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A preliminary study on the effect of the combination of *Blaptica dubia* (Order: Blattodea) and commercial fish feed on red hybrid tilapia, *Oreochromis* sp.

Munirah Mokhtar[1], Kumara Thevan Krishnan[1,*] and Pasupuleti Viswesara Rao[2]

[1] Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[2] Faculty of Science &Technology, REVA University, Rukmini Knowledge Park, Kattigenahalli, Yelahanka, Bangalore, Karnataka, India, 560 064

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☒*Corresponding author:
Dr. Kumara Thevan Krishnan
Faculty of Agro-Based Industry,
Universiti Malaysia Kelantan, 17600
Jeli, Kelantan, Malaysia
Email: thevan@umk.edu.my

Abstract

Fish meal and soybean meal are the main sources of protein in most fish feeds. Changes in feed and fish production costs are a concern for aquaculturists. Because they are a natural component of fish diets and have a high protein, vitamin, and digestibility profile, insects may be able to contribute to meeting the growing demand for fishmeal. This study was a preliminary investigation on the effect of various dietary combinations (*Blaptica dubia* + commercial pellet) on the weight of red hybrid tilapia, *Oreochromis* sp. fry. The *Oreochromis* sp. fingerlings were acclimatized to the laboratory condition for seven days. Five different mixtures of feed were concocted [T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% *B. dubia* + 30% commercial pellet), T3 (80% *B. dubia* + 20% commercial pellet), T4 (90% *B. dubia* + 10% commercial pellet) and T5 (100% *B. dubia*)]. The control group of *Oreochromis* sp. was fed with 100% commercial pellets. Throughout the experiment period, the growth of the fish was monitored weekly for 15 weeks. Then, the fish were euthanized, and the liver was subjected to histology analysis. The results show that T3, T4, and T5 feed mixtures promote the best growth and survival rate for *Oreochromis* sp. while causing liver damage. T1 and T2 fish feed ratios are better alternatives as they promote healthy fish and increased weight ($p < 0.05$).

1. INTRODUCTION

Blaptica dubia is a cockroach species under the Blaberidae family, the order of Blattodea, which is native to Central and South America. It has a large body size with a length of up to 4.5cm (Wu et al., 2013). They have a long-life cycle by which the adults can live more than two years, and males are usually shorter-lived (Alamer, 2013). Meanwhile, aquaculture production has been increasing over the last three decades, and its growth is predicted to continue. As the demand grows, the raw ingredients for fish feed need to be readily available. At the moment, fish meal and soybean meal are the requisite ingredients in most forms of fish feed as the source of protein (Lee et al., 2017). However, high demand for both plant-based and animal-based proteins in fish feed can led to the overuse of natural resources and subjected to price fluctuation in feed and fish production, which is an issue among the aquaculturist. The Food and Agriculture Organization of the United Nations (FAO) recommended insects as an alternative protein source in livestock feed (Van Huis et al., 2013). In this circumstance, insects have good potential to meet the rising demand for meat products and to replace fishmeal because they are a reliable source of protein, vitamins, high digestibility, and they are part of the natural diet of fish, human poultry, and pigs (Maria et al., 2017).

Oreochromis sp. or known locally as "tilapia," is from the Cichlidae family and order Perciformes, live in freshwater habitats including streams, ponds, lakes, and rivers. Tilapia farming is believed to have originated more than 4,000 years ago from Egypt (Gupta and Acosta, 2004). It has grown traction worldwide due to the efforts by development agencies to feed the rural poor (Fitzsimmons, 2001). In Malaysia, the market value of red hybrid tilapia is around USD 3.13-3.14 per kg, and the price is getting higher due to high demand from the market (Lembaga Kemajuan Ikan Malaysia, 2024). In addition, *Oreochromis* sp. were the cheapest fish in the market after African catfish which is affordable for the majority of consumers and with the introduction of genetically improved farmed tilapia (GIFT), the total production of tilapia increased in Malaysia (Siti et al., 2021; Nhuong et al., 2021). Besides, tilapia able to adapt to reproduce under a wide range of physical and environmental conditions, has excellent growth rates on a wide variety of natural and prepared diets, resistance to handling and disease-causing agents,

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and broad consumer appeal as a food fish, are the most successfully cultured fish species worldwide (Lim and Webster, 2006). Therefore, the objective of this experiment was to do a preliminary investigation on the potential of *B. dubia* as a value-added fish feed used in combination with commercial fish pellets.

