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Water hyacinth, *Eichhornia crassipes* (Mart.), leaf as an Alternative Protein Source for Siamese Gourami, *Trichogaster pectoralis*

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Abstract: In the present study, water hyacinth, *Eichhornia crassipes* leaf is attempt to be used as protein source in Siamese Gourami, *Trichogaster pectoralis* farming. Preliminary study revealed that *E. crassipes* leaf possess high protein content (12.1±1.81%). Therefore, further study was carried out to evaluate the potential of the plant as alternative protein source for Siamese Gourami farming.There were five treatments based on different percentage of combination of *E. crassipes* leaf and fish meal namelyT1 (20% of *E. crassipes* leaf + 80% fish meal), T2 (40% of *E. crassipes* leaf + 60% fish meal), T3 (60% of *E. crassipes* leaf + 40% fish meal), T4 (80% of *E. crassipes* leaf + 20% fish meal) and T5 (100% of *E. crassipes* leaf) with three replicates for each treatment. The control group of fish was fed with fish meal. The feeding trial is continuing until four weeks and the liver of fish of each treatment is subjected to histology study in order to evaluate the toxicity level of *E. crassipes* leaf against fish that received treatment. The results of the present study revealed that treatment T1 showed the best performance in term of growth rate and had significance difference ($p < 0.05$) compared to other groups. Furthermore, T1 treatment performed promising result in palatability and toxicity experiment reflect the huge potential of the plant as protein source in Siamese Gourami farming.

Keywords: *Trichogaster pectoralis*, water hyacinth,*Eichhornia crassipes*,nutrition

Introduction

Siamese gourami, *Trichogaster pectoralis*, is a native freshwater fish in Malaysia. However, this fish can also be found in USA (Courtenay *et al.*, 1984), Brazil (Magalhaes *et al.*, 2002), India (Welcomme, 1988), Indonesia (Kottelat *et al.*, 1993), Thailand (Amornsakun, 2004). The biggest size of Siamese gourami was reported 25 cm in body length and weight 200 g (Froese and Pauly, 2003). Traditionally, Siamese gourami was collected from its natural habitat such as paddy field, ditch, lake and river. Commonly, this fish is highly source out for pickling. Recently, the sudden demand from local market pushing Siamese gourami market price as high as USD 5/kg. In order to fulfil the demand from market, Siamese gourami need to be farmed and not only depend on natural source.

In the captivity environment, Siamese gourami was given commercial pellet which increased cost production of the fish. Hence, research was conducted to find the alternative protein source to feed farmed Siamese gourami to reduce production cost. Local ingredient was recognized can be used in formulating low cost fish feed. One of the local ingredient is water hyacinth where can be found widely in local natural water bodies. Water hyacinth, *Eichhornia crassipes*is known as weed and can be found abundant in the stagnant water bodies (Dipanjan, 2010). The fast growing of this weed create adverse impact to the environment (Dipanjan, 2010). The intense population of the aquatic plant restrict the penetration of sunlight into water body. Subsequently, most of aquatic organisms cannot survive in that environment. However, water hyacinth has been reported widely use as terrestrial and aquatic animal feed as early as 1970s (Sipauba and Braga, 2007). The results from the previous studies indicating application of the plant as feed is promising. Furthermore, preliminary experiment showed water hyacinth was rich of protein ranged from 6 to 12%. Since Siamese gourami was identified as algae/plant eater freshwater fish, in the present study, water hyacinth, *E. crassipes*, leaf, was used to evaluate its potential as alternative protein source for Siamese gourami farming.

