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The Optimizing of Growth and Quality of *Chlorella vulgaris* as ASUH feed supplement for Broiler.

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***Abstract*— Chlorella is spherical single celled freshwater micro-algae. Chlorella has potentials as element of ration or natural ASUH (safe, healthy, whole and halal) feed supplement for it contains nutrition and active component, decreases cholesterol level and resulting darker yolk. Chlorella vulgaris is type of green algae which, its economical potential need to be revealed. Variety of components of growing media is one of factors determining quality of microalgae. In terms of mass production, it is important to find correct, cheap and easy to feed nutrition for breeders. The objective of the research is to find out the optimizing of growth and quality of Chlorella vulgaris as ASUH feed supplement for broiler. Test using sedgwick rafter method conducted to find out the optimizing of growth and quality of Chlorella vulgaris while AOAC method applied to test quality of its nutrition. The result shows that. Chlorella vulgaris grew well at technical medium 10 % of Phyto-s, crude protein 57.63%, fat 5.84%, b Carotene 6.44 mg/gram, Vitamin C 4.12 mg/gram and vitamin E 1.32 mg/gram. We can say that Chlorella vulgaris potential to be natural and ASUH feed supplement and Phyto-s can be used as nutrition for mass production.**

***Keywords*— asuh, Chlorella vulgaris, feed suplement, phyto-s**

I. INTRODUCTION

Feed quality for food safety. To produce broiler chicken meat that fulfill the criteria of ASUH (safe, healthy, whole and halal) for the community, broilers should be fed with a quality ration that free from chemical residues.

The poultry sector faces expensive ration price as its main problem. Conventional ration is not easy to get and expensive. Other problem facing by the poultry is consumer's anxiety of poultry product such as broiler meat containing high cholesterol and chemical residue derived from additive component fed to broiler to fasten its growth. Chemical residue can trigger many diseases for people consuming this poultry product.

Microalgae potential to be natural and ASUH (safe, healthy, whole and halal) feed supplement as replacement of additive component since[1] it contains nutrient components (protein, fat, fiber, carbohydrate, vitamins and minerals) and active compounds (phycobilins, phenol, terpenoid, steroid and polysaccharide) which, role as antibiotic and anti oxidant. The result of research shows that [2] anti oxidant activity of local microalgae isolated from pond water has inhibition ability against free radical with concentration 80 µg/ml which, equal to 6 µg/ml synthetic vitamin C.

Microalgae is proven potential as ration for chicken, reduce cholesterol level in an egg and resulting darker yolk as impact of increasing carotene pigment [1]. Experiment of giving local microalgae to broiler as much as 1gram/kg of ration gave better performance and increase ration efficiency to 17.38% [3].

Chlorella is a green spherical single celled fresh water microalgae (smaller than human red blood cell) . approximately 60 % protein including 19 amino acids and all eight amino acids considered to be essential for man. More than 20 bio-avaiable vitamins and minerals, especially rich in beta –carotein and vitamin B12 (the plant source of B12). Beta-carotene is very easily converted into vitamin A. Beta-catene also act as a powerful antioxidant without having been converted into vitamin A [4].

Composition and nutrient of microalgae influenced by some factors such as nutrition difference, environment and growth phase [5]. Variety of components of growing media is a factor determines the quality of microalgae. There is difference of protein contained in microalgae grew in several waste water medium as its nutrition [2].

Chlorella vulgaris is type of green alga, *Chlorella vulgaris* isolated from pond water near to poultry farm in Nagari Mungka, Limau Puluh Kota District. Its economical potential has not yet been identified. It is hoped that *Chlorella* can be developed as ASUH feed supplement for poultry. For massive production, the use of chemical material Pro Analys (PA) is not economic. Therefore, we need to find cheaper and easy to apply fertilizer as alternative. The research aims to identify optimizing of growth and quality of *Chlorella vulgaris* as ASUH feed supplement for broiler.

