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The effect of house cricket (*Acheta domesticus)* meal on growth performance of red hybrid tilapia (*Oreochromis* sp.)

Seong Wei Lee[1], Huan Ching Tey[1], Wee Wendy[2] and Mohamed Wan Zahari[3]

1) Faculty of Agro Based Industry, Universiti Malaysia Kelantan Jeli Campus, 17600, Jeli, Kelantan, Malaysia.
2) Centre for Fundamental and Liberal Education, Universiti Malaysia Terengganu,
Kuala Terengganu, 21030, Terengganu, Malaysia.
3) Faculty of Veterinar Medicine, Universiti Malaysia Kelantan, Jalan Padang Tembak, Pengkalan Chepa, Kelantan, Malaysia.

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Abstract: In the present study, the effect of house cricket, *Acheta domesticus* meal on the growth performance of red hybrid tilapia, *Oreochromis* sp. was evaluated. There were five treatments with different combination of house cricket and rice bran, namely T1 (60% of *A. domesticus* + 40% rice bran), T2 (70% of *A. domesticus* + 30% rice bran), T3 (80% of *A. domesticus* + 20% rice bran), T4 (90% of *A. domesticus* + 10% rice bran) and T5 (100% of *A. domesticus*), each with three replicates. The control group of fish was fed with commercial pellet. The feeding trial was carried out for four weeks and the liver of the fish from each treatment was subjected to histology study in order to evaluate the toxicity level of house cricket against fish that received treatment. The results of the present study showed that there was significantly difference ($p < 0.05$) among the treatment diets towards the survival and growth rate of red hybrid tilapia, where Treatment T1 showed the best result in term of survival and growth rate. However, histology study revealed that the fish liver suffered from abnormal fatty changes as the concentration of *A. domesticus* increased in treatment diets.

Keywords: red hybrid tilapia, *Oreochromis* sp., house cricket, insect meal, rice bran, growth rate, histology

Introduction

Fish meal and soybean meal are the essential ingredients found in most forms of fish feed as the source of protein. However, high demand for both animal-based and plant-based proteins in fish feed have inevitably led to the overexploitation of natural resources and the subsequent price fluctuation in feed and fish production. As an alternative, insect meal was deemed to be an interesting source of protein to feed the cultured fish (FAO, 2013; Barroso *et al*., 2014). Fish meal contain high level of protein, complete amino acid profile, well balanced lipid profile including omega-3 fatty acid, high digestibility and palatability, low anti-nutritional factors and carbohydrate level (Barroso *et al*., 2014). In Malaysia, over 80% of fish meal supply is imported while the rest is locally sourced. The quality of local fish meal was comparatively inferior to those imported fish meal in several aspects such as lower crude protein (CP) (50-55%), higher ash (26-28%), higher salt (>3.5%) and fats (>10%). Moreover, inconsistency in the supply of thrash fish or fishery wastes has also been a problem faced by the local suppliers in quality assurance of the fish meal. Despite the nutritive value of fish meal, the escalated cost of fish meal has urged the industry to seek for alternative protein source to replace the costly fish meal. Recently, insect meal is gaining attention as one of the high quality protein source to feed the cultured fish (FAO, 2013; Barroso *et al*., 2014).

Red hybrid tilapia, *Oreochromis* sp. is famous freshwater fish not only in Malaysia but also worldwide. Sudden demand of red hybrid tilapia in the market leads to the intensive red hybrid tilapia farming throughout Malaysia. However, the increasing production cost of tilapia due to fish feed may set the price of tilapia become more expensive in the market and less affordable to the consumers. Therefore, potential of *A. domesticus* meal as an alternative protein source in promoting growth performance of red hybrid tilapia, *Oreochromis* sp. fingerlings were investigated in present study.

Materials and Methods

Experimental design

Treatments for this experiment consisted of five different combinations of house crickets, *Acheta*

(✉) leeseongwei@yahoo.com

domesticus and rice bran, namely T1 (60% A. domesticus + 40% rice bran), T2 (70% of *A. domesticus* + 30% rice bran), T3 (80% of *A. domesticus* + 20% rice bran), T4 (90% of *A. domesticus* + 10% rice bran) and T5 (100% of *A. domesticus*), each with three replicates. Meanwhile, the control group of fish was fed with commercial pellet (Cargill, Malaysia). All feed samples were subjected to crude protein (CP) analysis.

Fifty fingerlings with average initial weight of 1.427±0.037 g were put on feeding trials in respective tanks with approximately 25 L of water volume, for a period of 28 days. Growth and survival rate of experimental fish were monitored weekly for continuous four weeks. After the feeding trials, fish from each treatment was subjected to histology analysis on the liver to investigate toxicity level of *A. domesticus* and rice bran meal.

Fish tank set up

Red hybrid tilapia fingerlings were obtained from a private hatchery farm. All fish were acclimatized to the laboratory condition for seven days before experiment started. The experimental fish were fed with commercial diets twice daily at the rate of 2% of the total body weight and the palatability of the feed was recorded. Experimental tanks were equipped with aeration and water exchange was carried out every day. Fish growth and mortality rate were monitored and recorded weekly. The initial weights of the experimental red hybrid tilapia, *Oreochromis* sp. fingerlings were ranged from 1.175 to 1.480 g.