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Materials and Methods

Fish Feeding Experimental Design

In the present study, there are five treatments namely T1 (20% of *Eichhornia crassipes* leaf + 80% fish meal), T2 (40% of *E. crassipes* leaf + 60% fish meal), T3 (60% of *E. crassipes* leaf + 40% fish meal), T4 (80% of *E. crassipes* leaf + 20% fish meal) and T5 (100% of *E. crassipes* leaf) with three replicates for each treatment. Crude protein evaluation was carried out for the formulated feed of each treatment. The control group of fish was fed with fish meal (Cargill, Malaysia). 30 pieces of fish with the average weight 8.06 ± 0.023g was put in each experiment tank with the size of 40 L. The experimental fish were given feed at *ad libitum* and the palatability of the feed was recorded. The water exchange is conducted only when the water parameters are not in optimum range. Growth and survival rate of experimental fish were monitored weekly for continuous four weeks or 28 days. In the end of the experiment, the fish were sampled and subjected to histology analysis.

Formulated feed preparation

Eichhornia crassipes leaf was collected from its natural habitat where it can be found plenty on ditch, lake and river. Only young and succulent leaves were selected for further uses. The leaves were brought back to laboratory and oven dried at 40 ^{o}C after cleaning. The dried leaves were grinded into small particles and mixed manually according to the experiment design.

Water quality parameter

Water quality parameters of each experimental tank were monitored using multiparameters (YSP, USA). The temperature of each experimental tank was maintained at 25-28^{o}C; pH 6.0- 8.5 and dissolved oxygen ranged from 5 to 7 mg/L by supplied with aerator and fresh water exchange.

Crude protein analysis

In the present study, sample crude protein analysis was conducted as described by Hanan *et al.* (2011) and Jabir *et al.* (2012). Samples were digested using FOSS Tecator™ Digester (Chromscience, Malaysia) followed by kjeldahl test. Total nitrogen were calculated using formula as below:

$$\text{-Percent nitrogen} = \frac{(V_s - V_b) \times N \times 14.007}{W \times 10}$$

Where

V_s = volume of 0.1M HCl used to titrate sample

V_b = volume of 0.1 M HCl used to titrate blank

N = normality of HCl

14.007is the atomic weight of nitrogen

W = weight in g(s) of sample

-Percent crude protein = Percent nitrogen × F

Where

F = factor to convert nitrogen to protein (6.25)

Evaluation toxicity of *Eichhornia crassipes* leaf via histological analysis

At the end of experiment, five treated fish were sampled from each treatment to reveal adverse effect of the *Eichhornia crassipes* leaf to the experimental fish. The sampled fish were euthanized and their liver were fixed in 10% buffered formalin. The sampled liver were washed by using xylene and then embedded in paraffin wax. The waxed livers were then sectioned and subjected to hematoxylin and eosin staining. The sectioned slides were observed under light microscope (40 X) and captured by using Dino – Eye microscope eye piece camera (Dino Eye, Taiwan).Semi-quantitative scoring system was applied in comparison adverse effect of *E. crassipes* leaf to the treated fish.

Histopathology was determined based on severity of changes compared to control group. Scores were based on the severity as well as the number of images out of the total images of each treatment which the histological changes were observed (for more information see Tab. 3).

The histopathological changes refer to abnormal changes of liver tissues such as fatty infiltration, fatty degeneration, necrosis, lesion, inflammation, cellular degeneration and pigment (Ayoola, 2011).

Statistical analysis

The growth rate results in the present study were statistically analyzed and presented as mean ± standard error by using One way Analysis of Variance (ANOVA) test and followed by Tukey Post Hoc to determine the significant differences in mean ($P < 0.05$) using Software Program of Statistical Social Science Analysis (SPSS) 16.0.

Results

The findings of the present study showed crude protein of formulated feed by using combination of *Eichhornia crassipes* leaf and fish meal or *E. crassipes* leaf alone was ranged from 12.1 ± 1.81% to

25.6 ± 1.24% (Table 1). The major source of crude protein was derived from fish meal.T1 (20% of *E. crassipes* leaf + 80% fish meal) showed possess the highest crude protein (25.6 ± 1.24%) followed by T2 (40% of *E. crassipes* leaf + 60% fish meal) (22.8 ± 0.94%) and T3 (60% of *E. crassipes* leaf + 40% fish meal) (19.1 ± 1.06). T4 (80% of *E. crassipes* leaf + 20% fish meal) crude protein recorded as 15.2 ± 0.18% while T5 (100% of *E. crassipes* leaf) showed contain the lowest crude protein (12.1 ± 1.81%).

