Bio-fuel algal waste diet effect on growth and histological structure of Wader Pari (Rasbora lateristriata Bleeker, 1854) intestine

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Abstract. Sufficient availability of good food quality is important for fish aquaculture in order to increase fish production. Microalga is one of alternative protein sources, and one among those is Chlorella sp. Protein content of Chlorella sp. is very high, around 51-58% and consists of various kinds of essential fatty acids, which is important for fish nutrition. Recently, Algae also prospective source of ethanol biofuel. Ethanol extraction usually produce waste material, which still consist of high protein content, which valuable for fish feed sources. Therefore, the study aimed to examine the effects of fish food derived from algae biofuel-waste on growth and histological structure of wader pari (Rasbora lateristriata) intestine. Fish were divided into 5 groups of control negative (no additional protein), feed with waste algae, waste + Fresh algae, fresh algae and commercial fish food, respectively. The fish morphometry, weight, and intestine histological structure were examine. The results showed that fish feed treatment gave effect on fish growth and intestine histological structure. Algae waste feed showed similar performance to commercial fish feed on fish growth and showed no negatif effect on intestine villi length and goblet cells number, evidenced good prospect of algae waste as fish feed.

1. Introduction

Fish cultivation activities have been carried out both intensively and extensively. Wader fish are freshwater fish that have high economic value in terms of marketing and consumer demand [1][2], so it is necessary to conduct cultivation efforts and the availability of sufficient feed. To reduce production costs, one alternative is to use vegetable protein sources, namely microalgae. Microalgae have a very high protein content, it is are also known as single cell protein (SCP). Sources of SCP known to the public are Spirulina maxima and Chlorella vulgaris[3][4].

Fish body weight is proportional to the amount of protein absorbed by the body. The greater the protein content in the feed given, the higher the body weight of the fish [5]. Microalgae contain many valuable compounds such as lipids (2–46%), carbohydrates (8–64%), and proteins (6–71%). Algal waste products possessed high value as source of energy, food and various other applications [6][7][8][9].

Microalgal biodiesel production usually produced high portion of microalgal biomass waste. After lipid extraction for fuel production, around 65% of the microalgal biomass were remaining as left over
waste. Therefore, it is necessary to find appropriate use for micro algal waste after the fuel extraction [10].

The ability of fish to digest feed given is influenced by several factors, namely the chemical nature of water, water temperature, type of feed and the physical physics characteristics of feed digestive enzymes contained in the digestive tract of fish. Intestine is an important organ of the fish's digestive system. Intestine plays a role in the process of absorption of food. This study aims to investigated and compare the growth and histological structure of intestine wader pari (Rasbora lateristriata) treated with algae feed, with different concentrations.

2. Research methods

2.1. Design of the research
This study was conducted with Completely Randomized Design (CRD) comprising of 5 treatments with 3 repetition, of each. The group treatments were conducted as follows:
- P1: Feed I negative control with no microalgae
- P2: Feed II 35% of microalgal waste
- P3: Feed III 35% waste and fresh microalgae mixture
- P4: Feed IV 35% of fresh microalgae
- P5: Feed V Positive control of commercial feed

2.2. Fish acclimatization and maintenance
The samples used in this study were F1 of Indonesian native fish Wader pari (Rasbora lateristriata), which were reared in the laboratory. The research process was carried out at the Laboratory of Animal Structure and Development, Faculty of Biology, Gadjah Mada University.

2.3. Measurement of water quality
Water quality measurements are carried out once a week and the parameters measured are temperature, pH, and DO content is measured at the beginning of maintenance and at the end of maintenance.

2.4. Artificial feed preparation
The artificial feed ingredients were carefully measured to attain 35% of algae content on each feed and 65% of basic feed ingredient consist of 16,25% of cassava flour, 16,25% of corn flour and 32,5% of bran, respectively.

2.5. Fish treatment of feeding trial
Two weeks old fish were randomly stocked into 30 fish/tank. Fish maintained for 10 weeks and fed 3 times a day on the morning of 07.00-08.00, afternoon of 11.00-12.00 and late afternoon of 15.00-16.00, respectively. Each treatment was replicates three times. During the period of treatment, the aquariums were maintained by water replacement every once a week, with the composition of 25% : 75% of old and new water from the total volume in the aquarium

2.6. Survival rate of the fish
Survival rate is the number of fish, which were able to survive after 10 weeks of feed treatment. Survival rate was calculated by using the formula:
\[ S = \frac{N_t}{N_0} \times 100\% \]

\( S \) : survival (%) \\
\( N_t \) : fish number at the end of maintenance \\
\( N_0 \) : initial fish number during experiment period
2.7. Growth performance

The parameters, fish weight and length were measured at every 2 weeks. Formula for the calculation of Absolute Weight Growth (AWG), Absolute Length Growth (ALG), Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER) were as follows:

