



Response of fermented *Cladophora* containing diet on growth performances and feed efficiency of Tilapia (*Oreochromis sp.*)

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Abstract

Feed is still contributed the highest in the cost of aquaculture. Fermented *Cladophora* was applied to replace plant-based protein for tilapia. Five different diets, but all are iso-protein, were formulated, substituting 0% (as control) to 40% of plant-based protein with fermented *Cladophora*. The experiment was set up using 15 aquaria, each of 30 l and stocked with tilapia (initial individual size of 3.65 ± 0.26 g) at density of 1 fish l⁻¹. All the fish was fed at 3% of body-weight day⁻¹ and the experiment was ended at day 42nd. The result showed that tilapia fed with 10% of fermented *Cladophora* reached the highest in growth rate, feed efficiency, protein digestibility, and essential amino acid index. The growth rate of this treatment was 30% higher than the control diet (non substitution with fermented *Cladophora*). This means that by changing ingredients in the diet, it may affect in cost reduction or gain in production biomass.

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Introduction

Tilapia is widely cultured in many tropical and subtropical regions of the world. In Indonesia, tilapia production increased for about 134% in the last five years, from 206,904 mt in 2007 to 481,440 mt in 2011 (MMAF, 2011). A High demand of tilapia in Indonesia continues to increase as tilapia is a moderate priced-commodity and therefore it is affordable food fish for domestic consumption. In line with this, the growth of intensive farming in tilapia production is followed by the development of aquafeed or complete diet of this species.

Feed is the single largest expenditure (\pm 60% of total cost) in semi-intensive and intensive fish culture operations. Traditionally, fishmeal is preferred dietary protein source for many farmed fish and is appreciated for its amino acid balance (NRC, 1993). However, the world fishmeal production is not expected to increase further. Several strategies were applied to develop a sustainable and economical aquaculture system including finding alternate sources of high quality protein. Several studies in tilapia have been conducted to evaluate a variety of alternative protein sources such as fishery and terrestrial animal by-product meals (Hossain *et al.*, 1992; Fagbenro and Jauncey, 1994), oilseed meals and by-products (Jackson *et al.*, 1982; Pantha, 1982; Tacon *et al.*, 1983; Robinson *et al.*, 1984; Shiau *et al.*, 1989; Chiayvareesajja *et al.*, 1990; El-Sayed, 1990; Omoregie and Ogbemudia, 1993), aquatic plants (Almazan *et al.*, 1986; Klinnavee *et al.*, 1990; El-Sayed, 1992), single-cell proteins (Davies and Wareham, 1988; Chow and Woo, 1990), and legumes and cereal by-product (Jackson *et al.*, 1982; Wee and Wang, 1987; Martinez-Palacios *et al.*, 1988; Santiago *et al.*, 1988; Olvera *et al.*, 1990). Most studies were focused on plant by-product meals as they are quite rich in protein and favorable amino acid content. However, they are deficient in one or more essential amino acid and contain various quantities of anti-nutritional factors (NRC, 1993).

There is also a vital need to seek effective ingredients that can either partially or totally replace plant

ingredients as protein sources in plant feedstuffs aquafeeds. Algal products can be used to enhance the nutritional value of food and animal feed owing to their chemical composition; they play a crucial role in aquaculture. Green filamentous algae have shown to have low levels of protein (Boyd, 1973), but the very low cost involved in their culturing and harvesting made them potential as cheap sources of protein for fish.

This study aimed to investigate the effect of fermented cladophora formulated in the diets for tilapia on their growth rate, feed conversion efficiency and carcass parameters. The Experiment was set up to test the hypothesis that fish fed with fermented Cladophora-based diets would perform comparable to protein-based diets commonly used in commercial fish diets.

