Broiler Supplement by Optimizing the Growth of Local Microalgae in Various Wastewater

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Abstract. The variety of contents of the growing medium has become a factor to determine the quality of microalgae. Medium derived from the extract of bean sprouts waste, wastewater of tofu industry, wastewater of tempe industry, and wastewater of poultry feces have been tested to observe the growth of local microalgae. The culture placed in 250 ml Erlenmeyer flask aerated and lighted with a 40 watt TL lamp. Every type of waste with 6 concentrations was tested to see its growth. The observation has been conducted to observe population density within 20 days. The observed parameters are dry weight, protein, fat, Vitamin A, C, and E. All of them were tested at the end of the experiment. The result shows that the wastewater of tempe industry with a 4% concentration is the optimum medium for growing local microalgae. The population density was 1.02 x 104 cell/ml and attained on the 10th day of the experiment. The highest dry weight is bean sprouts extract, which is 13.03 mg/l. The content of crude protein ranging from 58.23 % - 63.07%, crude fat ranging from 20.88% - 21.56%, Beta carotene ranging from 6.3 mg/gram – 8.0 mg/gram, Vitamin C ranging from 2.24 mg/gram – 2.62 mg/gram, and the content of Vitamin E is 0.8 mg/gram – 1.14 mg/gram. It can be declared that the wastewater of tempe industry can be used as a medium for growing local microalgae. Local microalgae biomass can be the candidate of feed supplement for broiler since it contains nutritions.

Keywords: *local microalgae, wastewater, feed supplement, broiler*

1 Introduction

Microalgae or phytoplankton as microscopic algae are unicellular, photosynthetic microorganisms with grow in fresh or sea water[1]. Biomass produced in photobioreactors can be used for several purposes, including food and feed supplement, fertilizers, biogas, substrate, biofuels, which can be converted into packaging materials, and have the advantage of being renewable [2]. Microalgae have been widely developed in the food industry as supplements because it is rich source of nutrients and biologically active substances, including protein, amino acids, microelements, vitamins, antioxidants, carotenoids, and n-3 long-chain polyunsaturated fatty acids (LCPUFA n-3), they have a long history of application as a food for humans [3].

Several studies have shown that supplementation of microalgae on the layer can increase the DHA content of egg [4]. Similarly, supplementation of microalgae on the layer diets has been

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shown to reduce cholesterol levels of eggs[5]. Microalgae can increase endurance and act as biological antibiotics [6]. Microalgae has great potency as chicken feed, as when consumed by chicken would reduce cholesterol level in an egg and increase carotene pigment of egg yolk [5]. To grow microalga in the laboratory used nutrients from chemicals that cost relatively expensive if microalgae applied as a feed supplement for livestock is inefficient. Microalgae can grow well in wastewater because it can utilize CO2 from water and natural nutrients, growing well in the pH range of 5.2-8.3 with an optimum pH of 8.0-8.5 [1].

Local microalgae are able to grow in various wastewater and are instrumental in the CO fixation process. It has proven to be an economical method for wastewater treatment [7]. It has previously been reported that microalgae showed a significant increase in growth rate in a liquid medium containing peptone as an organic nitrogenous [7]. Some industrial food waste such as tempe, tofu, and chicken farm wastewater contain organic nitrogen which may also be utilized microalgae growth [8].

Tempe and tofu industry is very much in Indonesia. The consumption level of tofu in Indonesia reached 7.4 kg/person/year. Industrial tofu wastewater in large quantities. every 80 kg tofu produced, 2610 kg waste produced. Data obtained from BPPT (Agency for the Assessment and Application of Technology) [9]. The Tofu wastewater produced contains some organic materials which mostly consist of proteins and lipids about 40-60% (226.06 mg/L to 434.78 mg/L) and another compound which is carbohydrate (25%-50%), and fat (10%). The wastewater also contains nitrate and phosphate[9]. This led to a possibility for microalgae to grow in industrial wastewater medium, to produce microalgae massively then applied to livestock.

Problems in the livestock industry are the high price of rations feed, cholesterol and provision of uncontrolled antibiotics in poultry feed [5]. Suspected residue left behind in livestock products that is on meat and eggs [10]. Antibiotics are commonly used in the poultry industry for the treatment and prevention of respiratory diseases and other bacterial infections; often administered to groups of poultry via their drinking water [6].

Since 1 January 2012 the UK poultry meat industry has adopted voluntary measures proposed by the British Poultry Council on the use of antibiotics classed by the World Health Organisation as critically important for human health[6]. Successful antibiotic-free poultry production requires understanding producer and consumer perspectives including marketing, regulations, and science[6].

