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Mango seed meal as partial replacement in diet for red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*): Growth performance, feed utilization and economic efficiency

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Abstract The effects on the supplemention of mango seed meal (MSM) with different levels in diets for red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) was investigated . Five experimental diets were formulated containing the different levels of MSM from 0, 5, 10, 25, and 50%. Each group of 20 fish was randomly distributed into 15 glass tanks in triplicates. The fish were fed to apparent satiation twice daily for 8 weeks. The results showed that fish fed diet containing 10% MSM had higher final body weight (FBW), weight gain, average daily gain (ADG), specific growth rate (SGR), feed efficiency (FE) and feed efficiency ratio (FER) than those of other experimental diets. No significant difference was observed in growth performance in fishes fed with a diet containing 25% MSM compared with 10% MSM group. While FBW, weight gain, ADG, and SGR were significantly reduced with increasing MSM higher than 25% replacement. These results indicated that 10% of MSM replacement could improve growth performance without any adverse effects. Based on SGR and FBW, the optimum dietary MSM inclusion in diet was estimated to be around 17.14 – 17.46 g kg^{-1} of dry weight predicted by second-order polynomial regression analysis.

Keywords: Mango seed meal, Nile tilapia, Replacement, by-product, Polynomial regression

Introduction

Global aquaculture development has rapidly increased aquatic feed production due to the increase in fish protein consumption for humans. In order to reduce the cost of aquaculture feeding, the development of new feed ingredients for cost-effective production and satisfaction needs to be

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investigated (Tacon, 2004; Tacon and Metian, 2008). Several alternative ingredients have been investigated including plant and animal proteins. Among the plant protein sources, especially those that are not consumed by human, that are being used for aquatic animal feedstuffs, appear to be good sources for fish diets. Several studies revealed that plant feedstuff for fish diets have been successfully used in partial replacement (Monge-Ortiz *et al*., 2016; SÁNchez-Lozano *et al*., 2011) and total replacement (Ahmed *et al*., 2019; Kaushik *et al*., 2004; Regost *et al*., 1999) in several fish species.

Mangoes (*Mangifera indica*), belonging to the family Anacardiaceae, are one of the most important tropical fruits in the world, native to several countries such as India, China, Thailand, Philippines, and Mexico (Ediriweera *et al*., 2017; Hirano *et al*., 2010). The world mango production is approximately 42 million tons which is ranked as the second most productive fruit after bananas (Ediriweera *et al*., 2017). In Thailand, mangoes are processed to produce a variety of products in order to keep their shelf life longer. The mango processing generally generates wastes which are approximately 35-60% of the total fruit weight, consisting of peel, husk, and kernel. These wastes pose a serious problem with environmental impacts. In the particular case of mangoes, the seeds annually produced were more than a million tons (Torres-León *et al*., 2016). However, the mango seed meal (MSM) has a high nutritional composition, such as carbohydrates (58-80%), protein (6-10%), and fat (6-16%). MSM is a good source of the essential amino acid profile, especially lysine and methionine, and contains a high amount of fatty acids including stearic and oleic (Diarra, 2014; Sagar *et al*., 2018). However, it has been reported that MSM contains anti-nutritional factors as well such as tannin, cyanogenic glycosides, oxalates, and trypsin inhibitory activity (Ravindran and Sivakanesan, 1996).

Red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) is considered a promising candidate for the large commercial scale farming in Thailand and South East Asia. However, the feed prices have been continuously increasing, accounting for 45-85% of the total production cost in intensive tilapia farming (Ng and Hanim, 2007). This is an important financial factor in red hybrid tilapia production. The abundance of mango seeds in the mango processing industry provides an advantage in using MSM as a feed ingredient in fish. It would be sustainable, economical, and beneficial in intensive red hybrid tilapia farming if these by-products could be used in fish diets without any adverse effects on growth and physiological changes in the fish. The present study is to evaluate the use of MSM with increasing replacement at 0%, 5%, 10%, 25%, and 50% MSM. Growth performance, feed efficiency, protein utilization, and economic analysis were also investigated.