Materials and methods

Fish and experimental conditions

Tilapia (*Oreochromis* sp), with average individual body weight of 3.65 ± 0.26 g were obtained from local hatchery in East Java. The fish were acclimated and maintained in aquaria for approximately 2 weeks. During this period, all fish were given the experimental diets. Upon commencing of the experiment, fish were randomly placed into 15 aquaria (each of 30 l) at an initial density of 1 fish l⁻¹. The fish were manually fed twice a day with the experimental diet at optimum ratio (3% body weight day⁻¹). Every week, fish biomass were weighed to adjust the total feed needed for each aquarium. Water temperature maintained at 27-28°C.

Feed formulation and diet preparation

Prior to its used as ingredient, filamentous green algae, Cladophora, was made clean from contaminants (snails and weeds) and then fermented using EM-4, a local-commercially probiotics. The fermented algal protein source was then dried at 60°C for 48 hours, and ground into powder using a laboratory hammer mill. Five diets, each containing different proportion of fermented Cladophora (Table 1), were prepared by steam-pelleting machine, dried, and stored at -10°C before use. Each diet was assigned randomly to three aquaria, as replicate.

Table 1. Formulation and composition of five experimental diets.

Ingredients	Feed formulation (% fermented <i>Cladophora</i> in the diet)				
	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
Fish meal	30.0	30.0	30.0	30.0	30.0
Soybean meal	21.0	19.0	17.0	15.0	13.5
Fermented <i>Cladophora</i> (FC)	0.0	10.0	20.0	30.0	40.0
Corn starch	14.0	13.0	8.0	5.0	0.0
Rice bran	13.5	8.5	7.0	3.5	0.0
Tapioca flour	10.0	8.0	6.5	5.0	5.0
Fish Oil	4.0	4.0	4.0	4.0	4.0
CMC	3.0	3.0	3.0	3.0	3.0
Vit Mix	2.0	2.0	2.0	2.0	2.0
Min Mix	2.0	2.0	2.0	2.0	2.0
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5
Proximate Analyses (%dm)					
Dry matter	90.6	90.5	90.6	91.4	91.3
Crude Protein	29.9	29.9	29.4	29.2	29.1
Fat	7.2	7.2	7.2	7.1	7.1
Ash	15.0	16.5	18.1	19.8	20.1
Crude Fiber	5.8	5.6	5.6	6.3	6.7
NFE	32.6	31.4	30.3	29.1	28.6
Energy (kcal/g)	3.4	3.4	3.4	3.3	3.3

Sampling and chemical analysis

At the beginning of the trial, a fish was randomly sampled from each aquarium for determination of initial body composition. At the end of the study, all fish were harvested for counting and batch weighing to determine survival rate (SR), final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and protein digestibility (DP).

Dry matter analysis was conducted by drying to constant weight at 105°C, crude protein analysis was conducted by Kjeldahl method (AOAC, 1984), and crude ash by drying muffle furnace at 550°C for 4 hours. Lipid analysis was done with diethyl ether extraction method (AOAC, 1984). Energy analysis was determined by bomb calorimeter. Feed digestibility was measured by adding chromic oxide marker into the diet which gave a green appearance to pellet's feeds. Two hours after feeding, the excess feed and fish feces were removed through siphoning. The Essential Amino Acid Index (EAAI) was calculated using the method explained in Labuda *et al.* (1982); Ijarotimi and Keshinro (2011), based on the equation below:

$$EAAI = \sqrt[n]{\frac{100a \times 100b \times \dots \times 100j}{av \times bv \times \dots \times jv}}$$

where:

n = number of essential amino acids, a, b, ... j = represent the concentration of essential amino acids

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(lysine, tryptophan, leucine, isoleucine, valine, arginine, threonine, phenylalanine, histidine, and the sum of methionine and cystine) in test sample, and av, bv, ... jv = content of the same amino acids in standard protein (%).

Statistical analysis

Results were presented as mean ± standard deviation of three replicates. All data are subjected to one-way ANOVA and Tukey's multiple range tests using statistical software of SPSS v.16 for windows. The differences will be considered significant at p<0.05.