Microalgae can use chemical agents in several organic and inorganic waste due to its ability to conduct photosynthesis [9]. Wastewater of certain food products such as tempe wastewater, wastewater of poultry feces can be reused as natural media for growing microalgae. this study aims to determine the optimization of the growth of local microalgae in various wastewater as a medium and its quality as a feed supplement for the broiler.

2 Material And Method

2.1 Material

Local microalgae were collected using plankton net from chicken farm waste in NagariMungka, Lima Puluh Kota District, West Sumatra.

2.2 Method

2.2.1 Isolation.

Samples containing local microalgae are isolated and purified in laboratories.Isolates are cultivated in Erlenmeyer fed with Bold Basal Medium (BBM). Lighting uses a 40 watt TL lamp with a temperature of 23ºC and is aerated. Wastewater is used as a nutrient for mass production. Microalgae the research was conducted in Biology Laboratorium of Polytechnic Agricultural, Payakumbuh.

2.2.2 Growing Optimizing And Population Of Microalgae.

The first step is rejuvenation 2 ml of microalgae isolates were cultured in Erlenmeyer containing 500 ml BBM. The media is provided with aeration and lightning uses 40 watt at 23o C and is aerated. The culture can be used as the seed after 5 days for semi mass reproduction. The second step is to test microalgae growth in wastewater medium. The medium of culture filled with well water sterilized by using an autoclave. Every medium contents with nutrition of bean sprout extract (M1), tofu wastewater (M2), tempe wastewater (M3), wastewater of poultry feces (M4) with different concentration, which are: 1%, 2%, 3%, 4 %, 5 % dan 6 % and BBM (M5) used as comparison. The seed of local Microalgae with initial population number is 20.000 cell/ml poured into each medium and kept in a room with 23o C, using lighting from TL 40 watt lamp with continuous aeration. The observation was conducted for 20 days. A number of the cell was counted using Sedgwick rafter under a microscope.

The research is set in Factorial Complete Random Sampling with 4 main treatments that are 4 types of wastewater. The second treatment is concentration of nutrition which are 1%, 2%, 3%, 4 %, 5 % dan 6 %. The growth of algae was analyzed with the microalgae growth curve generated based on data derived from a number of cells per time unit. Analysis of a variety test used to find out the optimum concentration of local microalgae growth. Optimal growth was measured by comparing the value of relative growth between treatments. Relative growth was measured by using relative growth formula:

$$k = \frac{\ln Nt - \ln No}{t} \qquad (1)$$

Explanatory remarks:
Nt: number of the cell after time period t (peak)
No: number of inoculation cell at t = 0
t: time (day)
k: relative growth

2.2.3 Quality Of Chemical Contents Of Local Microalgae.

Determination of protein level. Kjeldahl semi-micro method applied to determine protein level. Sample weighed as mus as 0.1gr and poured into 100ml Kjedahl tube along with 2gr catalyst and 2.5mL high concentrated H2SO4.

The solution was heated on a water furnace for an hour and cooled at room temperature. After that, it was poured into the distillation tube and added with 15mL NaOH 50% and 10mL aquades. It was distilled for 10mL distillate remains. Destilat mixed ith 10mL 20% boric acid

solution indicator mixture of green Bromkresol and red metal. This solution then titrated with HCl 0.1N solution.

Determination of fat level using Soxhlet Method (SII 2453-90). One gram sample wrapped in the paper cover which layered with cotton. Both of its ends closed with cotton. Then, it dried inside the oven at temperature 80oC for 1 hour. The dried sample then inserted into a soxhlet tool connected with a fat tube containing dried heating stone and its weight measured. The next step is to extract it with hexane for about 6 hours. Fat extract dried in an oven at 105oC, then cooled off and weighted.

Determination of the level of Vitamin A, C and E. Vitamins A, and E analyzed separately by chromatography. The sample was saponifiable. Compound that can not be saponifiable was extracted using chromatography alumina and separated using chromatography magnesia. Then, it was analyzed using spectrophotometry.

Vitamin C analyzed using Iod titration method. 10ml filtrate was poured into a 100ml Erlenmeyer. Then, add 20ml aquades and 2ml 1% starch solution. Conduct titration with Iod 0.01N solution until in turns to blue. Every ml Iod is equivalent to 0.88 mg ascorbat acid.

