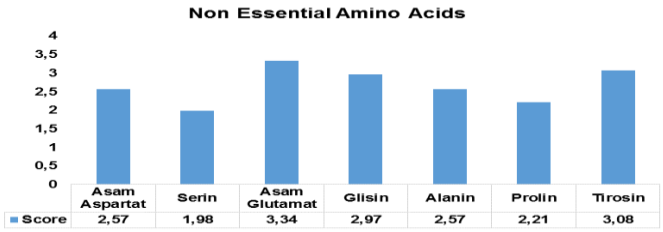

Figure 2. *Non Essential Amino Acids of Maggot Meal*

Biological Test of Feed in Carp

Based on the research results obtained survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), and protein eficiency ratio (PER) as shown in Table 3, below:

Table 3*. Average value and parameter statistical analysis.*

Parameter	Treatments A	B	C	D	E
SR (%)	97,78 ± 3,85^a	97,78 ± 3,85^a	100 ± 0,00^a	100 ± 0,00^a	100 ± 0,00^a
SGR (%)	1,54 ± 0,03^a	2,04 ± 0,02^d	2,39 ± 0,01^e	1,86 ± 0,03^c	1,60 ± 0,02^b
FCR	2,75 ± 0,04^d	2,03 ± 0,01^b	1,74 ± 0,012^a	2,29 ± 0,04^c	2,74 ± 0,02^d
PER	1,10 ± 0,017^a	1,49 ± 0,07^c	1,74 ± 0,01^d	1,32 ± 0,021^b	1,11 ± 0,05^a

Note: The same notation shows no difference, while different notation indicates a difference between treatments with a 95% confidence interval.

Survival rate (SR)

Carp after rearing for 30 days with experimental feed obtained the results that the treatment had no effect on the survival of carp (p> 0.05) (Table 3).

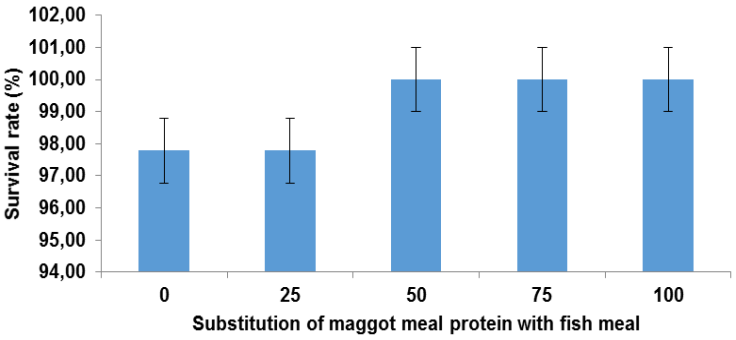

Figure 3. *Graph of average survival of carp.*

Spesific Growth Rate (SGR)

In Figure 4, it can be seen that the growth of carp which is maintained for 30 days is increasing and after the calculation is obtained the specific growth rate as shown in Table 3.

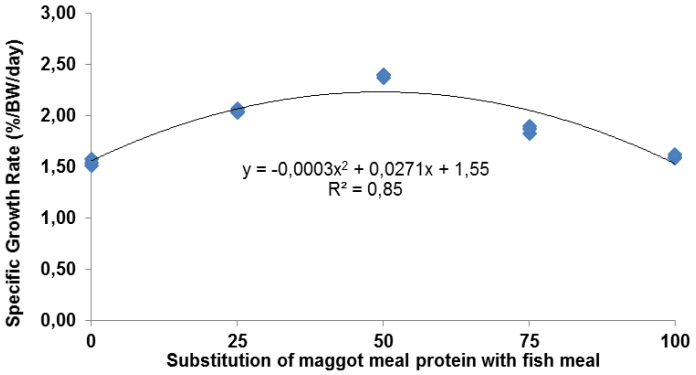

Figure 4. *Average growth rate of carp.*

Note :
A : Substitution treatment of maggot meal 0%
B : Substitution treatment of maggot meal 25%
C : Substitution treatment of maggot meal 50%
D : Substitution treatment of maggot meal 75%
E : Substitution treatment of maggot meal 100%

From Table 3 we can see that the maggot meal substitution treatment influences the specific growth rate for each treatment. The relationship between substitution of maggot meal and fish meal in feed formula (x) with a specific growth rate (y) quadratic pattern with the equation (Figure 5).

$$y = -0,0003x2 + 0,0271x + 1,55 ; R^2 = 0,85$$

From this equation, it is obtained that the substitution of maggot flour in feed formula which produces the highest growth rate of 2.16% / BW / day is 45%.

