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INTERACTION OF CULTURAL MEDIA COMPOSITION WITH EM4 DOSAGE ON POPULATION GROWTH, BIOMASS AND NUTRITIONAL CONTENT SILK WORM (TUBIFEX SP)

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Abstract. Culture media as a place for the maintenance of silk worms greatly affects the growth and nutritional content of silk worms. The purpose of this study was to analyze the interaction effect of the composition of culture media with a dose of EM4 on population growth, biomass and nutritional content of silk worms (Tubifex sp). This study used a completely randomized factorial design with 9 treatments and 3 replications each. Treatment D1M1, treatment D1M2, treatment D1M3, treatment D2M1, treatment D2M2, treatment D2M3, treatment D3M1, treatment D3M2 and treatment D3M3. Each treatment was kept in 27 trays. The resu its showed that the composition of the media material and the dose of EM4 had a very significant effect (P< 0.05) on population growth and biomass and nutritional content of silk worms. The highest average population and biomass growth was obtained in the D2M2 treatment, which was 27482.9 ind/m2 and 8.52 grams, and the best nutrient content was in the D3M3 treatment with a protein content of 50.88. Based on the results of the study, it can be concluded that the combination of media materials with a percentage of 50% azolla pinnata and 50% chicken manure can increase the population, biomass and nutritional content of silk worms.

Keywords: *tubifex SP*; chicken manure; *azolla pinnata*; rejected bread; EM4 dose.

INTRODUCTION

Cultivation activities, especially in hatchery activities, require natural feed which is an important supporting factor in the successful growth of fish larvae and fry (Silva-Aciares et al., 2013). Natural food is vegetable and animal plankton that is in accordance with the development of the digestive organs of larvae and fish seeds, one of which is silk worms (*Tubifex sp*) which are always moving and colored like blood red which will attract larvae and fish seeds to eat them. According to (T.-H. Liu et al., 2022), silk worms have an important role because they can stimulate fish growth faster than other natural foods such as water fleas (*Dapnia sp*. and *Moina sp*).

Culture media as a place for the maintenance of silk worms greatly affects the growth and nutritional content of silk worms. The growth of silk worms is influenced by bacteria and organic particles resulting from bacterial overhaul as food for silk worms (Chen et al., 2020). Bacteria need organic C and N content in the culture media for maintenance because by utilizing high-carbon and low-nitrogen materials, they can produce cell proteins so that silkworms use them in their growth (Fujita et al., 2020). One method that is often used is the fermentation process. The use of probiotic activator EM4 can be used in the fermentation process. (X. Liu et al., 2020) on the implementation method of making silkworm cultivation media using a dose of 1 ml EM4 with 200-250 ml of water and 25 ml of molasses drops for 1 kg of raw materials. The purpose of this study was to analyze the effect of the interaction of the composition of culture media with a dose of EM4 on population growth (Pujiastuti et al., 2018), biomass and nutritional content of silkworms

METHODS

This study used a Completely Randomized Factorial Design (RALF) consisting of 9 treatments and 3 replications. In this study, the treatment is the composition of the media material with the use of EM4 doses, namely:

The first factor is the difference in the composition of the material consisting of 3 levels of treatment, namely as follows: *Treatment M1* = Chicken manure 25% + *Azolla pinata* 25% + Bread rejected 50%. *Treatment M2* = Chicken manure 25% + *Azolla pinata* 50% + Bread rejected 25%. *Treatment M3* = Chicken manure 50% + *Azolla pinata* 25% + Bread rejected 25%.

The second factor is the difference in the dose of probiotic EM4 in the composition of the ingredients, as follows: *Treatment D1* = Dose 1 ml/kg *Treatment D2* = Dose 2 ml/kg *Treatment D3* = Dose 3 ml/kg *Working Procedures.*

Preparation Containers

Rearing silk worms using plastic trays of size (25×20×10) cm with a multilevel shelf model. The water channel uses a recirculation system with a shelter using a tarpaulin which is made to form a rectangle with a size of (100×50×25) cm.

