Effects of Moringa (*Moringa oleifera*) leaf meal on juvenile red hybrid tilapia (*Oreochromis sp.*)

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Abstract

The effects of moringa leaf meal inclusion were investigated on growth performance, feed utilization and body composition of juvenile red hybrid tilapia (*Oreochromis sp.*). Feeding trial was conducted for 12 weeks in a completely randomized design with five replications (3 treatments × 5 replicates × 30 fish per tanks). The fishes weighed ±31g were fed with isonitrogenous (32%CP) and isocaloric (13MJ/kg) diets containing moringa meal at the inclusion of 0%, 10% and 15%. The results showed that, during 12 weeks trial, there was no significant difference ($p>0.05$) observed on growth performance among treatments despite numerical increase in body weight ranged between 93.84 to 104.41 g. Meanwhile, it was found that the feed utilization was significantly affected by the feed given. The feed utilization of 10% moringa leaf meal was not significantly different to the control. The feed conversion ratio (FCR) recorded for the control, 10% and 15% of moringa leaf meal inclusion were 1.33±0.12, 1.44±0.13 and 1.75±0.15, respectively. The body composition also was not significantly different among treatments ($p>0.05$). The protein content of fish muscle ranged between 74.39±4.14 and 81.66±4.58%. In conclusion, moringa leaf meal could be incorporated in juvenile red hybrid tilapia diet with an optimum of 10% inclusion without any adverse effect on growth performance, feed utilization and body composition.

Keywords: Body composition, Feed uitilization, Growth performance, Moringa leaf, Oreochromis sp.,

Introduction

Soybean meal is a plant-based protein source which is commonly used feed ingredient in feed formulation. It is one of the major feed ingredients that were imported into Malaysia and consequently may lead to the increase of feed production cost during unfavorable import market price. Other than that, its importation is also affected by the world climate or weather, such as during the draught season in the US in the year 2012, where soybean production is depleted and exportation of this crop is restricted. The efforts of finding alternatives to replace or substitute soybean meal is needed and has to be continuously conducted. Studies have been conducted to evaluate plant-based proteins such as moringa leaf (Yuangsoi & Masumoto, 2012) and fenugreek seed meal (Sheikhlar *et al.*, 2018) as alternative protein sources. Although leaf meal may be a potential alternative, nutrient content and effectiveness of the meal in feed formulation should be well studied due to the presence of anti-nutritional factors that could have negative effects on the growth of the animals.

Moringa oleifera is a multipurpose plant, known as an excellent source of nutrition and energy booster (Paliwal et al., 2011). Every part of this leguminous plant

contains important nutrients including protein, fiber, fat, vitamins, minerals and phytochemicals (Nsofor *et al.*, 2012; Yuangsoi & Masumoto, 2012; Gopalakhrishnan *et al.*, 2016; Hasem *et al.*, 2017). According to Makkar and Becker (1996), the moringa leaf contains high protein and is rich in vitamins. In African countries such as Senegal and Benin, this plant received lots of attention, as the seeds, in particular, are consumed and functioning as remedy for malnutrition

Moringa leaves also contains significant level of essential amino acids including methionine, cysteine, tryptophan and lysine, and it is said that high levels of methionine in moringa leaf helps in protein synthesis of animals (Ferreira *et al.*, 2008). The essential amino acids in immature pods, leaves and flowers of Moringa respectively were 30, 40 and 31% (Sánchez-Machado *et al.*, 2010) and the patterns of essential amino acid of Moringa leave was similar to those of soybean seeds (Paliwal *et al.*, 2011). Since it is locally available with reasonably high yield, and relatively low in antinutritional component, high in protein, lipid and essential amino acids, Moringa has a potential to be used in fish (Lochman *et al.*, 2011). The current study was conducted to determine the effect of local *M. oleifera* on growth performance, feed utilization and body composition for juvenile red hybrid tilapia at two inclusion level.