2. MATERIALS AND METHODS

2.1. Rearing

Blaptica dubia colonies were reared at a mean room temperature of 26.16 ±2.5˚C with 75.34± 6.98% relative humidity. They were maintained in plastic boxes with the size of 35(h) x 45(w) x 60(l) cm. The plastic containers were covered with netted lids for air circulation and avoided pest infestations (Ø ~ 2 mm). As a shelter for hiding and breeding sides, the cardboard egg flats were provided and stacked horizontally inside the plastic boxes. The colonies reared using the protocol as suggested by Lam et al. (2018).

2.2. Stock pellet preparation

B. dubia was collected and dried in the oven at a temperature of 45°C for 24 hours. Then, the sample was fine-grained and filtered through a filter with a mesh size of 1 mm. To ensure the sample is homogeneous and to increase surface area for optimum contact with the binder. Later, the ground *B. dubia* (80%) were mixed with water (10%) and carboxymethyl cellulose (CMC) (10%) that act as binders. The mixtures were then shaped into small pellet sizes and air-dried for 24 h (Lee et al., 2017).

2.3. Fish tank set up

Oreochromis sp. fingerlings were purchased from a private hatchery farm. All fish were acclimatized to the laboratory condition for seven days before the experiment began. Acclimatization was carried out to lessen stress and familiarise the fish with the temperature and photoperiod in the laboratory. The fingerlings were fed with commercial diets two times per day at the rate of 2% of the total body weight (Taddase et al., 2014). The fish tanks were equipped with aeration, and water exchange was carried out twice a week. Growth rates were monitored and recorded weekly. The initial weights of *Oreochromis* sp. fingerlings ranged between 1.2g to 1.5 g.

2.4. Water quality parameter

Water quality parameters of each fish tank were monitored using multi-parameter sonde (YSI, USA). The temperature of experimental tanks was maintained at 25-28 °C, pH of water at 6.0 - 8.5, and dissolved oxygen ranged from 5 to 7 mg/L as suggested by Lee et al. (2017).

2.5. Preparation of experimental diet

This experiment consisted of five different combinations of *B. dubia* and commercial pellet; T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% *B. dubia* + 30% commercial pellet), T3 (80% *B. dubia* + 20% commercial pellet), T4 (90% *B. dubia* + 10% commercial pellet) and T5 (100% *B. dubia*), each treatment has three replicates. At the same time, the control group of fish was fed with 100% commercial pellet (KW Zone Fish Food, 38% crude protein). They fed twice daily, 10 a.m. and 6 p.m. *ad libitum*.

Fifty fingerlings of *Oreochromis* sp. with an average initial weight of 1.427±0.037 g were put on feeding trials in respective tanks with approximately 25 L of water volume. The growth rate of experimental fish was monitored weekly for continuous fifteen weeks.

2.6 Histological analysis

At the end of the experiment, all experimental fish were euthanized. Sampled fish liver from the experimental fish was subjected for histology analysis. The liver samples were fixed in 10% buffered formalin for less than 24 h. Then, the samples were washed with xylene and immersed in paraffin wax for thin sectioning at 5 µm thickness, followed by staining the thin sectioning of the liver with hematoxylin and eosin (H&E). The liver tissue was examined for abnormality using a compound light microscope (Leica, Germany) under 40x magnification, and images were captured using Dino-Eye microscope eyepiece camera (AnMo, Taiwan).

Tissues of the fish liver were compared using the semi-quantitative scoring system as suggested by Peebua et al. (2006) and Ayoola (2011), with modification. Micrographs of three serial sections of liver tissue from each treatment diet and control group were randomly selected. Histopathological changes on those samples from treatment groups were compared to representatives from the control group. Symbols (-), (+), (++), and (+++) represent the severity of the histopathological changes. The (-) 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 included fatty infiltration, fatty degeneration, necrosis, lesion, inflammation, cellular degeneration, and pigmentation (Ayoola, 2011).

2.7 Statistical analysis

Statistical analysis was performed on the different mixtures of feed were concocted *B. dubia* and commercial pellet. The treatments were subject to ANOVA test using SPSS ver 21 (IBM).

3. RESULT AND DISCUSSION

This research was the first attempt to apply *B. dubia* meal and commercial pellet combination to feed red hybrid tilapia, *Oreochromis* sp. fingerlings. Result shows there were significant differences among the treatment group ($p<0.05$). *Oreochromis* sp. fingerling obtained the

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maximum growth from T5, which consisted of 90% *B. dubia* diet. Researchers noted that the optimum growth for fry *Oreochromis niloticus* was obtained at a 45% crude protein diet (Tawwab et al., 2010). The weight gain increased from T1 to T5 (Table 1) as the percentage of *B. dubia* feed increased. In our previous study, the crude protein in *B. dubia* is 47-54% (Lam et al., 2018) compared with the crude protein from the commercial pellet, about 38%.

