Malaysian rubber (*Hevea brasiliensis)* seed as alternative protein source for red hybrid tilapia, *Oreochromis* sp., farming

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Abstract. Malaysia is one of the main rubber trees (*Hevea brasiliensis*) cultivating country with availability of rubber seeds in abundance throughout the year. Hence, this study was carried out to find out the potential of rubber seed as an alternative protein source for aquaculture uses. In the present study, there were five treatments namely T1 (20% of rubber seed + 80% fish meal), T2 (40% of rubber seed + 60% fish meal), T3 (60% of rubber seed + 40% fish meal), T4 (80% of rubber seed + 20% fish meal), T5 (100% of rubber seed) and a control group (commercial pellet). The feeding experiment was performed for consecutive eight weeks. Growth performance of experimental fish such as growth and survival rate was recorded. At the end of the experiment, liver sample of the fish from each treatment were sampled and subjected to histology analysis. The results of the present study showed that control, T1, T4 and T5 possessed significantly ($p<0.05$) higher survival rate compared to T2 and T3, whereas T1 recorded the highest growth rate among the treatment groups and control group. However, histopathological changes were present in the liver of the experimental fish from T3, T4 and T5 and it was mild for T1 and T2. On the other hand, T1 formulated feed was found to share similar palatability score index with commercial feed. The findings from present study indicate that rubber seed is suitable as protein supplement in feed formulation for fish when anti-nutritional factor of the rubber seed is eliminated.
Key Words: fish nutrition, toxicity, growth performance.

Introduction. Tilapia fish is the third important aquaculture species in the world. In Malaysia, red hybrid tilapia, *Oreochromis* sp. is a popular freshwater fish where growing demand lead to the development of intensive farming throughout Malaysia. However, production cost for tilapia is likely to increase in corresponding to the higher cost for feed. Nowadays, formulated feed in aquaculture industry is fully depending on fish meal as protein source and almost 80% of the fish meal supplied for fish feed formulation in Malaysia is imported (Lee et al 2016a). In the effort to reduce dependency on imported fish meal, local feed producer must seek for alternative protein source which is reasonable in price, consistent in supply and yet high in crude protein content compared to thrash fish and fishery wastes.

Rubber tree, *Hevea brasiliensis*, is an important commodity in Malaysia with estimated plantation area covered nearly 1.2 million hectares and able to produce 1.2 million kg of rubber seed (Malaysian Rubber Board 2009). Rubber tree is useful in producing raw materials in the form of latex and rubber wood for downstream rubber products manufacturing. Along with the production of raw materials, rubber seeds are also available abundantly but only small proportion of the rubber seeds is used for seedling and the rest is likely to be disposed as agriculture waste. According to Eka et al (2010), rubber seed was found to be rich in essential and non-essential amino acids and fat content. Rubber seed which underwent boiling process was also found can be used in formulated feed for livestock and poultry (Udo et al 2016). Hence, in the present study, rubber seed was evaluated to reveal its potential as alternative protein source for aquaculture uses.

Material and Method

Experimental design. The present study involved five treatments and one control group. These five treatments were T1 (20% of rubber seed + 80% fish meal), T2 (40% of rubber seed + 60% fish meal), T3 (60% of rubber seed + 40% fish meal), T4 (80% of rubber seed + 20% fish meal), T5 (100% of rubber seed), while the commercial feed with 30% crude protein (CP, Malaysia) was given to the control group. All treatments and control group were prepared in triplicate. After feeding trial, growth performance of the experimental fish was recorded and liver of the experimental fish was subjected to histological analysis.

Fish tank set up. All experimental fish with the mean weight of 1.34±0.104 g were purchased from a commercial farm and maintained in the Aquaculture Lab, Jeli Campus, University of Malaysia Kelantan for a month before the experiment was conducted. The experimental fish were given feed twice a day at the rate of 2% of the total body weight for eight weeks. Each experimental tank held 30 individuals, equipped with aerator and daily water exchange. Growth and survival rate of the experimental fish were monitored and recorded.

Feed preparation. Rubber, seeds were collected from local *H. brasiliensis* smallholder and brought back to Aquaculture Laboratory, Jeli Campus, University of Malaysia Kelantan for further processing. The shell of the rubber seeds were removed by using hammer and the seeds were chopped into small pieces. The processed seeds were oven-dried at 40°C in the oven (Red Line, Germany) followed by grinding using a blender (Panasonic, Malaysia). The rubber seed meal was then manually mixed and combined with fish meal at the desired concentration by the addition of commercial binder.