Materials and Methods

Moringa leaf meal and diet preparation

Moringa leaves were obtained from Kluang, Johor, dried at temperature of about 32-33˚C for 3-5 days, and ground into powder form. The moringa leaf meal was added into three isonitrogenous and isocaloric (32% and 13MJ/kg) diets at the inclusion levels; 0% (control), 10% and 15% (Table 1). The diet mixture was pelleted into 2mm mesh size using a lab scale extruder and was then dried in oven at 60˚C for 24 hours. After cooling to room temperature, the pellets were packed in plastic bags for storage until further use. The feeds were also analyzed for proximate values according to the standard method of AOAC (1999).

Table 1. Ingredients and chemical composition of experimental diets

Ingredient (g kg^{-1})	Control	10% Moringa	15% Moringa
Corn starch	150	150	150
Soy bean meal	342	242	192
Fish meal	150	150	150
Corn	100	100	100
Rice bran	100	100	100
Wheat pollard	100	100	100
Moringa	0	100	150
Palm oil	30	30	30
Limestone	5	5	5
Dicalcium phosphate	10	10	10
L-Methionine	5	5	5
Lysine	5	5	5
Vita-Min Premix	3	3	3
Total	1000	1000	1000
Nutrient composition by analysis (g kg^{-1} dry weight on fed basis)			
Protein	32.39±0.00	31.68±0.29	30.94±0.24
Fat	4.87±0.00	4.98±0.21	5.42.±0.08
Dry matter	94.29±0.01	95.38±0.02	94.53±0.17
Ash	7.36±0.01	7.78±0.05	7.78±0.05

Fish and tank preparation

The feeding trial was carried out at the Freshwater Fish Nursery of Livestock Science Research Centre MARDI, Serdang. Mixed sex red hybrid tilapia were purchased from local supplier in Balakong, Selangor and were acclimatized for a few weeks in fiberglass tanks (250 L) fitted with 24hr continuous aeration. The water exchange was done every alternate day. The fish were maintained at 27.14±0.24°C and other water parameters including dissolved oxygen and pH were kept at optimum levels according to the requirements of freshwater fish.

Feeding trial and rearing condition

Juvenile tilapia with body weight of ±31g each were used for this study. A number of 450 fish were randomly assigned into 15 fiberglass tanks with 5 replications. The fish were fed at 3% of body weight, twice a day (morning and evening) for 12 weeks. Every fortnight, the fish in each tank were weighed to readjust their daily feed. Mortality of fish was monitored and recorded every day.

Determination of growth and muscle composition

At the end of the study, all fish were counted and weighed in group for growth performance. Few fish were sampled from each tank and filleted for determination of dry matter, ash, fat and protein. The proximate analysis of fish muscle was done following the AOAC (1999) procedures.

Statistical analysis and calculations

All the collected data were analyzed using one-way analysis of variance using SPSS ver. 23.0 (SPSS Inc., Chicago, IL). Level of significance between individual treatments ($p > 0.05$) was evaluated by Tukey's test. The calculations of growth and feed utilization parameters are as the followings:

a) Weight gain (WG, g) = mean final weight – mean initial weight
b) Specific Growth Rate (SGR, % day^{-1}) = 100 [(ln initial live weight – ln final live weight) / experimental period (day)]
c) Feed intake (FI, g)= amount of feed consumed by fish within the feeding trial
d) Feed conversion ratio (FCR) = amount of feed consumed (g) / live weight gained (g)
e) Feed efficiency ratio (FER) = live weight gained (g) / feed intake (g)
f) Survival rate (SR, %) = (final no. of fish / initial number of fish) x 100

Results and Discussion

Growth performance and feed utilization

Growth parameters of red tilapia fed with different inclusion levels moringa leaf meal were shown in Table 2. All treatment groups showed increment in weight with the final weights were between 93.84 to 104.41 g. The results showed that there were no significant differences in weight gain, final weight and SGR of tilapia fish among treatment groups.

Table 2. Growth parameters in tilapia fed with diets containing different level of moringa meal for 84 days

Treatment	Control	10% Moringa	15% Moringa
Initial Weight (g)	31.67±0.00	31.67±0.00	31.67±0.00
Final Weight (g)	104.41±5.98	95.46±8.59	93.84±9.35
Weight Gain(g)	72.74±5.98	63.79±8.59	62.17±9.35
SGR	1.42±0.07	1.31±0.11	1.29±0.12

*Values are means of treatment groups±SD. Within a row, means with the same letters are not significantly different (P>0.05). Absence of letters indicates no significant difference between treatments.