1. AWG = Wt – Wo;
2. ALG = Lt – Lo;
3. SGR = [(lnWt – lnWo)/t]x100% and
4. PER = (Wt-Wo)/Pi

Wt: End of treatment fish average weight (g), Wo: Initial fish weight (g), Lt: end of treatment fish average length (cm), Lo: initial fish length (cm), lnWt: End of treatment Fish average weight (g), lnWo: initial fish weight (g), t: period of treatment (days) Pi: Amount of feed given x % of protein content of feed

2.8. Histology preparation

Fish intestines were collected for histological observation on the day of 60th. The organs were prepared following standard paraffin method. Prior dissection tricaine anesthesia were applied to the fish, which then sacrificed by dissection. Intestines were fixed with Bouin’s solution for overnight and immersed in 70% alcohol for fixative washing. Tissue dehydration were conducted with ethanol series followed with immersion at toluene solution for tissue clearing. The coupe of intestines was set on 6μm thickness, which be stained with Ehrlich Hematoxylin Eosin (HE).

2.9. Data analysis

Data were analyzed with ANAVA and continued with Duncan's multiple range test (DMRT) with the level of P ≤ 0.05. The intestine histological structure was analyzed qualitatively descriptive by comparing the histological structure of the intestine from each treatment

3. Results and discussion

3.1. Water quality during period of experiment

Water quality monitoring result of temperature, pH and dissolved oxygen were presented in table 1.

| Parameter   | Treatment | Optimal value |
|-------------|-----------|---------------|
| Temp. (°C)  | P1        | P2            | P3            | P4            | P5            |
|             | 27.7-28.5 | 27.6-28.3     | 27.5-28.4     | 27.6-28.3     | 27.7-28.4     |
| pH          | 7.78-8.41 | 7.93-8.44     | 7.9-8.43      | 8.41          | 7.87-8.43     |
| DO (mg/L)   | 6.2-9.5   | 6.2-9.2       | 6.6-9.6       | 6.8-9.6       | 8.1-9.2       |

Note: P1: negative control, P2: microalgal waste, P3: Waste and fresh microalgal mixture, P4: fresh microalgal, P5: commercial feed.

Result showed that water quality was at optimum range for wader pari (Rasbora lateristriata), which usually inhabit temperatures range of 25-33°C, dissolved oxygen of ≥ 5, and the pH ranged from 7-8. Therefore, it suggests that feed performant trial result was not influenced by water quality.

3.2. Fish growth

The study results showed that algae based meal had a significant effect on the fish growth performance, especially in the length and body weight of the fish. Data showed significant differences at some treatment (Table 2; Fig 1). The growth parameters (AWG, ALG, and SGR, and also Average weight, length growth) are shown in table 2 and table 3. Compared to all other treatments, a significant difference
at \( P < 0.05 \) was noted on fish diet P1 (control). However, fish diet P2, was showing best growth performance for the fish. In contrast, the p1 diet, which produced the poorest growth parameters.

**Table 2.** Average weight, length growth and Protein efficiency of wader pari (*Rasbora lateristriata*) during feed treatment

| Treatment | Fish body weight (g) | Fish body length (mm) | Protein efficiency (PER) |
|-----------|----------------------|-----------------------|--------------------------|
| P1        | 0.063±0.03a          | 5.29±2.41a            | 0.0034±0.00015a          |
| P2        | 0.084±0.05c          | 6.23±2.92c            | 0.0055±0.0011a           |
| P3        | 0.080±0.05bc         | 5.87±2.69bc           | 0.0050±0.0019a           |
| P4        | 0.075±0.05abc        | 5.65±2.74ab           | 0.0046±0.0017a           |
| P5        | 0.068±0.04ab         | 5.37±2.57a            | 0.0037±0.00026a          |

Description: Numbers followed by the same letters in the same column show no significant difference at the test level 5%. P2 treatment showed the highest average growth rate of weight and length of 0.084g and 6.23mm, followed by P3 treatment with the average of weight and length that did not differ greatly with P2 treatment of 0.080g and 5.87mm. Meanwhile, P1 treatment showed the lowest average weight and length growth rates of 0.063 g and 5.29 mm.

**Table 3.** The value of AWG, ALG, and SGR as the parameter to measure fish growth during 10 weeks of maintenance with feed treatment

| parameters | P1 | P2 | P3 | P4 | P5 |
|------------|----|----|----|----|----|
| AWG        | 0.093±0.004a | 0.149±0.02a | 0.136±0.05a | 0.124±0.04a | 0.099±0.006a |
| ALG        | 7.09±1.37a   | 9.28±0.40a   | 8.35±0.51a   | 8.19±0.80a   | 8.59±0.64a   |
| SGR (%)    | 0.103±0.005a | 0.166±0.03a  | 0.151±0.05a  | 0.138±0.05a  | 0.111±0.007a |

Description: Numbers followed by the same letters in the same column show no significant difference at the test level 5%. The result suggest that algal waste feed provide sufficient nutrient element for fish growth. These results also showed that *algal waste based fish* meal could be incorporated into wader pari (*Rasbora lateristriata*) diets.

