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Research Article

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Abstract.
The price of aqua feed containing *Dunaliella salina* meal was calculated. Also, the formulated feed's protein, lipid, and total carotenoid content were examined. The price of shrimp feed containing *D. salina* meal was higher than commercial feed with better protein and carotenoid content in the feed. This indicated that the inclusion of microalgae meal may increase coloration and growth in shrimp. We recommend using these formulated fish in shrimp aquaculture with necessary investment in microalgae meal and aqua feed production.

Keywords: microalgae, economic, aquafeed, shrimp

1. Introduction

Annual rate of global fish consumption increased 3.1 percent from 1961 to 2017, a rate almost twice that of annual world population growth (1.6 percent) for the same period, and higher than that of all other animal protein foods (meat, dairy, milk, etc.), which increased by 2.1 percent per year. These protein needs for human consumption from the fisheries sector will mostly be met from aquaculture[1]. Therefore, increasing production through aquaculture is necessary to meet these increasing needs in the future[2]. Thus, the provision of high-quality aqua feed is an important factor to be considered for high productivity of aquaculture particularly of those fed-cultured species such as shrimp. One factor that determines the quality of aqua feed for shrimp is the availability of essential nutrients needed for its coloration and growth such as carotenoids[3].

Carotenoids are natural pigments that can give orange, yellow, purple, blue, green color to vegetables and animals. Aquatic organisms cannot synthesize carotenoids in their bodies therefore, it is necessary to obtain supplements from the outside such as through feed[4,5]. One source of carotenoids that could potentially be used as an innovative feed additive for white leg shrimp ((Litopeneaus vannamei) is microalgae. Most species of microalgae produce distinctive products such as carotenoids, antioxidants and fatty acids. Microalgae Dunaliella salina (Dunal) Teodoresco 1905 is well known as a carotenoid producing species[6].

Primarily, D. salina produces β-carotene in addition to other pigments such as chlorophyll and lutein which can be accumulated in very high amounts in some stressful culture conditions such as nitrogen limitations, exposure to high light intensity and salinities. Potential and information on utilization of D. salina meal as a natural pigment enhancer and feed additive in shrimp feed is still limited. Although the cost of producing microalgae meal is still relatively high[6,7,8], but its

$$\%N = ml\frac{HCL\,(sample - blank)}{sample\ weight} \times NHCL \times 14,008 \times 100$$

potential to increase the nutritional value such as protein, lipid, carotenoid pigments for shrimp coloration may be compensated for these advantages[6]. Therefore, a price analysis of shrimp feed containing D. salina meal is necessary to estimate the price of shrimp aqua feed based on the ingredients price in the formulated feed. Additionally, proximate composition of the feed was also determined in this present study.

2. Material and Methods

Aqua feed was formulated for white leg shrimp with various concentration of D. salina meal (Table 1). Protein, lipid and carotenoid content of these formulated feed were analyzed[9,10,11].

Protein content of the feed was determined using Kjedhal method[9]. Pre-crushed feed samples were weighed to 1 g and inserted into the Kjeldahl pumpkin flask. 7 grams of K2SO4 (potassium sulfate) and 0.8 grams of CuSO4 were added to the Kjeldahl flask containing the feed sample and then 12 ml of H2SO4 was add prior to extraction. The destruction process was carried out in the acid chamber by heating the sample on the Kjeldahl pumpkin using an electric stove until it turned tosca green. The Kjeldahl pumpkin was the cooled down for 20-30 minutes before adding 25 ml distilled water to the Kjeldahl pumpkin. After that 50 ml of 40% NaOH and a few boiling stones were added to the Kjeldahl pumpkin. Erlenmeyer flask to collect the distillate from the distillation process was prepared with addition of H3BO3 solution and 3 drops of a BCG-MR indicator. The distillate was then titrated using a standard solution of HCl 0.1 N until

Table 1: Feed formulation for white leg shrimp.

Ingredients	Percentage (%) in aquafeed				
D. salina meal	0	3	5	7	9
Fish meal	25	25	25	25	25
Shrimp meal	20	20	20	20	20
Soybean meal	15	15	15	15	15
Wheat flour	10	10	10	10	10
Corn meal	8	8	8	8	8
Fine bran flour	12	9	7	5	3
Sago flour	4	4	4	4	4
Fish oil	1	1	1	1	1
Corn oil	1	1	1	1	1
Top mix	4	4	4	4	4
Total	100	100	100	100	100

the solution turned pink. The same procedure was performed to calculate %N blanks (the sample was replaced with distilled water). Feed protein content was calculated using the following formula:

% Crude Protein = %N × 6.25 (2)

The feed lipid content was extracted using the Soxhlet method[10]. Pre-crushed of 1-2 g (A weight) of feed samples was put into a thimble made of filter paper and then covered at the top using a fat-free cotton swab and the end of the thimble was folded tightly then inserted into a micro tube Soxhlet. The lower end of micro Soxhlet tube was then connected to pre-drained and pre-weighed lipid flask (weight B). The top of the Soxhlet micro extractor was connected to the back coolant that has been strung together on the water bath. After that petroleum benzene was poured approximately 2 times the volume of the tube (±15 ml) and flowed through the end of the back cooling. Extraction is carried out with a time variation of 0.5; 1; 1.5 and 2 hours. As a comparison, extraction was also carried out for 4 hours. The pumpkin flask that contained lipid extract then dried into the oven at a temperature of 105°C and walled in an excavator then weighed to constant weight (C weight). Lipid content was calculated using following formula:

where:

$$\%Lipid = \frac{C - B}{A} \times 100$$

A = Sample Wight (g)

B = Lipid flask weight (g)

C = Lipid flask weight + sample (g)