It was found that the optimal growth performance in this red hybrid tilapia was achieved at 10% inclusion. When fish were fed with 15% of moringa, the final weight and weight gain is slightly decreased. As more moringa is included in the diet, the performance becomes lower even though the feed intake increases. These results were in accordance with the study by Richer *et al.*, (2003) and Lochman *et al.*, (2011) using moringa leaf meal in Nile tilapia. The suggested inclusion level of moringa leaf meal was up to 10% and 30% substitution of soybean meal with moringa leaf meal gave similar fish growth and feed conversion ratio (Lochman *et al.*, 2011). Supplementation of moringa leaf powder to *Oreochromis mossambicus* at 5g of feed showed increment in weight higher than *Sesbania grandiflora*, *Coleus aromaticus*, *Ocimum basilicum* and *Solanum verbascifolium* treatment (Karpagam & Kreshnaveni, 2014). Based on their study, the acceptance of moringa leaf in aqua feed on fish may varies depends on the fish species.

Puycha *et al.*, (2017) reported that 10% moringa meal inclusion was the optimum level for *Pangasius bocourti*. The increment

of moringa inclusion beyond this optimum level reduced their growth performance. Apparently, partial replacement of moringa leaf meal with soybean for fancy carp recorded lower than 20g/kg (Yuangsoi & Masumoto, 2012). In another study using African catfish, *Clarias gariepinus*, substitution of fishmeal with moringa leaf meal at 20% replacement showed the best growth response (Nsofar *et al.,* 2012). The protein quality and the processing of moringa meal also may influenced the quality of feed itself (Sánchez-Machado *et al.,* 2010). For this study, the growth performance of juvenile red tilapia hybrid in all treatment groups were not significantly affected by the inclusion of moringa leaf meal.

Table 3 showed the feed utilization of fish fed with the experimental diets throughout the trial. The feed intake of tilapia was found to be varied between treatments, with fish fed with 15% of moringa has the highest intake and fish with 10% moringa was the lowest. Fish fed with 15% moringa recorded the lowest value of FER (0.58±0.05) and significantly different ($p<0.05$) compared to control (0.72±0.06) and 10% moringa leaf (0.70±0.06) treatment group. There was no significant difference on survival rate among the groups.

Table 3. Feed utilization parameters of tilapia fed with moringa leaf in feed for 84 days

Treatment	Control	10% Moringa	15% Moringa
Feed Intake (g)	100.83 ± 3.43^{ab}	91.02 ± 7.13^{a}	107.50 ± 6.33^{b}
FCR	1.33 ± 0.12^{a}	1.44 ± 0.13^{a}	1.75 ± 0.15^{b}
FER	0.72 ± 0.06^{b}	0.70 ± 0.06^{b}	0.58 ± 0.05^{a}
SR	95.83±4.19	91.11±5.09	95.33±2.98

Abbreviations used: FI: feed intake (g dry diet fish-1 for 84 days), FCR: feed conversion ratio (total feed consumed (g)/live weight gained (g)), FER: feed efficiency ratio (live weight gain (g)/dry feed intake (g)), SR: Survival rate (%)

*Values are means of treatment groups ± SD. Within a row, means with the same letters are not significantly different (P>0.05). Absence of letters indicates no significant difference between treatments.