Water quality parameter. The water quality of the experimental tanks was monitored using multiparameters (YSP, USA). During the experiment, water 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. In the present study, the samples of crude protein were analyzed as described by Hanan et al (2011), Jabir et al (2012) and Lee et al (2016a). Samples were digested using FOSS Tecator^{TM} Digester (Chromscience, Malaysia) with eight digestion tubes and followed by Kjeldahl test.

Histological analysis. In the present study, histology analysis was carried out on the liver samples obtained from the experimental fish which received treatments. The liver sample was fixed for 24 h using 10% buffered formalin before washing with xylene and embedded in paraffin wax. The samples were subjected to sectioning and staining with hematoxylin and eosin. The samples were then observed using compound light microscope under 40X magnification and photographed using Dino-Eye microscope eyepiece camera (Dino-Eye, Taiwan). Abnormality of the liver samples were carefully examined and recorded. Semi – quantitative scoring system was applied as the measurement for any abnormality of the liver tissue which included fatty infiltration, fatty degeneration, necrosis, lesion, inflammation, cellular degeneration and pigmentation (Oluwatoyin 2011). The score was determined as '-' for completely absence (0% of histopathological changes), '+' for present (<25% of histopathological changes), '+ +' for mild (<50% of histopathological changes), and '+ + +' for severe (>75% of histopathological changes).

Feed palatability score. Palatability of formulated feed was measured by using palatability score index and the scores were determined as follows (Lee et al 2016a):
\+ = 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.

Statistical analysis. The results of experimental growth and survival rate in the present study were presented as mean ± standard error and analyzed using one way analysis of variance (ANOVA) analysis followed by Tukey Post Hoc to reveal significant differences in mean ($P<0.05$) through Software Program of Statistical Social Science Analysis (SPSS) 16.0.

Results and Discussion. Crude protein analysis on feed samples applied in the present study showed that T1 has the highest crude protein content (35.6±1.8%), followed by T2 (32.2±1.3%), T3 (26.8±2.8%), T4 (23.6±4.5%) and T5 (18.8±1.3 %) (Table 1).

Table 1

Crude protein of formulation diet

Formulation diet	*Crude protein (%)*
Control	30.0 ± 0.2
T1	35.6 ± 1.8
T2	32.2 ± 1.3
T3	26.8 ± 2.8
T4	23.6 ± 4.5
T5	18.8 ± 1.3

T1 (20% of rubber seed + 80% fish meal);
T2 (40% of rubber seed + 60% fish meal);
T3 (60% of rubber seed + 40% fish meal);
T4 (80% of rubber seed + 20% fish meal);
T5 (100% of rubber seed).

Growth and survival rate of tilapia fish under five different treatments can be found in Table 2, where only T1 resulted positive growth rate at 28.3±0.31% with 88.1±2.7% survival rate. Stunted growth was resulted in fish under T2, T3, T4 and T5 treatments, where the growth rate ranged from - 3.25 to -13.8%. On the other hand, survival rate of the fish in all treatments was above 50%, in the range of 60.3% (T2) to 89.1% (T4).

Table 2

Growth and survival rate of tilapia received five different treatments

Treatment	*Survival rate (%)*	*Growth rate (%)*
Control	93.2 ± 2.6^{a}	13.4 ± 0.18^{a}
T1	88.1 ± 2.7^{a}	28.3 ± 0.31^{b}
T2	60.3 ± 1.4^{b}	-13.0 ± 0.14^{c}
T3	68.2 ± 1.7^{b}	-5.23 ± 0.15^{c}
T4	89.1 ± 1.2^{a}	-3.25 ± 0.13^{c}
T5	79.9 ± 2.8^{a}	-13.8 ± 0.12^{c}

T1 (20% of rubber seed + 80% fish meal);
T2 (40% of rubber seed + 60% fish meal);
T3 (60% of rubber seed + 40% fish meal);
T4 (80% of rubber seed + 20% fish meal);
T5 (100% of rubber seed).

Statistical analysis survival rate of the experimental fish results showed Group Control, T1, T4 and T5 were under same group which significantly higher ($p<0.05$) than Group T2 and T3. On the other hand, the growth rate of Group T1 is significantly higher ($p<0.05$) compared to control and the other treatment groups. Histological analysis showed experimental fish liver from group T2 and T3 suffered mild histopathological changes (Table 3) whereas liver of the experimental fish from group T3, T4 and T5 exhibited histopathological changes. Histopathological changes of the fish liver in the present study were referring to the fatty degeneration, cytoplasmic vacuolation, damage of nuclei and inflammation in the liver cells. The only normal liver sample was obtained from experimental fish of control group.