3 Result And Discussion

3.1 Optimizing Of The Growth Of Local Microalgae.

The result of the study shows that culture grew in bean sprout extract medium (M1), tofu waster water (M2), tempe wastewater (M3), wastewater of poultry feces (M4) and BBM (M5) produced a different population of the cell. The highest population was in tempe wastewater (M3) amounting 10139.77 cell/ml at 4% concentration on the 10th day. The increase of medium concentration for more than 4% did not increase the cell population. In fact, it tends to decrease. The illustration of relation between media variation and concentration is shown by Tabel 1 and Figure 1.

Tabel 1. Population of Microalgae (10^3)

Medium	Cell Population											
	K1		K2		K3		K4		K5		K6	
M1	3.03	d	4.75	bc	1.24	e	8.93	b	7.81	b	3.71	d
	E		C		F		A		B		D	
M2	2.71	e	3.02	d	4.15	c	4.21	e	2.97	e	3.42	d
	C		C		A		A		C		B	
M3	9.99	a	8.24	a	8.14	a	1.01	a	8.38	a	8.13	a
	A		B		B		A		B		B	
M4	5.21	b	5.02	b	2.81	d	5.86	c	6.51	c	6.40	b
	C		C		D		B		A		A	
M5	3.97	c	4.64	c	5.48	b	4.95	d	5.09	d	5.05	c
	D		D		C		B		A		B	

Remark :

1. Letter A-F in a row indicate the influence of concentration
2. Letter a-e in a collom indicate the difference in medium

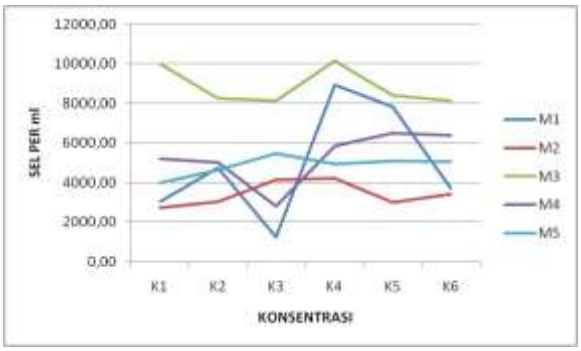

Figure 1. The relation between medium variation and concentration against cell population

The cell population increased up to the 10th day of observation. The highest increase of cell population in tempe wastewater (M3) indicates that microalgae cell content nutrient insufficient amount to support its growth such as carbohydrate, protein, and fat required as energy source for microalgae. It is possible that wastewater medium content several vitamins playing role as a growth factor in algae growth. The population at peak time was reached by using tempe wastewater (M3) with concentration by 4%. The lowest population was by using tofu wastewater (M2).

3.2 Dry weight of Microalgae

Media of bean sprout extract produces highest dry weight which is 13.03 mg/l while tempe wastewater was 12.68 mg/l, followed by waste water of chicken feces at 11.59 mg/l, 8.22 mg/l for tofu waste water and 8.19 mg/l fir BBM.

3.3 Nutrient contents of Microalgae

Result of analyzing nutrient contents of Microalgae at the laboratory presented in Table2.

Table 2. Nutrient Contents of Local Microalgae

Composition	M1	M2	M3	M4	M5
Protein %	61.62	58.23	63.07	62.65	60.27
Fat %	21.33	21.56	20.88	21.37	21.31
			Vitamin: mg/g		
Betacarotene	8.0	6.3	7.0	6.9	td
C	2.62	2.24	2.62	2.56	td
E	1.14	0.80	1.02	0.80	td

Table 2 shows that content of protein in Microalgae is 58.23 % -63.07 % , fat 20.88 % - 21.56 %. The protein was the highest content found Microalgae cultured in tempe wastewater which is 63.07%. The contents of beta carotene, vitamin C and Vitamin E are higher compare to Microalgae cultured in bean sprout wastewater.

Local Microalgae, can grow on the wastewater medium and optimum in tempe wastewater. The differences in the quality of fat, carbohydrate and protein levels indicate the variation of the nutrient content of each wastewater medium. Therefore, cultivating local microalgae on different mediums, yields different supplemental qualities

4 Conclusions

Wastewater such as tempe wastewater, tofu wastewater, and farm wastewater can be used as a medium to grow local microalgae because it still contains nutrients for its growth. In this study, tempe wastewater is the optimum for the growth of local microalgae and also its nutritional content, especially protein. Local microalgae that grow on tempe wastewater medium can be recommended to be a natural supplement for the broiler. Medium tempe wastewater is cheaper so it is easily applied to breeders.

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