From the results, it was found that higher inclusion of moringa leaf meal in tilapia significantly affects the feed utilization of the fish. The higher FCR value in 15% inclusion (1.75±0.15) indicating that although the intake was quite high, but the fish do not benefit in terms of weight gain. This indicate the use of moringa leaf meal is limited to 10 % inclusion and with higher inclusion rate, the performance of the fish will be negatively affected. This may be due to the presence of antinutritive factor in moringa leaf as reported by reported several authors (Makkar & Becker, 1996; Yuangsoi *et al.,* 2014; Gopalakrishnan *et al.,* 2016; Hasem *et al.,* 2017)

Polyphenols, consists of total phenols, and tannins are among the anti-nutritional factors that are normally present in plants. Egwui *et al.* (2013) reported the presence of phenol and saponin concentration in moringa leaf. These anti-nutritional factors may reduce the weight gain and inhibit the fish growth. A study done by Makkar & Becker (1996) reported that tannins concentration in moringa leaf was negligible (1.4%) and similar amount was found in soybean meal. Ferreira *et al.* (2008) also found that the concentration of tannins in moringa leaf was 12g/kg dry matter and was considered as relatively low. A study by Puycha *et al.,* (2017), however, indicated that the amount

of tannin was increased with the increasing level of moringa leaf in the diets of Bocourti's catfish (*Pangasius bocourti*). The optimal level of tannin in 100g/kg feed was 2.07±0.25% and beyond this level, the fish growth was reduced. Tannin levels in fish diets between 0.5% to 2.0% could depress the growth performance and above 5.0% often cause lethality (Giner-Chavez,1996).

Phytic acid is another anti-nutrient that present in moringa leaf. There was about 3.1% of phytic acid in moringa leaf (Makkar & Becker, 1996). The phytic acid in feed may react with protein to form phytic acid-protein complexes which will decrease protein digestibility in animal (Thomson, 1993). This is in accordance to the study reported by Puycha *et al.* (2017) when higher inclusion level of moringa leaf in diets reduced the digestibility coefficient and protein digestibility of catfish. Besides growth performance, histology and haematological parameters of fish fed with moringa leaf meal may also be affected (Hlophe & Moyo, 2014). As claimed by Lochman *et al.* (2011), the anti-nutritional factor can be reduced by soaking the plant in water for a few days. The processing of moringa seeds and leaves has also been reported to help maximize the nutrient utilization by reducing bioavailability of certain anti nutrients (Khachik *et al.* 1992). The concentration of anti-nutritional factor however differs with different parts of the plant with lower concentration was observed in moringa seed compared to other parts (Ferreira *et al.*, 2008)

Body composition

Fish in treatment of 15% moringa showed the lowest protein content and the highest dry matter, ash and fat content but it is not significantly differed than the other two treatments (Table 4).

Table 4. Body composition on dry matter basis of fish fillet fed with Moringa leaf

Treatment	Control	10% Moringa	15% Moringa
DM	21.03±3.68	22.90±6.81	24.88±4.55
Ash	5.72±0.12	5.71±0.36	5.91±0.34
Fat	8.77±1.74	8.33±0.02	9.32±0.95
Protein	81.66±4.58	78.44±3.47	74.39±4.14

*Values are means of treatment groups±SD. Within a row, means with the same letters are not significantly different (P>0.05). Absence of letters indicates no significant difference between treatments.

The body composition result found in this study was in agreement with the study done by Dongmeza *et al.*, (2006) using methanolic extract of moringa leaf where there was no significant difference among treatment groups. Hasem *et al.*, (2017) reported similar results when moringa seed meal was included in the diets of Nile tilapia (*Oreochromis niloticus*) fingerlings. However, the range of crude protein (61.50 to 63.00 %) in their study was lower compared to the current study. It was probably due to the different species of tilapia, digestive capability, feeding habit and environment of the study. While Yuangsoi and Masumoto (2012) observed that the essential amino acid compositions in muscle was not affected by the inclusion of moringa leaf extract in fish feed

Conclusion

In this feeding trial, it was found that there was no significant effect of moringa leaf meal inclusion on growth performance and body composition of red hybrid tilapia fingerling. On the other hand, the feed utilization was found to be significantly affected by the inclusion of moringa leaf meal with the optimal inclusion level of 10%. Exceeding the optimal inclusion level may lead to the decrease of the final weight of the juvenile fish. The moringa leaf meal showed a great potential to be used as an alternative protein sources in fish. However, more studies should be conducted particularly in terms on the effect of moringa leaf meal on fish physiology and the processing of the leaf meal in order to maximize its usage in aqua feed.

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