Media Preparation

Mud material taken from the rice fields of the Ulin River area was taken and sieved using a strainer/sieve with a mesh size of 1 mm. Chicken manure that will be used as a media material comes from chicken farms (Islam et al., 2021). *Azolla pinnata* which will be used as a media material comes from aquatic plant cultivators. The rejected bread that will be used as a media material comes from the seller of animal feed ingredients (Kuts et al., 2021). The three materials were dried in the sun to dry, then crushed or kneaded by hand and filtered using a 1 mm sieve to obtain a medium material in the form of a fine powder.

Preparation of Fermentation Materials

Media materials Chicken manure, *azolla pinnata* and rejected bread to be used were weighed according to each treatment with the total material for each treatment was 1 kg and mixed with fine mud. The mixed media material is given EM4 according to the dose that will be used for the fermentation process (Li et al., 2018). The use of EM4 as a fermentator is done by mixing EM4 with 250 ml of water according to the dose in the treatment, then mix it into the media material to be used. Fermentation media material with a predetermined dose is stored for 7 days in a closed container in the form of a jar.

Distribution of Silk

Worms Silkworms were first drained for about 1 minute and weighed on an analytical balance as much as 20 grams for each treatment as the initial weight of silkworm biomass. The maintenance of silk worms was carried out for 20 days. Re-fertilization was carried out after each sampling with the fermentation of the material used as much as 50 grams in each treatment.

Sampling of silkworms was carried out once every day. Silkworm sampling was carried out at 4 points in each treatment container. The sampling process uses a inch pipe with a diameter of 3 cm inserted into the substrate, then removed while closing the top hole with the palm of the hand. The taken substrate is accommodated in a fine mesh scoop then the substrate is washed in running water, then spread over a transparent plastic cover. Then the silk worms were separated using a pipette and counted one by one. The formula for population growth is based on (Ansyari and Rifa'i, 2005):

Total $^{=}$ × RJIS —

Description

LPP : Area of sample plots (m^2) LLP : Area of pipe circle (m^2)

RJIS : Average number of individual samples (Individual/m^2)

Biomass weight of silkworms

Observation of biomass weight gain was carried out by sampling method done once every day. Measurement of the weight gain of biomass by weighing using a digital scale from the sampling results of each treatment.

Daily Length Growth of Silk Worms

Sampling was carried out once every day by taking samples of 10 silkworms from the observation of biomass weight and measured using a digital ruler. The formula for finding length growth according to Effendie (2002) is:

$$P = Pt - Po$$

Description:

P = Length Growth

Pt = Final Length Growth (mm) P0 = Initial Length Growth (mm) Nutrient Content

Qualitative analysis consists of:

1. Nutrient content test Organic C, organic N and C/N ratio of material media before and after fermentation at the Soil Science Laboratory, Faculty of Agriculture, ULM.
2. Proximate test to determine the composition of protein, carbohydrates, fat

and water content contained in silk worms (*Tubifex sp*) after being cultured at the Nutrition and Animal Feed Laboratory, Faculty of Agriculture, ULM.

3. Test of Amino Acid Content of Silkworm (*Tubifex sp*) after being cultured at SIG Laboratory PT. Saraswanti Indo Genetech, Bogor.

Water Quality

Physical and chemical parameters of water measured include temperature, pH, dissolved oxygen, and ammonia. The research data were statistically analyzed using analysis of variance to determine the effect of the treatment given on the observed changes and if F count > F table 5% or 1% then the calculation will continue with further analysis with the provisions of the Small KK BNJ test (KK < 5%), medium KK BNT test (5% < HH < 10%) and large KK Duncan test (> 10%) (Hanafiah, 2013). Meanwhile, for water quality data, noanalysis of variance was carried out, but quantitative tests were carried out and will be explained descriptively.

RESULTS AND DISCUSSION

Nutrient Content of Silk Worm Material Media

The nutrient content of N, P, K and C/N ratio in silk worm material media by conducting laboratory tests on the media material. The results of the laboratory test of the material media can be seen in Table 1.

