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IDENTIFICATION OF SALMONELLA SP. BACTERIA, ANTIBIOTIC TETRACYCLINE IN SILK WORM (*Tubifex,* SP) CULTIVATION USING Chicken Manure

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ABSTRACT

Sustainability of farming activities is determined by the use of materials that are free from pathogens, residues of antibiotics and other chemicals. Prevention of pathogenic microorganisms can be done through the use of disease-free seed with a certificate Specific Pathogen Free guided by way of Good Aquaculture (CBIB). This study uses a completely randomized design (CRD) with 2 treatment (fermentation methods and non-fermented) and three replications. The identification results on the silkworms with fermentation methods and non-fermenting bacteria positive for Salmonella sp positive and tetracycline antibiotic residues (> 32 ppb) in the silkworm cultivation. Productivity silkworms in the fermentation medium are higher than in non-fermented in the amount of 267.91 ± 113.18 g / m^2 / cycle and for non-fermented 201.89 ± 16,85g / m^2 / cycle. Absolute growth fermentation medium peak at 30.92 ± 2.9 g / m^2 and non-fermented 9.12 ± 1.99 g / m^2.

***KEYWORDS*:** *Antibiotics, silkworms, chicken manure, Salmonella sp,(Tubifex sp)*

INTRODUCTION

Silkworm (Tubifex sp) cultivation techniques began in the 1980s (Hadiroseyani & Dana, 1994). Silkworms is very suitable for natural feed in fish larvae because they have a size that matches the larval mouth opening and contains 57% protein, 13.3% fat, 2.04% crude fiber, 3.6% ash, and 87.7% water (Pursetyo et al., 2011; Suharyandi, 2012). After the yolk mass is depleted, the larvae need food. Silkworms (tubifex sp) can be used as feed and can stimulate faster larval growth (Ngatung et al., 2017). Fish are aquatic organisms that are susceptible to microorganism infections such as viruses, bacteria, and parasites, and are susceptible to changes in environmental conditions. According to Karakassis (2001), aquaculture systems require monitoring fish health and disease, checking and identifying and managing risks, especially in drugs and chemicals in fish farming. Prevention of pathogenic microorganisms can be done by using disease-free seeds with the certificate of Specific Pathogen Free which is guided by the Way of Good Fish Farming (CBIB). Fish infected with bacteria, parasites, and viruses consumed by humans can be detrimental to health (Aloo, 2002). Provision of natural food that is free from bacterial contamination of *Salmonella sp*. and antibiotic residue content is necessary. According to SNI 01-6366-2000, the maximum limit of antibiotic residues is 0.1 mg / L. The use of antibiotics in the breeding of broilers is done to prevent bacteria and viruses and increase the immunity of chickens that are kept. Antibiotics given will accumulate in broilers that are kept (Nurhasnawati et al., 2017). According to Agustinus (2016), the use of chicken manure media as a substrate is suitable for the habitat of silkworm life because it produces a very high population within a span of 15 days of cultivation. Thus it is necessary to identify the antibiotic residues of chicken manure used as a medium for silkworm cultivation to ensure that there is no contamination by antibiotics carried by chicken manure. Increased fish consumption must be balanced with improved food quality and safety.

MATERIALS AND METHODS

The research was conducted from August to November 2019. Raising silkworms (Tubifex sp) carried out in the Laboratory of Aquaculture STP Jakarta. Testing *Salmonella sp*. and tetracycline antibiotic residues held in the Great Hall of the Laboratory of Freshwater Aquaculture (BBPBAT) Sukabumi. Measurement of water quality parameters carried in Aquaculture Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences Laboratory Loka IPB University and Fish Diseases and Environmental Inspection Attack (LP2IL).

Materials and Methods

Tools and materials used include incubators, ovens, autoclaves, analytical balance, laminar flow, test tubes, petri dishes, Bunsen, erlenmeyer, and loopful. Materials used are chicken manure, media SSA (*Salmonella Shigella Agar*), media SCA (Simmon's Citrate Agar), media SIM (Solid Indol Motility), media APW (Alkaly Peptone Water), reagent Gram, methyl red, glucose, sucrose, oxidase paper, and distilled water (BSN, 2006).

1) Samples

Silk worm samples from the cultivation by fermentation and non-fermentation methods were put into sterile plastic as much as 25 g and then observed in the laboratory.