Total carotenoid content of the formulated feed was extracted according to procedures in Sukarman et al., (2014) (11). The feed was crushed using mortar and pestle and then weighed to 0.04-0.05 g. The feed was put back in mortar and then 5 ml acetone was added and the mix was stirred until out of color. The mixture then was inserted into centrifuge tube and centrifuged for 10 minutes at a speed of 3500 rpm until homogeneous. The supernatant was put into the test tube and the remaining pellet was added another 5 ml of acetone and centrifuged for another 10 minutes at a speed of 3500 rpm. The total of 10 mL supernatant was read using spectrophotometer at 380, 450, 475 and 500 nm. The highest yield was the total value of carotenoids used in calculations, using the following formula:

where:

$$TC = \frac{abs\ max}{250} \times \frac{A \times 100}{sample\ weight\ (mg)} \times 100$$

TC = Total Carotenoid (%)

abs = Absorbance

A = Acetone 10 mL

Total price for 1 kg of formulated feed was the sum of all feed ingredients price. Price analysis for each feed ingredients in formulated feed was carried out using following equation:

$$Feed\ price\ (IDR) = \frac{WI\ (g)}{WT\ (g)} \times PI\ (IDR)$$

where:

WI = weight of feed ingredients

WT = Weight of total formulated feed

PI = Price of feed ingredients

3. Results and Discussion

The prices of formulated feed for white leg shrimp ranged from IDR 52,000-100,920 per kg. Higher price was found in formulated feed with D. salina meal inclusion compared to commercial feed (CF) (Table 2).

D. salina is one of the mot cultured microalgae species for its high carotenoid content and mostly used for food production as colouring agent. Therefore, this study is a pioneer in using *D. salina* as feed additive in aqua feed for shrimp aquaculture. The main bottleneck for large scale production of microalgae meal is high production cost from culturing, harvesting and dewatering of the biomass which in turn increase the aqua

Table 2: Total feed price for white leg shrimp based on feed ingredients compared to CF.

Feed Ingredients	Price of feed ingredients (thousand IDR) based on *D. salina* meal inclusion (%)					
	0	3	5	7	9	CF
D. salina meal	0	12	20	28	36	-
Fish meal	6.3	6.3	6.3	6.3	6.3	-
Shrimp meal	37.8	37.8	37.8	37.8	37.8	-
Soybean meal	14.9	14.9	14.9	14.9	14.9	-
Wheat flour	1.5	1.5	1.5	1.5	1.5	-
Corn meal	1.3	1.3	1.3	1.3	1.3	-
Fine bran flour	0.3	0.4	0.3	0.2	0.1	-
Sago flour	0.5	0.5	0.5	0.5	0.5	-
Fish oil	1	1	1	1	1	-
Corn oil	1	1	1	1	1	-
Top mix	0.6	0.6	0.6	0.6	0.6	-
Total Price	65.3	77.1	85.1	93	101	52

feed price with microalgae meal inclusion. Therefore, the current practice in aqua feed industry is using lost cost synthetic feed additives[6]. However, production of microalgae biomass in the tropics is considered more profitable as the availability of sunlight all year round for photoautotroph production of microalgae. These operational modes may reduce the production cost thus enable economic production of low-value food and feed products going down to fishmeal replacement in the future economy[12]. Additionally, factors such as customer preference for natural products can enable algal pigments to penetrate the market[6].

Increasing percentage of D. salina meal inclusion in aqua feed for white leg shrimp was able to increase protein and total carotenoid content of the feed (Table 3). However, at 9 % D. salina meal inclusion, protein content of formulated feed for white leg shrimp decreased but total carotenoid content was the highest. The lipid content of formulated white leg shrimp feed was remaining stable around 9-10% that was similar to lipid content in commercial feed (Table 3).

Table 3: Protein, lipid and total carotenoid content of formulated white leg shrimp feed.

D. salina meal (%)	Protein (%)	Lipid (%)	Total Carotenoids (mL.mg$\cdot^{-1}$)
0	27.25	10.25	7.50
3	24.19	10.07	13.86
5	38.86	10.13	29.50
7	47.07	10.27	39.60
9	32.11	8.78	41.93
CF	22.78	9.14	6.37

This study confirms that *D. salina* may be used as feed additive in shrimp feed to supply carotenoid and at the same time increasing protein content of the feed. *D. salina* is well known for its carotenoid (up to 21.28 ± 2.88 μg.g-1 wet biomass) particularly of β-carotenoid (up to 13 % of dry weight) and protein content (up to 57 % of dry weight) with values higher than soybean, corn, and wheat[12,13]. Furthermore, *D. salina* contains the highest amount of n-3 fatty acids (ALA, DHA) compared to other well-known microalgae species such as *Chlorella vulgaris* and *Arthrospira platensis*[14].

Roles of carotenoids as strong antioxidants in protecting broodstock nutrient reserves and developing embryos from oxidation are reported. Also, this compound is pigment reserves in embryos and larvae for the development of chromophores and eyespots, and as a vitamin A precursor. Carotenoids accumulate in the hepatopancreas during sexual maturation and they are transported in the hemolymph as carotenoglycolipo proteins to accumulate in the eggs as part of the lipovitellin protein during vitellogenesis. Moreover, carotenoids enhance egg quality, perhaps by protecting against the damaging effects of UV radiation or other environmental pro-oxidants[3]. Therefore, supplementation of microalgae carotenoid in feed may increase growth and survival of shrimp juveniles because of its antioxidanto properties.

High production cost of *D. salina* meal can be reduced by culturing the microalgae using natural sunlight in tropics and necessary technology improvements in harvesting using high efficiency harvesting techniques such as centrifugation or flocculation but low cost and energy.

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