II. MATERIAL AND METHOD LAYOUT

A. Material

Chlorella vulgaris is isolated from fresh water pond near chicken farm in Nagari Mungka, Lima Puluh Kota District, West Sumatra. After purification process completed, isolates was grow in 500ml erlenmeyer under the light from TL 40 watt lamp and at 23^{o}C temperature with aeration. Bold Basal Medium (BBM) served as nutrition while medium Phyto-s used for semi-mass production.

B. Method

1) Growing Optimizing and Population Determination

The seed of *Chlorella vulgaris* with initial population number is 20.000 cell/ml poured into 500ml erlenmeyer tube containing sterilized water from well and fertilized with modified Phyto-s (consist of Urea, ZA, SP36, sugar, vitamin B1 and B12). For testing growth level 4 concentration of Phyto-s fertilizer was given and BBM fertilizer used as comparison. The treatment was repeated 4 times and each culture kept in a room with 23 ^{o}C temperature using lightning from TL 40 watt lamp with continual aeration. Observation was conducted for 5 days. The count of cells was done by using sedgwick rafter under microscope with the help of hand colony counter.

2) Research Design and Data Analysis.

The research is set in Complete Random Sampling with 4 treatments and 1 control. The treatment is concentration of nutrition which, are 5 %, 10%, 15% and 20%.

3) Data Analysis

The optimizing growth of algae was analyzed with microalgae growth curve generated based on data derived from per time unit. Quantitative data collected was transformed into a graphic. Analysis of variety test used to find out the optimum concentration of *Chlorella vulgaris* growth. These data can be used to calculate relative growth of culture. Formula of relative growth [6].

$$K = \frac{\text{Log (Nt/No)}}{\text{Tt - To}} \times 3{,}22 \qquad (1)$$

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

4) Culture of Algae

Semi mass culture was conducted in Biology Laboratory of *Politeknik Pertanian Negeri Payakumbuh*. It was done to get microalgae samples to analyze nutrition contents such as protein, fat, and vitamin. Semi mass scale culture conducted using 10 liter light trough plastic bag as much as 5 bags. Water from well was sterilized and poured into the bag as 9 liter each and added 1liter of nutrition. Each bag gets fertilizer using the best concentration from first experiment. Initial population is 20.000cell/ml. Sun light was used during the day, while light from TL lamp was used in the night. Continual aeration provided. After reaching the peak of population *Chlorella vulgaris* harvested using filter with 30 μm hole diameters.

5) Quality of chemical contents of Chlorella vulgaris Determination of Protein Level.

To determine protein level Kjedahl semi-micro method applied. Sample weighed as much as 0.1gr and poured into 100ml Kjedahl tube. Then, 2 gr catalyst and 2.5mL high concentrated H_2SO_4 was added. It was heated on water furnace for an hour and cooled. After that, it was poured into distillation tube and added with 15mL NaOH 50% and 10 mL aquades. It was distilled until we get 10 mL destilat and collected in distillate tube containing 10 mL 2% boric acid solution mixed with indicator mixture of green Bromkresol and red metil, titrated with HCl 0,1 N solution.

6) Determination of Fat Level using Soxhlet Method (SII 2453-90)

Sample amounting 1 gr wrapped in paper cover which layered with cotton. Both of its end closed up with cotton. Then, it dried in an oven at temperature not more than 80ºC for about 1 hour and put into soxhlet tool connected with 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.

7) Determination of the Level of Vitamin A, C and E.

Vitamins to be analyzed separated by using chromatography. The principle is that sample containing fat was lathered and compound which is not lathered or vitamins dissolved in water (Vitamin C) extracted by using alumina chromatography. Then, both of it were analyzed using spectrophotometer.

III. RESULT AND DISCUSSION

A. Optimizing the growth of Chlorella vulgaris.

Requirements of microalgae for supplement of livestock ration are containing nutrients and able to be produce in mass scale. Optimizing of microalgae growth signify by population rate of microalgae at treatment medium. Average population of microalgae at treatment medium in the first 10 days were shown in Table 1.