2) Isolation, selection and identification of bacteria *Salmonella sp* (ISO 01-2332. 2- 2006)

Pre-enrichment, enrichment stage, selection on selective media, Qualitative test *Salmonella spp*, *Salmonella spp* biochemical test

3) Parameter Observation of Tetracyclines antibiotic residues Elisa Kit

a. Stages of sample handling

A total of 10g samples of each method smoothed until homogeneous and then added 3 ml of 80% methad, and stirred with a shaker for 20 minutes. The solution was then centrifuged for 10 minutes as much as 2000x, centrifuge results taken as 2 ml and 1 ml of the supernatant was transferred into a new tube.

b. Testing procedure

A total of 50 µL of buffer solution was added to each of 100 µL of standard solution and 50 µL of enzyme conjugate solution. The solution was then incubated for 1 hour at room temperature and rinsed using a 150 µL wash buffer for 30 minutes at room temperature. The solution was then added with 100 µL stop solutions Elisa photometer with absorbance at a wavelength of 450 nm.

Research Design

This research was conducted with a completely randomized trial design (CRD) consisting of 2 treatments and 3 replications. The types and descriptions of treatments used in the study are as follows:

a) Treatment A: 50% chicken manure fermentation + 25% bran + 25% tofu waste + 10 ml molasses + 1 ml probiotics + 100 ml water.

b) Treatment B: Non-Fermentation 50% chicken manure + 25% Bran + 25% Molasses tofu waste 10 ml + 1 ml Probiotic + 100 ml water.

Procedure

1) Making Culture Containers

The silkworm maintenance container has a size of 20 × 25 × 8 cm^3. The containers used were 6 plastic trays. 2 aerated faucets are installed in containers for air circulation. The container is perforated for the manufacture of outlets with a water discharge of 0.35 liters/second.

2) Preparation of Culture Media

Silkworm cultivation media was usesds ingredients such as chicken manure, tofu dregs, bran, probiotics, and molasses. Making media was done by 2 methods, namely fermentation and non-fermentation. In the fermentation method using probiotics EM4, molasses 10 ml, probiotics 1 ml and 100 ml water then given aeration for 2-6 hours (Febrianti, 2004). Mixing was carried out for the activation of probiotics with a ratio of 1: 1 media material with an amount of 16.19 kg / m^2

(Masrurotun et al., 2014). The fermentation results were mixed with 22.93 kg / m^2 of fine sludge that had been cleaned and sifted, the media good ones have 4-6 cm mud height (Febrianti, 2004).

3) Silkworm Culture

Spreading is done on fermented and non-fermented media that has been prepared. The seeds was used are from Bogor and have been quarantined using running water for 3 days. Spreading the seeds using the planting method with a distance of about 7-10 cm with the number of seedlings planted as much as 2.5 g / point. In the research container, there were 6 planting holes so that the total seedlings planted were 15 g / m^2 (Effendi & Tiyoso, 2017).

4) Feeding

Feed on silkworm culture is made with chicken manure, fine bran, and tofu dregs. The fermented material is then given to silkworms at a dose of 0.25 kg / m^2. At the time of feeding, the flow of water is shut off for 10 minutes so that the feed given does not carry water flow (Masrurotun et al., 2014).

5) Water Management

Silkworm maintenance is carried out with a recirculation system where water continues to flow 24 hours a day. Measurement of temperature, pH and dissolved oxygen parameters is done every 7 days in the morning, while measurements of nitrite, nitrate, and ammonia are carried out at the beginning and end of the study. Water discharge in the culture medium is regulated so that it remains at a speed of 0.35 liters/minute.

6) Harvest

Harvesting is done on the 21st day when the worm's growth reaches its peak. Silkworm harvesting is done by turning off the flow of water for 1 hour before the harvest process so that the worms come to the surface and form a colony.

Data analysis

1) Absolute Weight Growth

Absolute weight growth was calculated based on Effendi (1997),

$$G = Wt - Wo$$

G = absolute growth (g),
Wt = Average Final Weight (g)
Wo = Average initial Weight (g)

2) Productivity

Silkworm productivity was calculated based Alatorre-Jacome et al., (2012)

$$\text{Productivity} = \frac{BT}{L}$$

Produtivity = Productivity Biomass (kg / m^2)
BT = biomass at the end of maintenance (kg)
L = area of container surface (m^2)

3) Proximate analysis

Ash Content Analysis (AOAC 923.03, 1998), analysis of protein content (AOAC 960.52 in 1998), the analysis of Water Content (SNI 01-2891-1992), Fat Content analysis (SNI 01-2891-1992), Carbohydrate Content analysis 100% - (moisture + ash + levels of protein + fat + fiber content).

4) Statistical analysis

Analysis of the diversity of the data carried by T test (T-test) for comparison between two treatment. Several software such as Ms. Excel, and SPSS 25 was used to analyze the data.