Table 1. Elemental Content of N, P, K and C/N Ratio

Treatment	Nutrient content				
	N	C	P	K	Ratio C/N
M1	1.23	9.18	0.67	0.74	7.46
M2	1.32	9.03	1.22	1.13	6.84
M3	1.28	8.14	1.46	0.92	6.35
D1M1	1.30	10.74	0.55	0.39	8.26
D1M2	0.83	4.45	0.34	0.45	5.36
D1M3	1.25	8.01	1.12	1, 09	6.40
D2M1	0.97	8.17	0.45	0.24	8.42
D2M2	1.10	5.41	0.36	0.33	4.91
D2M3	1.04	7.82	1.07	1.02	7 ,51
D3M1	1.08	8.25	0.59	0.36	7.63
D3M2	1.00	4.79	0.39	0.39	4.79
D3M3	1.23	9.03	1.55	1.23	7.34

The C/N ratio in this study is the result of analysis before and after the fermentation process. The principle of fermentation for media materials is to reduce the C/N ratio of organic matter (Hastuti et al., 2022). This can be seen in Table 1. which shows that before and after the fermentation process the organic material has completely decomposed or degraded so that the material media can be used in the maintenance of silk worms. During the fermentation process, microorganisms utilize only a small amount of phosphate and potassium elements for their metabolic activities. The increase in the growth of silk worms in the culture material media plays a very important role in the success of silk worm cultivation. Likewise, the nutritional quality of silk worms from cultivation is largely determined by the material media that the silk worms feed on to live during the maintenance period (Sudarman & Velina, 2021).

The increase in the average growth of biomass in the D2M2 treatment was 8.52 gr which can be seen in Table 4.8. assumed to be due to the presence of a higher number of bacteria and organic particles, which became food for silk worms. According to Anggraini (2017), fermentation of different media materials can affect the increase in organic matter in the media, so that it can increase the amount of food ingredients and subsequently have an impact on the population and biomass of silk worms.

In the D2M1 treatment where the growth in length, population and biomass were found to be low, it showed that the use of 25% chicken manure + 25% *pinnata* azola + 50% rejected bread caused the growth of weight and biomass to be less than optimal. Allegedly this is because the combination of media materials is quite difficult to digest, besides the D2M1 treatment contains a higher C/N ratio than other treatments, so it takes longer time for silkworms to digest.

Daily Length of Silk Worm

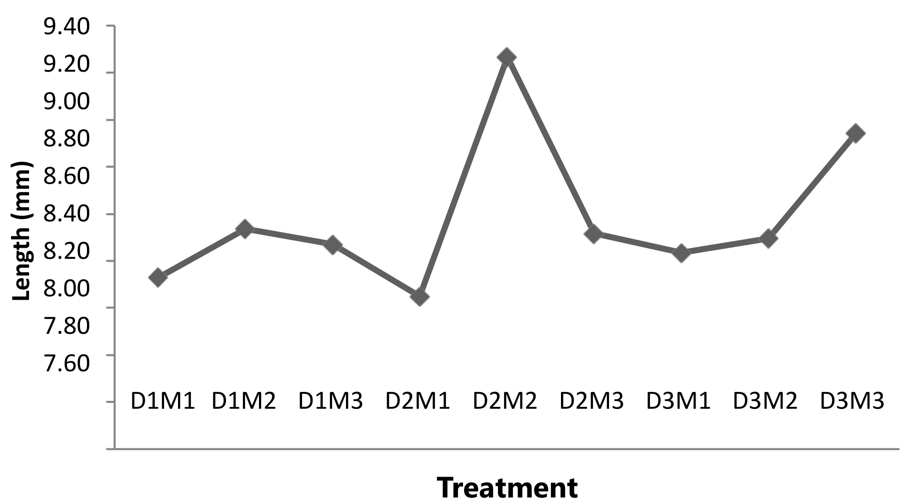

Figure 1. Graph of Average Daily Length of Silkworms

The figure above shows the daily length growth of different silkworms during 20 days of rearing. The highest daily length growth was obtained in the combination treatment of 25% chicken manure + *azolla pinnata* + 25% rejected bread with a dose of EM4 of 2 ml (D2M2) with an average length growth of 9.27 mm. While the lowest length growth results were achieved in the combination treatment of 25% chicken manure + *azolla pinnata* + 50% rejected bread with a dose of EM4 of 2 ml (D2M1) with an average length growth of 8.25 mm.