Table 1. shows average population *Chlorella vulgaris* which, the highest is at medium of 10% concentration of Phyto-s and it show significant difference from which with BBM. It means that Phyto-s 10% medium is suitable for

growing *Chlorella vulgaris*. The increasing of Phyto-s nutrition concentration definitely do not increase microalgae population. The characteristic of growth determines time to harvest. Table 2 shows the characteristics of relative growth and time to reach population peak. Rate of initial growth shows that microalgae adapting to the culture quickly [7].

TABLE I
CHLORELLA VULGARIS POPULATION GROWTH AVERAGE

Day	Concentration				
	P 5%	P 10%	P15%	P20%	MBB
0	20000	20000	20000	20000	20000
1	50396	47531	40221	28552	96178
2	83471	78991	68471	35134	131365
3	120536	124531	95116	87461	136324
4	149273	156443	123915	102415	142765
5	168214	203103	147116	201576	143016
6	307315	474441	394817	437403	498403
7	605972	715271	696138	649394	703518
8	719113	901021	701153	**741952***	**796648***
9	**917541***	973205	781163	736837	694117
10	903331	1059641	**819663***	681900	683816
11	858341	**1078392***	807439	582734	528762
12	857642	989740	800692	496816	500751
13	715406	887169	624615	376548	328621
average	462611 b	550677.1 a	437179.9 c	369908.7 d	386020.3 d

Note: * the peak density of cell of *Chlorella vulgaris*

TABLE II
RATE OF RELATIVE GROWTH

Day	Type of Medium			
	Phyto-s 10 %		BBM	
	Number of cells/ml	k	Number of cells/ml	k
0	20000	-	20000	-
1	47531	1.211	96178	2.196
2	78991	0.710	131365	0.436
3	124531	0.637	136324	0.052
4	156443	0.319	142765	0.065
5	203103	0.365	143016	0.002
6	474441	1.186	498403	1.746
7	715271	0.574	703518	0.482
8	901021	0.323	796648	0.174
9	973205	0.109	694117	-0.193
10	1059641	0.119	683816	-0.021
11	1078392	0.024	528762	-0.360
12	989740	-0.120	500751	-0.076
13	887169	-0.153	328621	-0.589

Figure 1 and 2 depict the curves of growth and relative growth of *Chlorella vulgaris* cultured in Phyto-s 10% and BBM medium.