Parameter Test

Test of parameters was observed and measured in the study of which is test bacteria *Salmonella sp.* tetracycline antibiotics, growth in absolute weight, productivity, proximate testing, and quality of water. Testing of bacteria, *Salmonella sp*. (SNI 01-2332-2006)

RESULTS AND DISCUSSION

Testing of *Salmonella sp.*

Starch Sulfuric Acid (SSA) changed color from pink to yellow and there are black colonies. According to Zaraswati (2006) the SSA media that experienced discoloration and black colonies marked the presence of *Salmonella sp.*

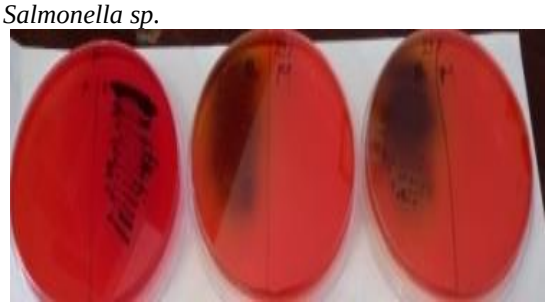

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Figure 1. Cultures of *Salmonella* sp, (a) Scratches on SSA, (b) Color Change Media SSA from Red to Yellow.

Biochemical observations made using TSIA showed the formation of H_2S and CO_2 gas in a test tube where it appears that the media so that the tube was raised. Based on observations by using Test Triple Sugar Iron Agar (TSIA) test showed a change in color to black so that it shows that there is H_2S in the tube, as the results obtained by Afriyani et al., (2016) and Arifah (2010).

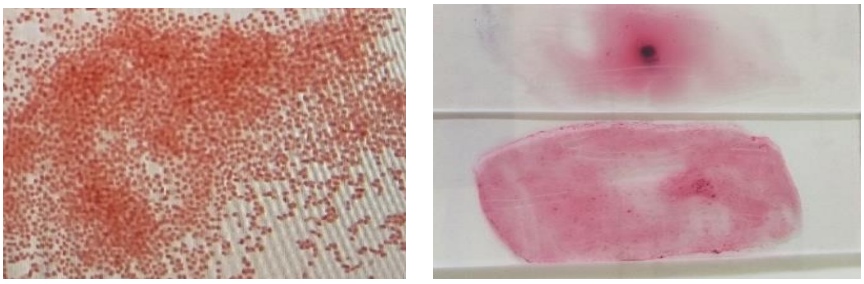

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Figure 2. Gram-Negative, (a) The form of bacterial stems, (b) Staining of the sample.

Based on observations of samples of silkworms (*Tubefex s*p) cultivated using chicken droppings show the presence of pink areas (Figure 2). This showseds that the detected bacteria included in the bacteria was (Gram-negative). Microscope observations with an enlargement of 1000 lux of bacteria are marked with a stem and are pink. These result are similar to those reported by previous studies (Afriyani, et al., 2016; Amiruddin et al., 2017; White et al., 2001; Yuswananda, 2015). Fish larvae given silkworm feed contaminated by *Salmonella sp*. can be dangerous to human health with a characteristic feature of diarrhea, abdominal cramps, fever 8-72 hours (Srigede, 2015). According Bhunia (2008),Salmonella sp. can spread through the air, seawater, freshwater, brackish water, and soil sediments contaminated by dirt and feces. Salmonella sp. can live in water sediments for a long time, especially if environmental factors support the life of the bacteria.

Tetracycline antibiotics

The results of research tests on cultivated silkworms using chicken manure media with positive fermentation and non-fermentation methods containing antibiotic residues of tetracycline of> 32 Ppb, Lukistyowati & Syawal (2013) stated that the accumulation of antibiotic residues in organs or tissues occurred due to low supervision in use of antibiotics. Regulation of the Minister of Maritime Affairs and Fisheries of the Republic Indonesia Number PER. 19/MEN/2010 concerning controlling the quality assurance system and safety of fishery products, as well as the application of Good Fish Culture Methods (CBIB). Antibiotics in animal husbandry are used to prevent and treat disease and become additional substances in the feed, but in giving the wrong dose will cause the chicken to be exposed to residues. According (Saniwati & Agustina, 2015), there were no tetracycline antibiotics in the results of the study because the farmed broilers were not fed after approaching slaughter. This results in antibiotics being consumed out together with feces. The maximum residue limit set by SNI No. 01-6366-2000 is a maximum of 0.1 mg / L.

Absolute growth of silk worms

The results showed that the use of fermentation and non-fermentation was significantly different.