Materials and methods

Fish culture technique

Red hybrid tilapia fingerlings (*Oreochromis niloticus* × *O. mossabicus*) weighing approximately 0.20 g/fish were purchased from a local hatchery farm (Dokdin hatchery farm, Phitsanulok province, Thailand) and transported to the Fish Nutrition Laboratory of the Naresuan University, Phitsanulok province, Thailand. The fishes were acclimatized in the laboratory rearing conditions for a month. During this period, the fish were housed indoors in plastic tanks (500 liters capacity) with a gravity biofilter and aeration. The red hybrid tilapia fishes were hand-fed with commercial diets containing 40% crude protein, 4% crude fat, and 4% crude fiber (High grade9961, Samutprakarn, Thailand) to apparent satiation 3 times daily (08.00, 12.00, and 16.30 h).

An 8-week growth trial was started with approximately 2.72 ±0.04 g/fish. After fasted for 24 h, fish were randomly distributed to each tank (20 fishes/tank) and anesthetized with 30 mg L^{-1} clove oil containing 90% ethanol. Each experimental diet was randomly assigned in triplicates. The fishes were manually fed by hand twice daily (09.00 and 16.00 h) to apparent satiation. During this period, the fishes and consumed diets were bulk weighed every 2 weeks. Water qualities during feeding trial were approximately 30±3˚C, pH 6.89 ± 0.97, DO 3.87±1.24 and with a natural photoperiod of approximately 12:12, light: dark conditions.

Experimental diets

Mango seeds were obtained from the local small and medium-sized enterprises in Phitsanulok Province, Thailand. The seeds were thoroughly washed with tap water. The seed kernels were removed, dried at 60˚C using hot air oven, ground, and sieved through a 300 µm mesh. The MSM were then collected in polyethylene bags and stored at – 20˚C until use.

The feed formulations of the experimental diets are shown in Table 1. A total of five isonitrogenouse and isolipidic diets were formulated containing approximately 300 g kg^{-1} crude protein and 70 g kg^{-1} crude lipid. Fishmeal and soybean meals were used as the sole protein source and fish oil as the energy source. A basal diet was formulated to contain 40% cornmeal and then four other diets were replaced by 5, 10, 25, and 50% of corn meal with MSM (referred to 5% MSM, 10% MSM, 25% MSM, and 50% MSM diets, respectively). All coarse dry ingredients were finely ground and sieved through a 300 µm mesh. All dry feed ingredients were well mixed and after the addition of fish oil and distilled water (approximately 35%), the feeding dough was pelleted to a 2 mm diameter. The experimental feeds were dried at 80˚C in a hot air oven overnight and kept in –20˚C in polyethylene bags until use.

Table 1. Feed formulation and proximate composition of experimental diets used in the present study

Ingredients	**Graded levels of mango seed meal in experimental diets**				
	Control (0%)	5% MSM	10% MSM	25% MSM	50% MSM
Fishmeal	17	17	17	17	17
Soybean meal	29.2	29.2	29.2	29.2	29.2
Corn meal	40	38	36	30	20
Mango seed meal	0	2	4	10	20
Rice flour	6.6	6.6	6.6	6.6	6.6
Vitamin mixture*	1	1	1	1	1
Mineral mixture**	1	1	1	1	1
Fish oil	5.2	5.2	5.2	5.2	5.2
Total	100	100	100	100	100
Chemical composition (%)					
Moisture	5.44 ±0.01	5.74 ±0.67	5.54 ±0.65	5.66 ±0.07	5.77 ±0.39
Crude protein	30.02 ±0.61	30.28 ±0.69	30.17 ±0.86	30.15 ±0.22	30.92 ±1.03
Crude fat	7.94 ±0.29	7.07 ±0.07	7.55 ±0.90	7.99 ±0.20	7.90 ±0.21
Crude fiber	3.45 ±0.80	2.17 ±1.00	1.99 ±1.43	2.91 ±0.30	2.98 ±0.12
Ash	6.40 ±0.34	6.51 ±0.10	6.37 ±0.01	6.59 ±0.11	6.57 ±0.07