The results of analysis of variance in daily length of silk worms for 20 days of maintenance showed that the combination of media material and dose of EM4 obtained ($P < 0.05$) so the treatment had a very significant effect. In figure 4.1. It can be seen that the daily length of silk worms ranged from 8.25-9.27 mm, where observations on days 6-10 the average length of silk worms decreased because on that day silk worms had already laid eggs and silkworms were seen inhabiting colored substrates. smaller redness than other silkworms.

Silkworm Population

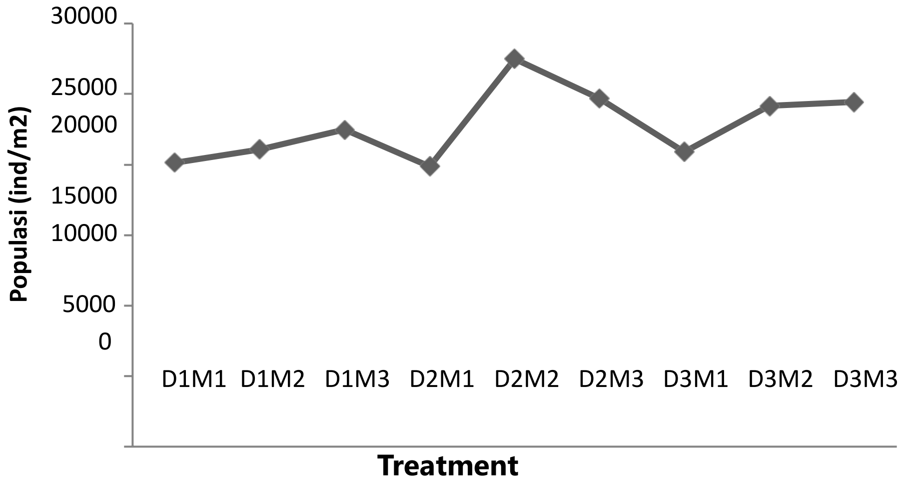

Figure 2. The Average Silkworm Population

Graph Figure 2. The above shows the total population of silk worms for all treatments with a combination of different media materials and EM4 doses used during the study. The highest population was achieved in the combination treatment of 25% chicken manure + *azolla pinnata* + 25% rejected bread with a dose of EM4 as much as 2 ml (D2M2), obtaining a population average of 27428.9 ind/m^2. While the lowest population results were achieved in the treatment of a combination of 25% chicken manure + 25% *azolla pinnata* + 50% rejected bread with a dose of EM4 as much as 2 ml (D2M1), obtaining a population average of 19863.1 ind/m^2. The results of the analysis of variance of the silkworm population during 20 days of rearing showed that the combination of media material and EM4 dose obtained ($P < 0.05$), the treatment had a very significant effect.

Silkworm biomass

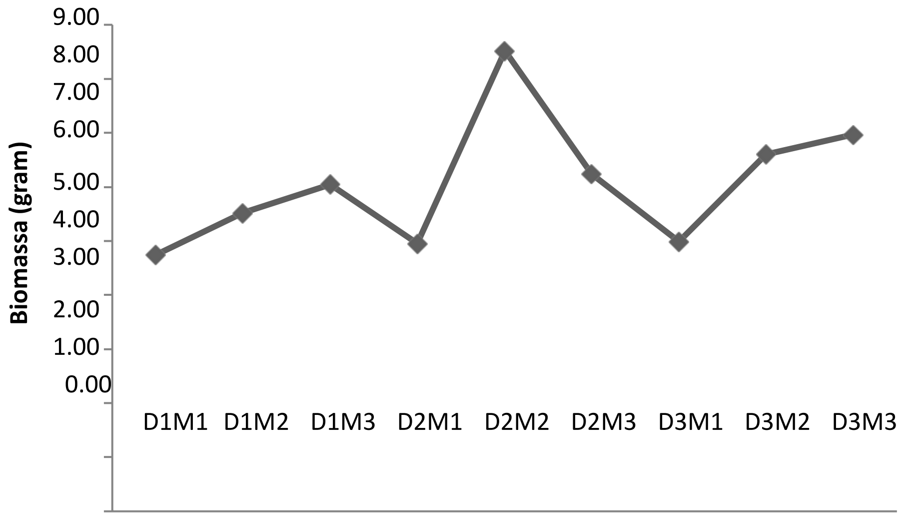

Figure 3. Graph of Average Silkworm Biomass

Figure 3. The above shows that the silkworm biomass reared for 20 days with a combination of different media materials and EM4 doses increased until the last day of rearing. The highest biomass growth was in the treatment of a combination of 25% chicken manure + *azolla pinnata* + 25% rejected bread (D2M2) with a dose of EM4 of 2 ml to obtain 8.52 grams of biomass. This is because the nutrient content