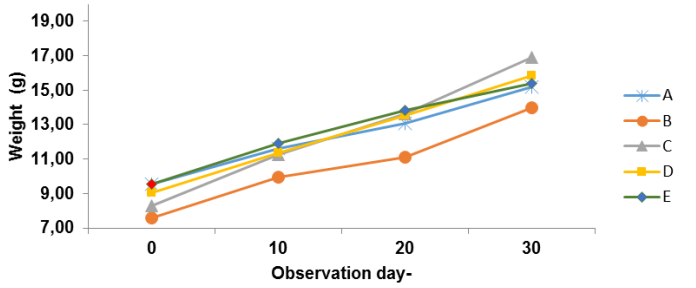

Figure 5. *The relationship between substitution of maggot meal to fish meal in feed formula on the specific growth rate of carp.*

Feed Conversion Ratio (FCR)
From Table 3 it can be seen that the treatment has an influence on the feed conversion ratio in fish. In the results of the study gave significantly different results between treatments B, C and D, where A and E did not different. The relationship between substitution of maggot meal with fish meal in feed formula (x) with feed conversion ratio (y) with quadratic pattern and equation (Figure 6).

$$y = 0,0004x2 - 0,0352x + 2,71 ; R^2 = 0,92$$

From these equations it was found that substitution of maggot flour in the feed formula which produced the best feed conversion ratio of 1.94 was 44%.

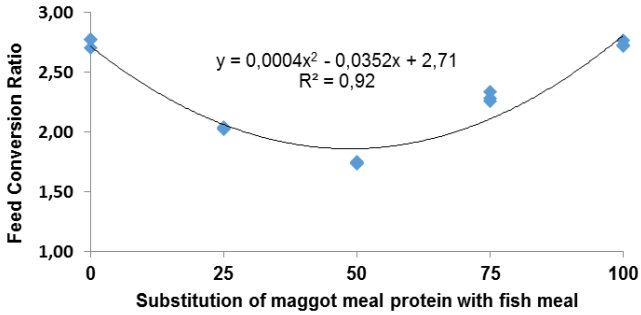

Figure 6*. The relationship between substitution of maggot meal to fish meal in the feed formula to the conversion ratio of carp feed.*

Protein Efficiency Ratio (PER)
From Table 3 it can be seen that the treatment gives the effect of the ratio of the efficiency of carp protein. From the results of the research conducted gave significantly different results on treatments B, C, and D, while treatments A and E did not different. The relationship between substitution of maggot flour and fish meal in feed formula (x) with the ratio of efficiency of protein (y) quadratic patterned with the equation (Figure 7).

$$y = -0,0002x2 + 0,0208x + 1,114 ; R^2 = 0,86$$

From these equations it was found that substitution of maggot meal in the feed formula which produced the best protein efficiency ratio of 1.65 was 52%.

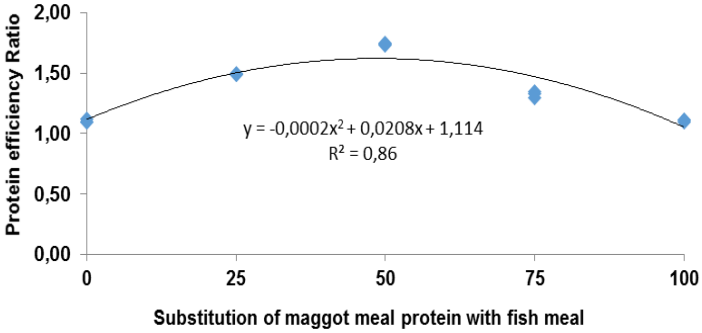

Figure 7. *The relationship between substitution of maggot meal to fish meal in feed formula to the protein efficiency ratio of carp.*

4. DISCUSSION

Biomass and Proximate Analysis of Maggot

The protein contained by maggot Hermetia illucens is sourced from proteins found in growing media, because maggot Hermetia illucens utilizes the protein present in the media to form proteins in the body. Metabolically, maggot will convert proteins and various nutrients into maggot biomass. Protein is digested in the stomach by the enzyme pepsin. Pepsin is able to digest all types of proteins that are in the growth media. One of the most important things about digestion by pepsin is

its ability to digest collagen. Collagen is the main ingredient in connective tissue in the skin and cartilage. Pepsin starts the process of protein digestion, the breakdown of these proteins is the hydrolysis process that occurs in the polypeptide chain. Most protein digestion takes place in the intestine. When protein leaves the stomach, it is usually protein in the form of proteoses, peptons, and large polypeptides. After entering the intestine, most broken products will be mixed with pancreatic enzymes under the influence of proteolytic enzymes, such as trypsin, kimotripsin, and peptidase. Both trypsin and kimotripsin break down protein molecules into small polypeptides. The peptidase will then release amino acids. Amino acids that are synthesized in cells as well as those produced from the process of breaking down proteins in the liver are carried by the blood for use in system.