*Vitamin mixture (mg or IU/kg diet): A, 5,000 IU; D3, 1,000 IU; E, 5,000 mg; K, 2,000; B1, 2,500 mg; B2, 1,000 mg; B6, 1,000 mg; B12, 10 mg; inositol, 1000 mg; pantothenic acid, 3,000 mg; niacin acid, 3,000 mg; C, 10,000 mg; folic acid, 300 mg; biotin, 10 mg
**Mineral mixture (g/kg feed); calcium phosphate, 80; calcium lactate, 100; ferrous sulfate, 1.24; potassium chloride, 0.23; potassium iodine, 0.23; copper sulfate, 1.2; manganese oxide, 1.2; cobalt carbonate, 0.2; zinc oxide, 1.6; magnesium chloride, 2.16; sodium selenite, 0.10

Sample analysis

After 24 h deprivation period at the end of the feeding trial, fish were sacrificed by an overdose of clove oil (approximately 100 mg L^{-1}). The fish were then counted and bulk weighed in each replicate to determine survival rate and growth performance as follows: weight gain (WG, %) = [final weight (g) – initial weight (g)]/initial weight (g) × 100, average daily gain, (ADG, g/day) =

final weight (g) – initial weight (g)/days, specific growth rate (SGR) = ln [final weight (g) – initial weight (g)]/days × 100, survival rate (%) = (number of final fish)/initial number of fish × 100.

To determine the experimental feed utilization, the weight of feed consumed was recorded to determine nutrient utilization of each experimental diet as follows: feed conversion ratio (FCR) = total feed intake (g)/weight gain (g), rate of feed intake (%/fish) = [F/(W0 + Wt) × (N0 + Nt) × t] ×100, Where F = dry weight of consumed diet (g), N0 = number of initial fish, W0 = average weight of initial fish (g), Nt = number of final fish, and Wt = average weight of final fish (g), feed efficiency (FE, %) = (1/FCR) x 100, and feed efficiency ratio (FER) = weight gain (g)/dry feed intake (g).

To determine protein utilization of fish, experimental diet, and initial and final whole body samples were incubated at 105˚C until constant dry weight. Samples were then finely ground using pestle and mortar. Protein from whole-body fish samples was determined according to the standard procedure with four replications (AOAC, 1997). Whole-body protein content was used to calculate protein utilization parameters as follows: protein efficiency ratio (PER) = wet weight gain (g)/protein intake (g) and protein productive value (PPV) = protein gain (g)/protein intake (g).

Chemical analyses

Chemical analysis of dry matter, crude protein, crude fat, ash, crude fiber of feed ingredients, experimental diets, and fish samples were analyzed according to the standard methods (AOAC, 1997). Briefly, dry matter was determined by air-drying the samples using a hot air oven (Memmert model UL50, Germany) at 105˚C until it reached a constant weight. Crude protein was analyzed by the Kjeldahl method (N × 6.25) with a Kjeldatherm® block heating system (Gerhardt Vapodest, 45s, Germany) and distillation units using a semi-automatic Kjeldahl (Gerhardt Vapodest, 45s, Germany). Fat was conventionally extracted with petroleum ether using the soxhlet apparatus (Gerhardt, Germany). Ash content was measured using the combustion method in a muffle furnace (Carbolite ELF 11/14, England) at 550˚C for 8 h. Crude fiber was digested with acid and basic digestion (Fibertec System M., 1020 Hot Extractor, Tecator). All samples were determined in quadruplicate.