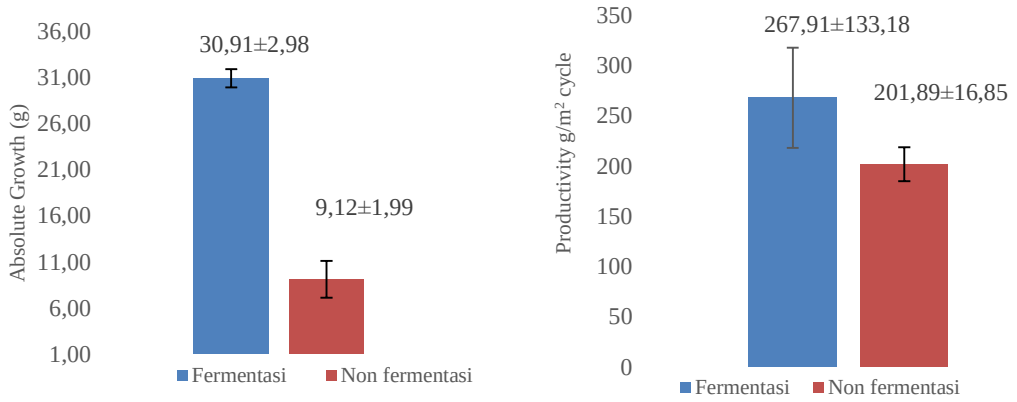

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Figure 3 Average absolute growth of silkworms in each treatments (a) Productivity of silk worms in each treaments (b).

The highest average absolute growth of silkworms is 30.90 ± 2.98 g / m^2 in the fermentation media. Whereas the non-fermentation media showed an absolute growth yield of 9,11.46 ± 1.99 g / m^2 (Figure 3a). The results of this study indicate that the absolute growth in the fermentation media is higher than the results of the study by Kusumorini et al. (2017) which is 17.32g (figure 3b). Productivity in the study showed that silkworm cultivation in the fermentation media was 267.91 ± 113.18 g/m^2 / cycle and the non-fermentation productivity was 201.89 ± 16.85 g/m^2/cycle after 21 days of maintenance.(Muria, 2012) Agustin's research results (2016) showed that the value of productivity was lower at 99.88 g / m2 for 20 days maintenance using chicken manure media.

Nutrient Organic Materials

Table 1. Proximate values media raw silk worm in each treatment.

Treatment	Proximate content (%)				
	Ingredients	Protein	Fat	Ash	Carbohydrate
Non-Fermentation	Tofu	12,75	3,3	3,41	80,0
	Bran	2,43	0,5	19,25	68,93
	Chicken manure	8,77	0,75	48,35	33,34
Fermentation	Tofu	14,14	1,66	3,3	9,23
	Bran	2,4	1,11	9,65	35,04
	Chicken manure	9,7	0,79	20,25	17,54

The results of the proximate analysis found differences in the nutritional value of each organic material (Table 1). The nutritional value of protein and fat is found in fermented tofu waste which is 14.14% (protein) and 3.3% (fat), while the highest nutritional value is shown in non-fermented tofu waste by 80.04%. The highest ash percentage was seen in chicken manure, which was 48.35%. The nutritional value of aquaculture worms is shown in Table 2

Table 2. Proximate test results on silk worms in each treatment.

Treatment	Proximate Test			
	Protein (%)	Fat (%)	Ash (%)	Carbohydrate(%)
Fermentation	42.8	2.7	1.89	3.36
Non-Fermentation	40.67	1.33	2.09	5.49

The highest silkworm test results were seen in the treatment of fermentation 42.80%, and 40.67% while the results of non-fermentation. The results in this study are slightly higher than the results of the study of Muria (2012) who suggested that the *Tubifex sp*. has 41.1% protein content and 20.9% fat. The role of silkworms as larval feed cannot be replaced by artificial feed.

Water Quality of silkworm cultivation.

Table 3. Water quality parameters result study and followed by several references.

No.	Parameter	Result study	Range the result by reference	
1.	Suhu (˚C)	25.80-26.70	23 - 27	(Hossain et al., 2011)
2.	Power of Hydrogen (pH)	6.70-7.20	6.50-8.00	(Pursetyo et al., 2011)
3.	Dissolved Oxygen (mg/L)	3.50-4.20	2.50-7.00	(Utami, 2018)
4.	Nitrite (mg/L)	0.06-0.26	1.00-0.30	(Suharyandi, 2012)
5.	Ammoniac (mg/L)	0.30-1.30	1.94-2.22	(Anggaraini et al., 2017)

CONCLUSIONS

Manufacture of fermented and non-fermented for silkworm growth with positive chicken manure material containing *Salmonella sp.* and tetracycline so that chicken manure is not recommended in the manufacture of silkworm cultivation media.

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