### 3.3. Protein efficiency ratio (PER)

Protein efficiency ratio (PER) was the amount of fish weight produced from each unit of protein in the feed [11]. In fish feed, protein is an important component because of its influence on growth. Insufficient protein levels in the diet have an effect on growth [12]. Protein is also needed for body maintenance, tissue formation, replacement of damaged body tissue and as a source of energy [13]. The protein efficiency ratio (PER) results, which representing feed utilization was presented in table 2 and Figure 1A. Based on the results of the PER calculation, P2 treatment showed the highest PER value of 0.0055 followed by P3 at 0.0050, P4 treatment at 0.0046 and P5 treatment at 0.0037. Meanwhile, the P1 exhibit lowest PER value of 0.0035 compared to all other results, respectively. The statistical analysis showed that the PER value of feed treatment was not significantly different on each treatment (\( P > 0.05 \)) for all diet.
3.4. Survival rate of the fish
The results showed that the percentage of survival in each treatment varied, ranging from 43-77%. Fresh microalgae feed showed the highest percentage of survival rate was 77% and the lowest percentage was positive control at 43%. The result indicated that percentage mortality was higher in the control group that was not fed with microalgae, suggest that microalgae feed performance was outpace the commercial food performance in term of fish survival rate. The result is inline with the previous research [14] on the effects of short term feeding on *Dicentrarchus labrax* post larvae with marine microalgae.

3.5. Histological structure of intestine
Intestine is an organ that plays an important role in various physiological functions and is also a major part of the process of digestion and absorption of food. Figure 2 shows the intestine histological structure consists of 4 layers / tunica which are generally the same as in vertebrates such as mammals, birds, amphibians and reptiles.
The four layers / tunica are the mucosal tunica which consists of epithelial layer, lamina propria and mucosa muscularis; submucosal tunica consists of connective tissue; muscularis tunica consists of muscle layers; Serous tunica is a large part of the intestine[15]. Villi are protrusions of the mucosa (epithelium and lamina propria) and are coated by a layer of columnar epithelium that forms enterocyte cells (absorptive cells) and goblet cells[16].

**Figure 2.** Intestine histological structure of wader pari (*Rasbora lateristriata*) from each treatment by staining Hematoxylin Eosin (HE). 40x magnification. Note: Lumen (L), Villi (Vil), Tunica Mucosa (M), Goblet Cells (GC), Tunica Submucosa (Sm), Tunica Muscularis (Mus), Serosa (S).
Figure 3. Histological structure of wader pari (*Rasbora lateristriata*) intestine from each treatment by staining Hematoxilin Eosin (HE). 10x magnification. Note: Liver (Hp), Proximal Intestine (Prx), Middle Intestine (Mid), Distal Intestine (On).

Table 4. Micrometry data on the length of villas of wader pari (*R. lateristriata*) during maintenance with feed treatment.

| Treatment | Villi length μm    |
|-----------|--------------------|
| P1        | 60.36±8.9          |
| P2        | 138.29±22.2        |
| P3        | 132.68±10.6        |
| P4        | 126.27±10.6        |
| P5        | 100.71±5.2         |

Note: Numbers followed by the same letters in the same column show no significant difference at the 5% test level. The treatments were P1 (protein 5.3%), P2 (protein 17.445%), P3 (protein 26.24%), P4 (protein 13.595%), P5 (protein ± 30%).

The P2 treatment showed the largest villi length is 138.29 μm and the number of goblet cells are 15.67, this indicates that the influence of the protein contained in P2 feed in addition to provide good growth is also resulted in good development of villi. The length size of the villi will expand the surface...
of the intestine to absorb nutrients or food to optimize the fish growth. The results of research on *Satanoperca pappaterra*, showed that intestinal length greatly influences the aspects of digestion and absorption of food as well as the presence of long villi will directly affect the absorption process[17]. The number of goblet cells produced will increase the content of mucus and intestine activity. Intestine walls that are coated by columnar epithelium have many mucus and secrete goblet cells[18]. Columnar epithelial cells from the intestine mucosa also function in the absorption of food.

4. Conclusion

The conclusion of this study is that utilization of microalgae waste on fish feed, can be considered as very promising for fish flour ingredients, since the feed showed good result of fish growth performance and exhibit no negative effect in respect to nutrient utilization or histological structure of the intestine.

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