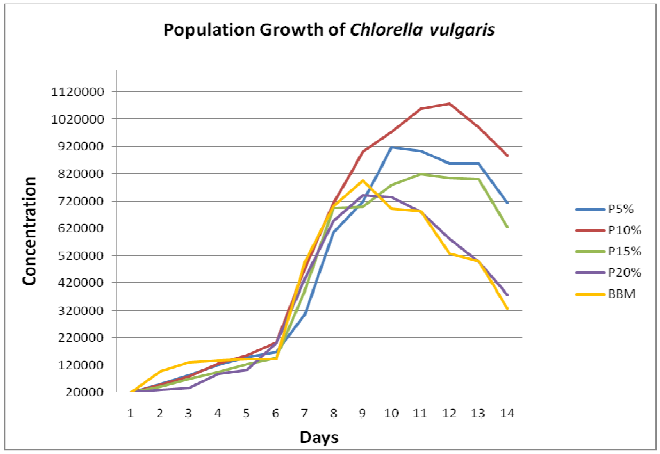

Fig. 1. Population growth of *Chlorella vulgaris*

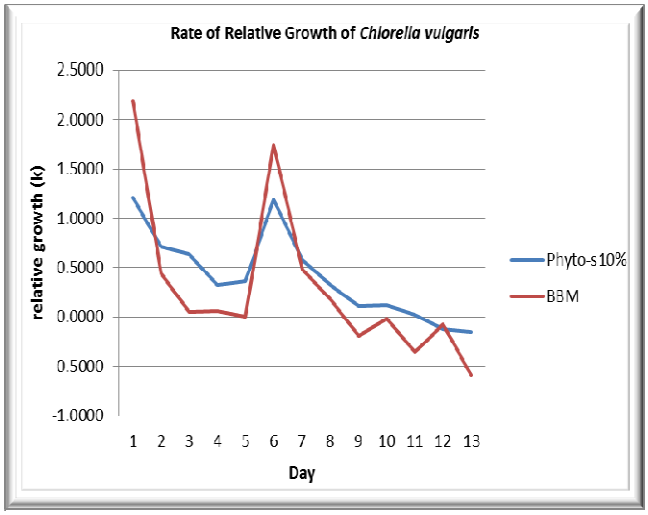

Fig. 2. Relative growth of Chlorella vulgaris.

Result of observation of the growth depicted in the curve of population growing at difference nutrients concentration shows that the higher nutrient concentration, the slower its growth. It shows that high nutrient concentration can inhibit the growth of *Chlorella vulgaris* . Figure 1 depicts that microalgae grow fast in the first and third day in BBM but it grew slowly after that. This fact may be caused by the availability of nutrient needed at initial phase (lag) is higher in BBM rather than in Phyto-s 10%. However, at the growing phase (log) nutrient in Phyto-s 10% is more sufficient. Microalgae have 4 different phases of growth such as lag phase, log phase, stationer phase and death phase [8]. Fugure 2. shows relative growth of *Chlorella vulgaris.* that grew in Phyto-s 10% and BBM. *Chlorella vulgaris* . grew relatively faster in Phyto-s 10% medium or in other words the growth of *Chlorella vulgaris.* relative fast in technical medium rather than in pro-analys medium. The price of technical medium is cheaper and easy to get and apply by the breeder. It supports the cultivation or production of ASUH feed supplement.

B. Quality of chemical contents of Chlorella vulgaris.

Table 3. display the results of test of level of protein, fat and vitamin of *Chlorella vulgaris.*

TABLE III
ANALYSIS OF NUTRITION CONTENT OF CHLORELLA VULGARIS.

No.	Medium	Crude Protein %	Crude Fat %	Vitamin mg/gram		
				beta Carotene	C	E
	Phyto-s 10%	57.63	5.84	6.44	4.12	1,32
	BBM	33,32	1,74	7,44	2.99	0,89

Based on data inTable 3, the content of protein of *Chlorella vulgaris* cultured in different medium result in different protein level where in Phyto-s 10% medium is 57.63 % and in BBM medium is 33,32 %. We can say that nutrient contents of media influenced the content and composition of nutrition of microalgae. High protein level is one requirement for *Chlorella vulgaris.* to be treated as ration or natural supplement. Based on Table 3 the average level of fat is not that high. *Chlorella vulgaris.* also contains Vitamin A, C and E. stated by [1] that *Chlorella vulgaris.* classified as green algae that is rich with chlorophyll and beta carotene. *Chlorella vulgaris* . can be recommended as raw material of livestock ration or supplement for poultry. *Chlorella vulgaris* . can adapt to in short time and grow faster and easy to be cultured [8].

There is difference in the level of fat and protein in previous research. It shows that different location and culture will result in different level of fat protein. This fact supported by statement [9] that salinity, pH, nutrient compound, temperature, carbon source and light influence the growth of microalgae. Therefore, culturing similar species of microalgae at different environmental condition and place will result in different nutrition composition and contents.

IV. CONCLUSIONS

Based on test of optimizing growth and quality of *Chlorella vulgaris* . we can say that it is the best to give Phyto-s 10% as nutrition for optimal growth *Chlorella vulgaris* . quality of chemical contents of *Chlorella vulgaris* and nutrient composition will be different when it is cultured in different media. *Chlorella vulgaris* . can be treated as supplement for livestock due to its rapid growth and fulfill the requirement of ration supplement.

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