Result and discussion

The Mean Body-Weight of *Tilapia*

The mean body-weight of *tilapia* fed with various diets were presented in Fig. 1. Growth responses of fish for different diets during the experimental period were shown in Table 2. Diet with 10% of fermented *Cladophora* resulted in the highest growth response. It also showed significantly the best (lowest) FCR with the other diets. The apparently poor performance of fish fed with diets containing higher inclusion levels of filamentous algae may be due to several factors. Appler (1985) observed that most of the aquatic plants including algae contain 40% or more of carbohydrate, of which only a small fraction consists of mono- and di-saccharides. The 10% fermented *Cladophora* may reach an optimal ratio

between mono- to poly-saccharides that provide optimal growth.

Table 2. Growth responses and feed utilization of tilapia fed at different levels of fermented *Cladophora* substituting diets.

Parameters	Level (%) of Fermented <i>Cladophora</i> in the diet				
	1 (0%)	2 (10%)	3(20%)	4 (30%)	5(40%)
SR (%)	94.44±1.92 ^a	95.56±1.92 ^a	88.89±1.92 ^a	88.89±1.92 ^a	90.00±1.92 ^a
SGR (% WG/day)	1.13±0.08 ^b	1.51±0.06 ^c	1.23±0.08 ^b	1.13±0.07 ^b	0.99±0.05 ^a
FCR	2.21±0.16 ^b	1.90±0.04 ^a	2.13±0.14 ^b	2.28±0.07 ^b	2.60±0.10 ^c
PER	1.52±0.11 ^b	1.76±0.04 ^c	1.60±0.10 ^b	1.50±0.05 ^b	1.32±0.05 ^a
Protein Retention (%)	18.22±1.09 ^b	22.06±0.41 ^c	18.63±0.49 ^b	17.39±1.29 ^b	14.37±0.46 ^a
Protein Digestibility (%)	74.62±1.94 ^b	78.99±1.25 ^c	74.83±2.22 ^b	74.09±1.18 ^b	71.05±1.46 ^a

Note: symbols after the value indicated statistical difference or P < 0.05.

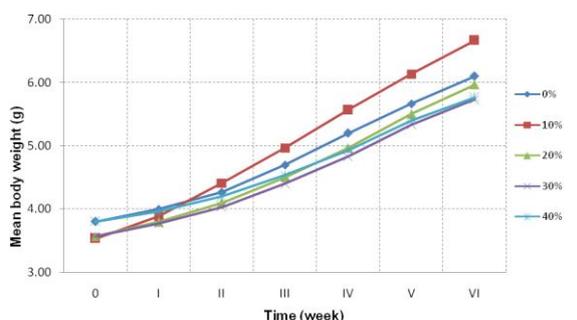


Fig. 1. Mean body-weight of tilapia at the different levels (%) of fermented *Cladophora* in the diets.

Protein Efficiency Ratio (PER)

Diet added with 10% fermented *Cladophora* resulted in statistically the highest Protein Efficiency Ratio (PER). However, adding more fermented *Cladophora* into the formulated diet will reduce the PER, given 40% fermented *Cladophora* the lowest PER. The more the filamentous algae added into the diet, the more difficulty the fish would utilize the protein from feed. However, the protein utilization reached its optimal level at 10% fermented *Cladophora*. This means that different sources of plant-based protein can be used and reach effective utilization at certain concentration. Taking into account the price of soybean is very high compared to this filamentous algae, a new diet for tilapia can be designed at lower price and with potentially provide higher growth response and PER.

Protein Retention (PR)

The protein retention (PR) revealed similar trends decreasing from diet 2 (10%) to 5 (40%). Diet 2 produced slightly higher values for PER and PR than the control (diet 1) but still lower PR (22.06%) than

that of 27% reported for *Cladophora* fed to tilapia (Appler and Jauncey, 1983), which was probably due to the higher protein content of *Cladophora* in the ingredient. Diet 3 and diet 4 produced PER and PR values intermediate between those of diets 1 and 5. This was probably because of its lower protein level (29%) in the diet. Numerous authors have reported increasing PER and PR with decreasing dietary protein level (Ogino and Saito, 1970; Dabrowski, 1977; Mazid *et al.*, 1979; Jauncey, 1981, 1982). The accumulation of reserved lipid was generally well controlled and the reserved lipid was first mobilized into energy prior to muscle protein degradation when fish fed with algae-supplemented diets.