Table 3
Histology analysis of red hybrid tilapia, given five different concentrations rubber seed using semi-quantitative scoring system

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

T1 (20% of rubber seed + 80% fish meal);
T2 (40% of rubber seed + 60% fish meal);
T3 (60% of rubber seed + 40% fish meal);
T4 (80% of rubber seed + 20% fish meal);
T5 (100% of rubber seed);
- =completely absence (0% of histopathological changes), + = present (< 25% of histopathological changes), + + = mild (< 50% of histopathological changes), + + + = severe (> 75% of histopathological changes).

On top of that, commercial feed and T1 formulated feed shared similar palatability score index (+++), as well as the highest score in the present study (Table 4). T2 scored the second highest (++), followed by T3, T4 and T5 which were under similar palatability score index (+).

Table 4
Palatability of red hybrid tilapia received five different concentrations of rubber seed and Control group

Treatment	*Palatability score index*
Control	+++
T1	+++
T2	++
T3	+
T4	+
T5	+

T1 (20% of rubber seed + 80% fish meal);
T2 (40% of rubber seed + 60% fish meal);
T3 (60% of rubber seed + 40% fish meal);
T4 (80% of rubber seed + 20% fish meal);
T5 (100% of rubber seed);
\+ = 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.

Malaysia rubber plantation is well known and established same goes with its the by-product, rubber seed. Rubber seed was found rich in fat (68%) and protein (17%) where reported widely used as biodiesel (Morshed et al 2011), feed and food (Eka et al 2010). In the present study, rubber seed was processed and used in formulated fish feed for red hybrid tilapia to reveal its potential as alternative protein source for aquaculture uses and fish meal replacement.

In the present study, experimental fish that received low concentration of rubber seed (20%) was performed significantly higher growth and survival rate. However, the experimental fish suffered mild adverse effect. Although, rubber seed is rich in nutrients but also contain toxic compounds such as hydrogen cyanide (HCN) and cyanogenic glucoside at high concentration (Eka et al 2010). Furthermore, high fat content of rubber seed may lead to fatty acid degeneration and vacuolation incidences in the experimental fish which received fish feed formulated by using rubber seed. However, Deng et al (2015) claimed that rubber seed meal at lower than 30% in tilapia formulated feed will not gave obvious adverse effects on the growth; at the meantime it may increase non-specific immune response of tilapia against *Aeromonas hydrophila*. Many plants possess medicinal property which has a huge potential as food supplements for fish diets. For instance, *Andrographis paniculata* leaf extract (Lee & Wendy 2011); *Michelia champaca* leaf and flower extracts (Lee et al 2011) and *Peperomia pellucida* (Lee et al 2016b). However, almost all plants possess toxic compounds which are harmful to fish. Sharma et al (2014) claimed application of isoproteinous and isocaloric diet can reduce 85% of toxic compounds in rubber seed meal, and the treated rubber seed meal can be a good source of protein for *Labeo rohita*. On the other hand, Suprayudi et al (2015) reported that common carp, *Cyprinus carpio*, which received defatted rubber seed meal at concentration more than 50 % in the feed suffered abnormalities in the intestine and kidney as well as decreased haematological indicators. More studies need to be carried out as the effect of rubber seed meal may vary in different fish species.

The low survival rate and negative growth rate of experimental fish from group T2, T3, T4 and T5 may due to high concentration of rubber seed used in feed formulation. The major toxic compound in the rubber seed which is extremely poisonous is the HCN. This compound may be responsible to low survival rate and negative growth rate of experimental fish that given feed more than 60% of rubber seed. However, this poisonous compound can be gradually lost if rubber seeds are kept for minimum four months in dark conditions (Fuller 1988). Other methods to eliminate toxic elements (HCN and cyanogenic glucoside) in the rubber seed were roasting at 350°C for 15 min and soaking in hot water. Therefore, there are many constraints that hindering the application of rubber seed commercially as feed. In seeking new raw materials for feed formulation several factors should be consider such as allergy hazard, microbial hazard, parasitical hazard and chemical hazard (Belluco et al 2013) because these hazards may pose a threat to fish and consumer health. In spite of the fact, additional process need to be underwent in order to minimize potential hazards in those raw materials.

Conclusions. The findings of the present study revealed that formulated feed using rubber seed as protein source have adverse effect to *Oreochromis* sp. fish due to anti nutritional factor in rubber seed. However, further study should be carried to minimize toxic elements (HCN and cyanogenic glucoside) in the rubber seed in order to ensure treated rubber seed safe for aquaculture uses.

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