Tab. 1: Crude protein of formulation diet

Formulation Diet	Crude protein (%)
Fish meal (Control)	30.1 ± 0.31
T1	25.6 ± 1.24
T2	22.8 ±0.94
T3	19.1 ±1.06
T4	15.2 ± 0.18
T5	12.1 ± 1.81

- T1 (20% of *Eichhornia crassipes* leaf + 80% fish meal)
- T2 (40% of *Eichhornia crassipes* leaf + 60% fish meal)
- T3 (60% of *Eichhornia crassipes* leaf + 40% fish meal)
- T4 (80% of *Eichhornia crassipes* leaf + 20% fish meal)
- T5 (100% of *Eichhornia crassipes*leaf)

Treatment T1 showed the best treatment in terms of survival (88 ± 3.8%) and growth rate (14.3 ± 0.31%) and significantly different ($p < 0.05$) compared to control and other treatments (Table 2). Experimental fish in treatment T2, T3, T4 and T5 showed reverse growth rate but T4 (91 ± 0.2%) performed the highest survival rate.

Tab. 2: Growth and survival rate of Siamese gourami received five different treatmentsof *Eichhornia crassipes* leaf (Abbreviations are available in Tab. 1).

Treatment	Survival Rate (%)	Growth Rate (%)
Fish meal (Control)	86 ± 4.6^a	6.6 ± 0.13^a
T1	88 ± 3.8^a	14.3 ± 0.31^b
T2	63.5 ± 2.1^b	- 4.0 ± 0.12^c
T3	73.5 ± 2.4^b	- 8.1 ± 0.31^c
T4	91 ± 0.2^a	- 4.5 ± 0.56^c
T5	81 ± 2.2^a	- 4.8 ± 0.17^c

Histology analysis on the liver of the experimental fish showed that T2 suffered the most where all the sampled livers exhibited less than 50% hispathological changes. In comparison, hispathological changes present in liver samples from other treatments were only less than 25%. Hispathological changes was completely absent in the liver samples of the control group. In the present study, experimental fish from group of control and treatment T1 were the most prefer to the given feed (Table 4). This was followed by treatment T2. Experimental fish from treatment T3, T4 and T5 showed not interested to the given feed.

Tab. 3: Histology analysis of Siamese gourami, given five different concentrations of *Eichhornia crassipes* leaf using semi-quantitative scoring system (Abbreviations are available in Tab. 1).

Formulation Diet	Percent of liver affected (%)
Fish meal (Control)	-
T1	+
T2	++
T3	+
T4	+
T5	+

- =completely absence (0% of histopathological changes)
+ = present (< 25% of histopathological changes)
+ + = mild (< 50% of histopathological changes)
+ + + = severe (> 75% of histopathological changes)

Tab. 4: Palatability of Siamese gourami received five different concentrations of *Eichhornia crassipes* leaf (Abbreviations are available in Tab. 1).

Treatment	Palatability score index
Fish meal (Control)	+++
T1	+++
T2	++
T3	+
T4	+
T5	+

+ = fish consumed less than 25% of given feed in 5 min
++ = fish consumed less than 50% of given feed in 5 min
+++ = fish consumed more than 75% of given feed in 5 min

Discussion

Malaysia is a tropical country blessed with abundant of natural resources. One of the plenty resources is plant. Emmanuel and Harinder (2012) reported that there were lesser known and underutilized plant which possess huge potential to be ingredient for animal feed production. Water hyacinth is recognized as weed and create problem to the environment due to its fast growing character. This plant is labelled as worst aquatic plant in the world as its pose a threat to ecological and economic as well (Dipanjan, 2010). However, this plants is claimed to be used as feed to various animals including fish. Thus, the present study is attempted to reveal the potential of *Eichhornia crassipes* leaf as feed for Siamese Gourami.