Protein Digestibility

Results indicated that the apparent digestibility for crude protein was significantly affected by the substitution of fermented *Cladophora* in the ingredients. Protein Digestibility was varied from 71 to 79% for all of the diets. Diet 5 (40% substitution) resulted in the lowest digestibility than other diets. The value of 71% for diet 5 is slightly lower than that of 74% reported for carp fed on diet with an algal meal as the sole protein source (Hepher *et al.*, 1978). Decrease in protein digestibility for tilapia was recorded with increases level of fermented *Cladophora* containing diet. The decreases is, however, very pronounced on substitution with digestibility values recorded being reducing up to 11% for level of substitution reaching 40% fermented *Cladophora*. Due to the presence of indigestible filament algal materials, the more algal content in the diet, the poor the digestibility and consequently the

feed utilization for tilapia. Pantastico, *et al.* (1985) reported that newly hatched Nile tilapia fry was able to digest diet from uni-algal cultures of *Euglena elongata* and *Chlorella ellipsoidea*. Both algal culture do not contain celluloses as such in *Cladophora*.

Essential Amino Acid Index (EAAI) and Biological Value (BV)

The availability index for essential amino acids tended to reflect digestibility coefficients for protein among highly digestible diet. The disadvantage of these well-digested high-protein *Cladophora* in the diet compared with full soybean meal was that the essential amino acid content, amino acid profile, and

amino acid availability of these ingredients were inferior to soybean meal.

The essential amino acid index (EAAI) and biological value (BV) of the fermented *Cladophora* were 15.66% and 5.4%, respectively. While the control and fermented *Cladophora* containing diets, the EAAIs and BV were presented at Table 3. From the present study it is observed that the BV and EAAI values were generally low; and these could be attributed to the fact that fermented *Cladophora* is low in protein content and deficient in essential amino acids, tryptophan, compared to that of soybean. Biological value and EAAI more than 80% were stated as a good nutritional quality of the diet (Ijarotimi and Keshinro, 2011).

Table 3. Amino acid composition (mg 100g⁻¹ protein) and Nutritional Quality in the Feed and Fermented *Cladophora*.

Nutritional Quality	Cladophora Fermented Flour	Feed 2 (10%)	Feed 3 (20%)	Feed 4 (30%)	Feed 5 (40%)
Essential Amino Acid					
Arginin	2.39	1.20	1.20	1.19	1.19
Histidin	0.06	0.55	0.53	0.51	0.49
Isoleusin	6.08	0.97	1.02	1.08	1.14
Leusin	2.84	1.43	1.38	1.35	1.31
Lysin	1.87	1.52	1.50	1.47	1.46
Methionin	0.19	0.83	0.80	0.78	0.76
Phenilalanin	1.81	1.13	1.11	1.08	1.06
Threonin	1.75	1.13	1.10	1.08	1.06
Valin	2.39	0.29	0.28	0.27	0.26
Triptopan	Nd*	1.06	1.06	1.06	1.06
Nutritional Quality					
EAAI** (%)	15.66	24.58	24.57	24.56	24.55
BV*** (%)	5.37	15.09	15.08	15.07	15.06

* Nd = not detected

** Essential Amino Acid Indeks

*** Biological value

Water Quality

During the experiment, water temperature was maintained at the levels of 27-28°C, and with oxygen levels of between 5-6 mg l⁻¹. Webster and Lim (2002) found the temperature for tilapia was between 25-30°C, and with oxygen level should not fall below 3 mg.l⁻¹. So, all water quality during experiment were considered optimal for tilapia growth.

Summarizing all the results, it showed that tilapia fed with 10% of fermented *Cladophora* reached the highest in growth rate, feed efficiency, protein

digestibility, and essential amino acid index. The growth rate of this treatment was 30% higher than the control diet (non substitution with fermented *Cladophora*). This means that by changing ingredients in the diet, it may affect in cost reduction or gain in production biomass.

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