The T1, T2, and Control treatments showed the normal liver condition (Figure 1-A, B, C). However, T3, T4, and T5 exhibited a histological change in the fish liver (Figure 1- D, E, F). The fish liver in T3, T4, and T5 treatments shows (Table 2) blood congestion, accumulation of melanomacrophage, and enlargement of hepatocyte size. Many authors explained morphological alterations to the liver caused by Vitamin E (Huang and Huang, 2004), non-ideal diet (Mumford et al., 2007), toxic substances (Abdelwarith et al., 2011), aluminum (Hadi and Alwan, 2012), cadmium sulphate (Jalaludeen et al., 2012), trichlorfon (Xu et al., 2012), overfeeding (Taddase et al., 2014) and high-fat diet (Jia et al., 2020).

Figueiredo-Fernandes et al. (2007) suggested that an increase in the hepatocytes size may be due to the high content of lipids and Lam et al. (2018) found high-fat content in *B. dubia*, which is between 35-44 %. It is known that free fatty acids from ingested food are normally transported into hepatocytes, where they are converted into cholesterol or phospholipids or oxidized to ketone bodies (Taddese et al., 2014). Hence, oxidation of the fatty acids and other nutrients probably causes a stress response in the fish liver. At the same time, there was an increase in melanomacrophage in the group T3, T4, and T5. Melanomacrophages are aggregates of highly pigmented phagocytes found in the kidney, spleen, and liver of many invertebrates (Steinal and Bolnick, 2017). An increase in the density of the melanomacrophage aggregates is generally related to significant hepatic lesions (Pacheco and Santos, 2002). The function of the melanomacrophages in the liver of fishes remains uncertain, but some studies have suggested that it is related to the destruction, detoxification, or recycling of endogenous and exogenous compounds (Haaparanta et al., 1996; Steinal and Bolnick, 2017). Generally, the functions of the fish liver include assimilation of nutrients, production of bile, detoxification, maintenance of the body metabolic homeostasis, and at the same time, plays a significant role in the synthesis of plasma proteins (Taddese et al., 2014). Furthermore, Camargo and Martinez (2007) explained fish liver damages could be categorized into three stages; those changes in Stage 1, such as nuclear hypertrophy, irregular shaped nucleus, irregular shaped cells, and melanomacrophages aggregates, do not alter the normal functioning of the liver tissue. This could be explained by the increased weight of fish fed B. dubia insect protein, which results in weight gain at the expense of liver abnormalities in T3, T4, and T5. As a result, T1 and T2 are better choices because they promote weight gain and healthy fish. One of the significant advantages of using histopathological biomarkers is that it allows examining specific target organs such as kidneys and liver responsible for vital functions such as excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001; Camargo and Martinez, 2007). The morphological altercation in these organs serves as warning signs of damage to animal health (Hinton and Lauren, 1990). Therefore, the histological changes observed in the liver of the *Oreochromis* sp. in the present study indicate that the fish responded to the direct effects of the fish feed.

Table 1: Weight of *Oreochromis* sp. before and after treatment

Treatments	Initial weight (g)	Final weight (g)
C	1.37±0.157	24.327±3.624
T1	1.263±0.143	28.76±4.994
T2	1.283±0.162	25.427±3.750
T3	1.457±0.148	31.163±1.224
T4	1.45±0.113	34.75±1.051
T5	1.423±0.190	44.39±8.769

T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% *B. dubia* + 30% commercial pellet), T3 (80% *B. dubia* + 20% commercial pellet), T4 (90% *B. dubia* + 10% commercial pellet) and T5 (100% *B. dubia*)

Table 2: Histology analysis of the liver of tilapia fingerlings given five different concoctions of *B. dubia* using a semi-quantitative scoring system**.**

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

The (-) 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).

4. CONCLUSION

In aquaculture, fish health is of the utmost importance that leads to profitability, and fish feed is one of the significant components that contribute to the operation cost. Therefore, besides providing optimum feeding, alternatively, aquaculturists can opt for alternative feed resources. The present study revealed that mixtures of *B. dubia* between 60-70% (T1 and T2) with commercial pellets could promote healthy and increased weight in *Oreochromis* sp. Hence, this shows the potential of *B. dubia* to be used with the combination of commercial fish feed.