in the media material can increase organic matter as a food source for silk worms. Provision of 50% azolla pinnata as the media is suspected as the largest supplier of N elements because it has a high protein content, so it can add food to the media. According to Lestari *et al*. (2020), *Azolla pinnata* is a plant that has the privilege of being able to bind free N from the air due to its symbiosis with blue algae (*Anabaena azollae*) so that it contains high protein. According to Alalade and lyayi (2006), the crude protein content of *Azolla pinnata* is 21.4%, this can be seen in Table 4.1. The amount of N content in the combination media with a dose of EM4 amounted to 1.10. The results of the analysis of silkworm biomass variance for 20 days of rearing showed that the combination of media material and EM4 dose obtained ($P < 0.05$) so the treatment had a very significant effect.

The content of the proximate analysis of silk worms

The results of the proximate analysis of silkworms reared for 20 days on a combination of media materials with different doses of EM4 can be seen in Table 3. following.

Table 3. Analysis Content of Silk Worms Reared on Different Media Materials and Doses of EM4

Treatment	Proximate content (%)				
	content Ash	content Protein	Fat Crude	content fiber	content Carbohydrate
D1M1	12.63	46.19	16.06	2.43	38.75
D1M2	11 ,48	42.45	15.33	2.31	43.76
D1M3	10.19	42.67	16.42	2.28	48.98
D2M1	11.09	42.07	17.94	1.62	45.21
D2M2	13.55	43.47	17.34	1.38	41.60
D2M3	12.82	45.33	17.11	1.63	40.21
D3M1	13.85	49.11	24.03	1.94	35.09
D3M2	14.99	47, 17	20.08	1.81	36.03
D3M3	15.05	50.88	20.66	1.56	32.51

Amino acid content of silk worms Amino acid

content analysis was carried out to determine the amino acid composition of silk worms (*Tubifex sp*) reared with a combination of media materials with a dose of EM4. The amino acid content of silk worms reared in a combination of media materials with a dose of EM4 can be seen in Table 5.

Table 5. Amino Acid Content of Silk Worms Maintained on Different Media Materials Aad EM4 Doses

Test Parameters (%)	D1M1	DIM2	D1M3	D2M1	D2M2	D2M3	D3M1	D3M2	D3M3
L- serine	0.31	0.22	0.18	0.35	0.45	0.25	0.25	0.28	0.30
Acid	0.60	0.39	0.39	0.71	Glutamic	0.60	0.57	0.57	0.64

L- Phenylalanine	0.38	0.23	0.18	0.45	0.48	0.26	0.24	0.27	0.30
L- Isoleucine	0.19	0.17	0.30	0.37	0.24	0.23	0.24	0.27	L-
Valine	0.33	0.23	0.21	0.38	0.46	0.30	0.29	0.30	0.33
L- Alanine	0.34	0.24	0.23	0.46	0.43	0.27	0.23	0.34	0.36
L-Arginine	0.41	0.19	0.16	0.49	0.63	0.28	0.28	0.33	0.35
Glycine	0.35	0.26	0.21	0.42	0.52	0.31	0.29	0.31	0.34
L-Lysine	0.32	0.21	0.21	0.31	0.36	0.24	0.29	0.28	0.26
L-Aspartic Acid	0.41	0.28	0.27	0.34	0.54	0.34	L	0.34	0.34
L-Leucine	0.46	0.32	0.29	0.51	0.63	0.41	0.39	0.41	0.46
L-Tyrosine	0.27	0.15	0.12	0.30	0.32	0.18	0.16	0.18	0.21
L-Proline	0.22	0.15	0.14	0.26	0.30	0.23	0.19	0.21	0.23
L-Threonine	0.33	0.23	0.19	0.39	0.47	0.26	0.26	0.28	0.31
Hisidine	0.19	0.12	0.09	0.20	0.20	0.11	0.1 1	0.12	0.13