Based on the present finding, crude protein in *E. crassipes* leaf is quite high but can not be used alone as main ingredient in fish feed formulation. From literature survey, there are many plants and plant

parts possess higher crude protein compared to *E. crassipes* leaf. For instance, *Psophocarpus scandens* leaf possess crude protein from 28 to 30%, *Urtica dioica* (28%) and *Colocasia esculenta* (20-25%) (Emmanuel and Harinder, 2012). Hence, we suggested that this plant can only be used as supplement to the fish as the crude protein cannot fulfil requirement for a fish need.

The highest growth rate of the experimental fish in the present study was achieved from Treatment T1. The high growth rate may be supported by palatability score of the experimental fish to the formulated feed. The fish preferred to feed from control and Treatment T1. This was followed by Treatment T2. Treatment T3, T4 and T5 shared similar score of palatability. Palatability of the formulated has direct influence on the growth rate of the experimental fish. Less consumption of the formulated feed was observed among experimental fish in the treatment of T3, T4 and T5. Hence, we may conclude that high concentration of the plant extract led to the reverse growth rate of the experimental fish from the mentioned treatment. Anti-nutrition factors that may exist in the plant can consider as the reason of the fish refuse to consume of the given formulated feed.A lot of uneaten feed can be observed in the Treatment T3, T4 and T5 where the fish seem refuse to consume the given formulated feed. The study of application of *E. crassipes* leaf in aquaculture has been recorded as early as 1971 where Liang and Lovell (1971); Little (1979) claimed that *E. crassipes* leaf was use as supplement to the feed of channel catfish at the rate of 5 to 10% can increase the growth and survival rate of the fish. Therefore, we proposed that *E. crassipes* leaf can only be as supplement to boost the fish growth at low concentration. Other plants were also found to be useful as supplement to boost growth and survival rate of the fish. For instance, *Citrus microcarpa* extract was reported can increase immune system of African catfish (Lee *et al.*, 2014). In earlier, the study of Lee and Najiah (2009) claimed that the bioactive coumpund of *C.microcarpa* extract is 2-hydroxypropane-1,2,3-tricarboxylic acid that possess huge antimicrobial property as can inhibit the growth of various pathogenic bacteria. Besides that *Cymbopogon nardus* citronella essential oil (Lee and Wendy, 2013) and *Allium sativum* (Lee and Najiah, 2008) were found to enhance the immune system of the fish.

E. crassipes leaf was found to possess anti nutrition factor which may lead to low survival of the experimental fish. Mild histopathological changes was found in the liver experimental fish in the Treatment T2 while histopathological changes was found present in the experimental fish from Treatment T1, T3, T4 and T5. Although higher concentrations of *E. crassipes* leaf was applied in the treatment of T3, T4 and T5, however, less adverse effect was found due to low score of the palatability was observed in those treatments. On the other hand, high score of palatability in the Treatment of T1 but less adverse effect was probably due to the low concentration of the plant in the formulated feed. Saha and Arun (2011) reported that fermented *E. crassipes* leaf was found to be able to increase the growth rate of Rohu, *Labeo rohita*, without any adverse effect to the fish. The fermented plant can fully substitute fish meal as main ingredient and providing protein to the fish.Other study of Sipauba and Braga (2007) found that application of *E. crassipes* as organic fertilizer in the *Colossoma macroponum* larvae farming can enhanced the abundance of phytoplankton community in the aquaculture sites. In spite of the fact, *E. crassipes* leaf has huge potential to be used as feed to fish, however, the plant must undergo treatment such as fermentation to eliminate anti nutrition factors before it can be used as feed. Hence, further study should be carried out to identify the anti nutrition factors that responsible to the adverse effect to Siamese Gourami and the treatment to eliminate the anti nutrition factors before it can come to a commercial sense.

Conclusion

The findings of the present study showed that application of *Eichhornia crassipes* leaf had adverse effect to the fish. However, at low concentration, the plant in the formulated feed was found able to increase the growth performance of Siamese Gourami. Further study should be carried out to reveal on how to reduce toxic elements from the plant before this finding can come to a commercial sense.

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