House crickets, *Acheta domesticus* and rice, *Oryza sativa* L. bran preparation

House crickets, *A. domesticus* were purchased from commercial farm in Kelantan. Rice bran was collected from local rice mill in Kelantan. The house crickets, *A. domesticus* were gathered and frozen at -20^{o}C in a freezer (Snow, Malaysia) for further uses. Thawed house crickets were oven-dried at 40 ^{o}C using oven (Red Line, Germany) followed by grinding process using a blender (Panasonic, Malaysia). The powderized house crickets, *A. domesticus* were then manually mixed with rice bran and commercial binder to process into fish feed.

Water quality parameter

Water quality parameters of each experimental tank were monitored using multiparameter sonde (YSI, 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.

Crude protein analysis

Crude protein analysis of samples in the present study was according to Hanan *et al*. (2011) and Jabir *et al*. (2012). Samples were digested using Tecator™ Digester (FOSS, USA) with eight digestion tubes. The samples were then subjected to Kjeldahl test. Total nitrogen was calculated using formula as below:

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

Where
V_s = volume of 0.1M HCl used to titrate sample
V_b = volume of 0.1M HCl used to titrate blank
N = normality of HCl
14.007 is the atomic weight of nitrogen
W = weight of sample, in g

Crude protein (%) = nitrogen in sample x F
F = 6.25, factor to convert nitrogen to protein

Histological analysis

At the end of experiment, the fish were euthanatized. For each treatment, liver sampled from the experimental fish was examined by histology study. The liver samples were fixed with 10% buffered formalin for less than 24 h. The samples were then washed with xylene and embedding in paraffin wax for thin sectioning at 5 µm thickness. This is followed by staining with hematoxylin and eosin (H&E). The liver tissue from fish that received different treatment diets were examined for abnormality using compound light microscope (Leica, Germany) under 40x magnification and images were captured using Dino-Eye microscope eyepiece camera (AnMo, Taiwan). The results were compared between control group and treatment groups.

Histopathological changes found on the liver tissues of the fish were compared using semi-quantitative scoring system by Peebua *et al*. (2006) and Ayoola (2011), with modification. Micrographs of three serial sections of liver tissue from each treatment diets and control group were randomly selected. Histopath-ological changes on those samples from treatment groups were compared to samples from the control group. Scores in the form of symbols (-), (+), (++) and (+++) were based on the severity of conditions found on the micrographs, where '-'indicated completely absence (0% of

histopathological changes), (+) indicated present (<25% of histopathological changes), (++) indicated mild (<50% of histopathological changes), and (+++) indicated severe (75% of histopathological changes). The histopathological changes on liver tissues were such as fatty infiltration, fatty degeneration, necrosis, lesion, inflammation, cellular degeneration and pigmentation (Ayoola, 2011).

Statistical analysis

The results 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 Statistical Package for the Social Sciences (SPSS) 16.0.

Results

In the present study, crude protein of the commercial pellet was 30%, followed by 28% in T1, 33% in T2, 36% in T3, 40% in T4 and 43% in T5 (Tab. 1). Positive growth rate were recorded from control (5.6 %) and T1 (33.3%) whereas other treatments resulted negative growth rate, where T2, -3%; T3, -7.09%; T4, -3.53% and T5, -3.8% (Tab. 2). The highest survival rate was recorded from T4 (90%) whereas the lowest was T2 (62.5%). Overall, survival rate ranged from 62.5 to 90% (Tab. 2). Histological study revealed that control treatment showed normal liver condition whereas T1 and T2 recorded mild histological change. T3, T4 and T5 exhibited the presence of histological change (Tab. 3). As for the palatability assay, commercial pellet and treatment T1 were similar and shared the highest palatability score, whereas treatment T2 was found to score the second highest. This was followed by treatment T3, T4 and T5 which shared the lowest score (Tab. 4). Examination of liver tissues from red hybrid tilapia, *Oreochromis* sp. fingerlings after 28 days exposure to different concentration of experimental treatment feeds showed that some hepatic cells were vacuolated. Closer examination on the liver abnormalities of the fish in present study indicated that the increasing amount of house crickets, *A. domesticus* in feed formulation possibly caused cytoplasmic vacuolation, damage of nuclei, fatty degeneration and inflammation in liver cells. The liver is composed of hepatic lobule in which the central vein obscure. The parenchyma of the hepatic lobule is formed from hepatocytes with the surrounded by blood sinusoid in cord-like structure known as hepatic cell cord. The bile ductile between the cords of hepatic cells is directed toward the periphery of the lobule to open in the bile duct (Lee *et al*., 2016). Based on statistical analysis, there was significant difference in terms of growth and survival rate at $p < 0.05$ where Treatment T1 showed the best result in terms of growth and survival rates.

Tab. 1: Crude protein of formulation diet.