Based on the amino acid content test of silkworms reared for 20 days in a combination of media materials with a dose of EM4 showed that each treatment had different amino acid content. The amino acid content in the D2M2 treatment looked better than the other treatments. The different amino acid content of silkworms is thought to be due to differences in the nutrient content of nitrogen, carbon, phosphorus and potassium which can be seen in Table 4.1. The difference in potassium content in the combination of material media affects the amino acid content of silk worms where the nitrogen and potassium content of the D2M1 treatment is lower than that of the D3M3 treatment. It is presumed that in the D2M1 study with the lowest population and biomass, silk worms fully utilized the nitrogen content for growth, so that the amino acid content obtained was higher than in the D3M3 treatment, but because the potassium content in the D2M1 treatment medium was lower than D3M3 and D2M2 then the formation protein N levels will be disrupted and can produce high ammonia content and cause death in silkworms.

In the feed laboratory, protein is separated from carbohydrates and lipids due to the nitrogen content of the protein.

According to Syam *et al.* (2011), sufficient N content will increase the activity of microorganisms so that the decomposition process can take place effectively. Organic C is the main constituent of carbohydrates and fats which are an energy source for the metabolism of silk worms, so that a balanced content of organic N and C can affect the high population growth and biomass of silk worms (Anggaraini, 2017). The important role of nutrient K in determining products with chemical composition and physical appearance. For example, in plants that lack K, protein formation will be disrupted so that protein

N levels decrease and non-protein N levels increase (Wiraatmaja, 2017). If the K deficiency has reached a serious level, plant tissue contains a lot of nitrate and free ammonium, amides and organic acids which will reduce the quality of the product.

Table 4. Water Quality Parameter

Treatment	Parameter			
	Temperature	pH	DO	Ammonia
D1M1	27.65	6.52	4.43	1.6
D1M2	27.79	6.59	4.21	1.8
D1M3	27.59	6.46	4.55	1.6
D2M1	27 ,71	6.42	4.21	2.4
D2M2	27.81	6.48	4.45	2.4
D2M3	27.85	6.44	4.71	2.3
D3M1	27.65	6.62	4.56	2.4
D3M2 27.87	6.39	4.47	2.4	D3M3
27.67	6.45	4.73	2.4	The

Results of the water temperature measurement in Table 4.13 above are suspected to be the optimum temperature for good metabolism and nutrient absorption in organic matter media. maximum. Lestari *et al* (2020), said the optimum range of temperature in the growth of silkworms was 25–28 ^{0}C. The optimum range of dissolved oxygen could meet the needs for the growth of silkworms range. Dissolved oxygen for silkworm growth is 2.5-7 mg/L (Effendi, 2013). It is also stated by Lestari *et al* (2020) that the dissolved oxygen requirement for the growth of silk worms is in the range of 2.24-6.48 mg/L. the pH value during the research on the maintenance media was still in the optimal value, namely 6.39-6.59. Safrudin *et al* (2005) stated that at neutral pH, bacteria can break down organic matter normally into simpler ones that are ready to be used by silkworms as food, ranging from 6.02-7.76. The ammonia content was still at the optimum limit for the growth of silk worms. Ammonia content during the study ranged from 1.6 to 2.4 mg/L. This is in accordance with Anggraini's (2017) statement that the optimum range for the growth of silk worms is <3.6 mg/L.

CONCLUSIONS

The interaction of the composition of the culture media with the dose of EM4 on population growth and biomass of silkworms (*Tubifex sp*) had a very significant effect. The highest daily length, population and biomass growth was obtained in the combination treatment of 25% chicken manure + *azolla pinnata* + 25% rejected bread with a dose of EM4 of 2 ml (D2M2) on day 20 with an average length growth of 9.27 mm. , the average population was 27482.9 ind/m2[and] the average biomass was 8.52 grams, while the nutrient content of silk worms (*Tubifex sp*) reared with different compositions of culture media and EM4 doses obtained results. D3M3 treatment obtained the highest protein content. ie 50.88, then D2M2 obtained a protein content of 43.47.

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