Amino Acid of Maggot Meal

The quality of proteins is generally measured based on the amino acid profile they contain. Amino acids can also be used for the synthesis of body proteins or other nitrogen components (nucleic acids, amines, peptides, hormones, etc.), provide a carbon source for intermediate metabolism or become oxidized to provide energy [5]. Based on the results of amino acid testing on maggot flour derived from pollard flour media obtained the highest amino acid yield in the leucine type (Figure 1) and non-essential amino acid type glutamic acid (Figure 2). Leucine is an important molecule that can stimulate muscle protein synthesis [6]. As for glutamic acid in feed given to livestock can increase metabolism in the digestive system [7]. That is because glutamic acid has a role as a specific precursor for the formation of arginine, proline and glutathione in the intestinal mucosa. So, this can show that glutamic acid also plays an important role in increasing the formation of amino acids and maintaining intestinal function.

Biological Test of Feed in Carp

The survival of common carp for 30 days with experimental feed using maggot meal substitution on fish meal in feed formula did not have a significantly different effect with values ranging from 97.78% -100% (Table 3). Survival is caused by several factors, one of which is when the first sampling is not careful so that the fish experience stress and then die, or it could also be in maintaining the condition of the water in the aquarium. The high value of goldfish survival obtained in this study was due to the quality of water during maintenance is still within the optimum conditions for the cultivation needs so that it is suitable for goldfish survival (Table 4). Factors that can affect the level of survival of an organism are biotic and abiotic factors. Biotic factors include competitors, population density, age and ability of organisms to the environment while abiotic factors such as temperature, dissolved oxygen, and pH.

Table 4. *Observation Results of Carp Water Quality*

Water Quality Parameters	Water Quality Value	
	During maintenance	According to literature
Temperature (^{0}C)	25,17 – 26,83	22–30 ^{0}C [8]
pH	7,68 – 8,18	6-8,5 [9]
DO (mg/L)	6,10 – 8,20	1-10 [10]

Fish growth is closely related to the feed given, because feed provides nutrients and energy that are needed for growth. In addition, fish growth can occur if the amount of feed nutrition that is digested and absorbed by fish is greater than the amount needed for maintenance of the body. Specific growth rates occur because of the ability of fish to absorb and utilize the protein feed provided. Cr2O3 in feed can be absorbed by the digestive tract and can then be accumulated by the body. Chromium is part of a chromodulin compound that can activate insulin receptors, and will further make insulin work to transfer glucose and amino acids into cells [11] [12]. This means that the presence of Cr2O3 in feed will increase the utilization of feed carbohydrates so that the digestibility of feed protein can be increased by cells for protein synthesisThe protein in maggot flour has been broken down into simple compounds that are more easily absorbed by the fish's body. These simple compounds are amino acids. This amino acid has a strong relationship with metabolism that will later affect hormones or the immune system in the fish's body. Amino acid metabolism is the process of exchanging compounds or substances that occur in the body of fish chemically with the aim of forming proteins in the body. The digestion process of feed protein begins in the fish's stomach. Protein in the stomach will undergo a denaturation process by the action of HCl and hydrolyzed by the enzyme pepsin, so that the protein is turned into a peptide. Furthermore, the digestive process in the intestine, peptides will undergo hydrolysis with the enzyme carboxypeptidase, trypsin, chimotripsin and elastase as the catalyst. Furthermore, this oligopeptide will be hydrolyzed with the enzyme peptidase into a form of tripeptide, dipeptide and amino acids [13]. The reaction shown by amino acid metabolism will initially involve the process of transferring amino groups which will then proceed with the process of changing carbon compounds contained in amino acid compounds. This amino group transfer process is divided into 2 processes, namely the process of transamination and deamination. Transamination process is a process of catabolism which involves the transfer of amino groups to one another. The result of the transamination reaction is glutamic acid. The higher of protein contained in the feed, the more the growth rate will decrease. This is because the excess of this protein can stimulate the metabolic system of carp (Cyprinus carpio) to carry out the process of deamination in synthesizing protein in the body into ammonia as a toxic compound and will be able to inhibit the absorption of nutrients in the body of the fish, causing growth in fish to become slow. Fish growth is relatively slow due to the energy content of feed, especially those from carbohydrates and fats which are not sufficient for the metabolic process. As a result, protein is used for this process through the mechanism of gluconeogenesis in the body, so that the protein in the feed is not sufficient for fish to grow. With an increase in growth rate, the use of feed will be more efficient. Because the lower the value of feed conversion shows the efficiency of the better utilization of feed by the body is used to increase growth. Due to the complexity of feed substances and the limited ability to digest, not all feed consumed can be absorbed by the body of the fish.

5. CONCLUSION

And the results use of maggot meal substitution in feed did not have an effect on the survival of carp, but had a very significant effect on the specific growth rate of 45%; 2.16 BB / day; 44 % for feed conversion ratio 1.94, 52% for protein

efficiency ratio 1.65.

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