Formulation Diet	Crude protein (%)
Control	30 ± 0.3
T1	28 ± 2.1
T2	33 ± 2.3
T3	36 ± 1.8
T4	40 ± 2.1
T5	43 ± 2.6

Tab. 2: Growth and survival rate of tilapia fingerlings that received five different treatments

Treatment	Initial Weight (g)	Final Weight (g)	Survival Rate (%)	Growth Rate (%)
Control	1.480±0.20	1.563±0.32	85±5.6^a	5.6±0.1^a
T1	1.175±0.23	1.508±0.12	87±4.8^a	33.3±0.3^b
T2	1.405 ±0.41	1.363±0.42	62.5±3.1^b	- 3.0±0.1^c
T3	1.453±0.13	1.350±0.69	72.5±3.4^b	- 7.09±0.3^c
T4	1.445±0.12	1.394±0.23	90±1.2^a	- 3.53±0.5^c
T5	1.350±0.15	1.299±0.39	80±3.2^a	- 3.8±0.1^c

T1 (60% of *A. domesticus* + 40% rice bran), T2 (70% of *A. domesticus* + 30% rice bran), T3 (80% of *A. domesticus* + 20% rice bran), T4 (90% of *A. domesticus* + 10% rice bran) and T5 (100% of *A. domesticus*)

Tab. 3: Histology analysis of the liver of tilapia fingerlings given five different concentrations of *A. domesticus* using semi-quantitative scoring system.

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

Tab. 4: Palatability of *A. domesticus* formulation diets fed to tilapia fingerlings.

Formulation Diet	Palatability Score Index
Commercial pellet	+++
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 less than 75 % of given feed in 5 min

Discussion

The increasing cost and limited resources of raw material to produce fish feed has become major constraint to the development of aquaculture industry as one of the food security tool to generate nutrition for human being. As a result, scientists around the world are urged to find alternative protein source for aquaculture instead of dependence on fish meal to formulate fish feed. One of the suggestions is to derive protein source from insect. A review by Belluco *et al*. (2013) suggested that insect possesses huge potential not only as food for human but also to be applied as raw material in feed for animal husbandry including fish. Hence, this study was carried out to evaluate the feasibility of house cricket, *A. domesticus* meal as feed replacement for fish meal in tilapia farming.

This study was the first attempt on the application of house cricket meal and rice bran combination to feed red hybrid tilapia, *Oreochromis* sp. fingerlings. Partial replacement of protein source in fish feed is possible as revealed by the findings in present study, where T1 which consisted of 60% house cricket meal in feed formulation and 28% ± 2.1 CP was found to gain higher growth rate compared to the control group fed with commercial pellet, while having almost similar survival rate and palatability score. However, in the present study, the hybrid red tilapia fingerlings were notably showing stunted growth when fed with formulation diet consisted of more than 70% house cricket meal. This finding is in agreement with the previous study by Hanan *et al*. (2011), whereby cricket meal found to reduce the growth of Nile tilapia, *O. niloticus*. Similar growth pattern was also found in the later study by Jabir *et al*. (2012), where Nile tilapia was fed with super worm meal.

Findings from this study also found that there were some limiting factors which need to be resolved to ensure the feeding of house cricket meal does not cause indigestibility and health impairment to the fish. For instance, the presence of chitin in fish feed may cause poor digestibility in tilapia (Shiau and Yu, 1999). Furthermore, Blue Gourami, *Trichogaster trichopterus*, was also found unable to digest cricket meal that was rich in chitin compound (Kedar *et al*., 2013). These may explain the low palatability score in T2, T3, T4 and T5 and the stunted growth pattern in present study when feed formulation consisted of more than 70% of house cricket meal.

Besides the presence indigestible compound in the cricket meal, Giaccone *et al*. (2005) described that cricket meal which contain high microbial count may led to microbial infection to the fish. High mortality rate recorded from T2 and T3 may be associated with the microbial infection that occurred. However, low mortality rate from the treatments (T4 and T5) that received higher concentration of cricket meal can be explained as the fish refused to consume the given feed. The presence of high microbial community in the cricket meal can be reduced by using chemical or by mixing cricket meal with natural antimicrobial agent such as plant extract which is environmental friendly and safe to the end user. In previous study, plant extract from *Citrus microcarpa* juice was found not only to eliminate microorganism growth (Lee and Najiah, 2009; Lee *et al*., 2014) but also can enhance the immune system of the fish (Lee *et al*., 2014). Other potential plant extracts as antimicrobial agent in feed inlcuded *Cymbopogon nardus* (Lee and Wendy, 2013) and *Allium sativum* (Lee and Najiah, 2008).

Due to the fact that application of insect as ingredient in formulating feed may develop several risks such as allergy hazard, microbial hazard, parasitical hazard and chemical hazard (Belluco *et al*, 2013) where these hazards may pose a threat to fish and consumer health, hence, we have to be more cautious when producing insect meal as alternative protein for aquaculture uses.

Conclusion

The present study revealed that formulated feed using *A. domesticus* in high amount (>60%) can cause adverse effect to red hybrid tilapia fingerlings, especially to the liver and growth performance. Therefore, fully replacement of fish meal by house cricket meal is not recommended. The use of house cricket meal in feed formulation will require further study to eliminate the chitin component and to detect hazardous compounds in the cricket meal.

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