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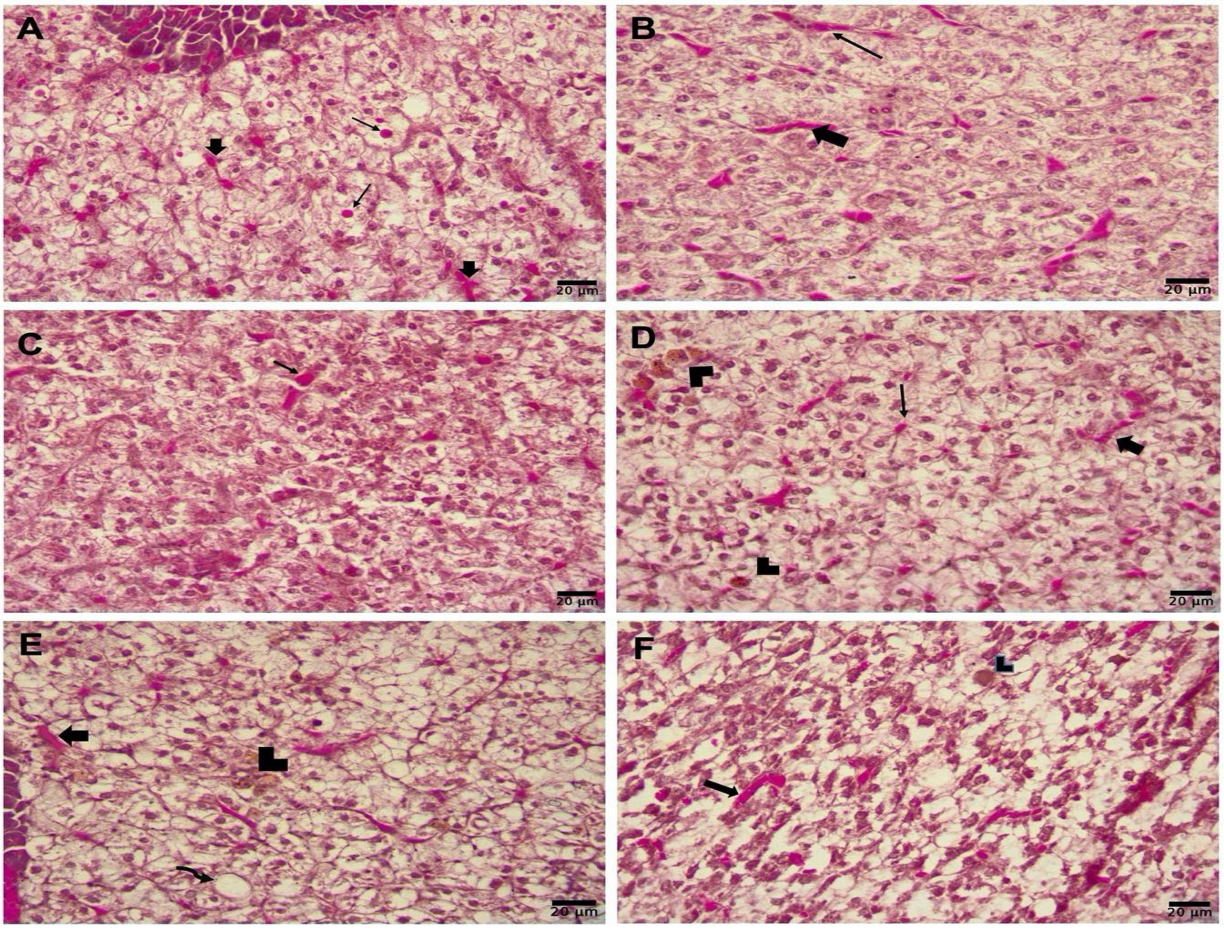

Figure 1: Liver section micrography of *Oreochromis* sp.; **A** - Control group, showing normal shaped hepatic cells, sinusoids (arrows), Kupffer cells (thick arrows); **B** - T1 group, showing normal shaped hepatic cells, dilated sinusoids (arrows), Kupffer cells (thick arrows); **C** - T2 group, showing normal shaped hepatic cells, sinusoids (arrows)**; D -** T3 group, showing normal shaped hepatic cells, sinusoids (arrows), Kupffer cells (thick arrow) but there are present of accumulation of melanomacrophage (arrowhead); **E** - T4 group, showing not normal shaped hepatic cells, Kupffer cells (thick arrow) but there are present of accumulation of melanomacrophage (arrowhead) and vacuolar degeneration (nonstraight arrow); **F -** T5 group, showing not normal shaped hepatic cells, Kupffer cells (thick arrow) but there are present of accumulation of melanomacrophage (arrowhead), hematoxylin and eosin, × 400.

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