Economic analysis

The experimental values were economically determined by calculating the cost of the feeding in Thai bahts (฿, currency in 2019, 1 US$ = 30.37 ฿). The feeding cost of each experimental diet was calculated using the following equation: feeding cost (baht kg^{-1}) = ∑ (ingredients used in Table 1 × prize per kg of each ingredient/100). The price per kg of each ingredient was as follows: fish meal = 42 ฿, soybean meal = 16 ฿, cornmeal = 11.4 ฿, MSM = 4 ฿, rice flour 30 ฿, vitamin mix 60 ฿, mineral mix 60 ฿, and fish oil 400 ฿. The economic conversion ratio (ECR) = FCR (kg diet kg of $fish^{-1}$) × diet price (฿ kg of $diet^{-1}$). The economic profit index (EPI) was calculated following the equation EPI (฿ $fish^{-1}$) = [weight gain (kg) × selling price (฿ kg^{-1})] – [weight gain (kg) × diet price (฿ kg^{-1})]. Red hybrid tilapia sale price was calculated at 35 ฿ kg^{-1} (Mart ńez-Lloren *et al*., 2007).

Statistical analysis

All treatments were laid out a completely randomized design and analyzed using one-way analysis of variance (ANOVA). Differences among the mean values were carried out using Duncan's Multiple Range Test (DMRT). Statistical analysis was carried out using SPSS statistical package version 17.00 (SPSS Inc., Chicago, IL, USA). Statistical significance was determined at $P < 0.05$. Data were represented as mean ± SD.

Results

Growth performance

The red hybrid tilapia was observed in good condition, including growth performance, health, and survival rate. A significant difference was observed in growth performance, feed utilization and nitrogen utilization in red hybrid tilapias fed the MSM containing in diets (Table 2- 4). The results of growth performance for 8 weeks are shown in Figure 1. Individual fish weights for all diets showed no significant difference during the first 2 weeks. After this, FBW was significantly different from week 4 to week 8 ($P < 0.05$). The average weight at week 4 was markedly decreased when MSM replacement level exceeded 25% ($P < 0.05$). A similar trend was also observed from week 6 to week 8 (Figure 1). The growth performance of red hybrid tilapia fed with the experimental diets is shown in Table 2. The results on growth performance revealed that 10% MSM could replace cornmeal in terms of increasing FBW, weight gain, ADG, and SGR. Although the fish fed with 25% MSM diet showed slightly higher growth performance than the control group, the observed differences were not statistically significant ($P > 0.05$). The survival rate did not significantly differ among dietary treatments (Table 2).

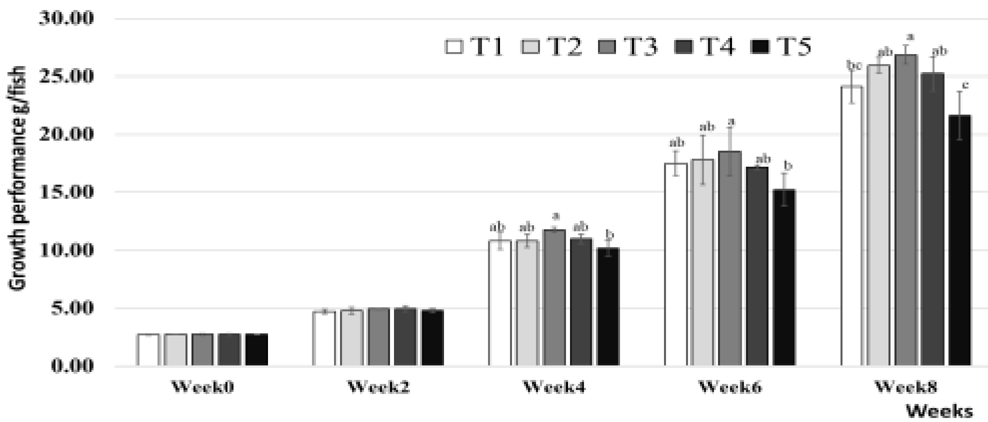

Figure 1. Average body weights of red hybrid tilapia fishes fed with different levels of mango seed meal for 8 weeks

The second order polynomial regression was employed based on dietary MSM replacement to estimate the optimum MSM content for predicting the maximum MSM replacement. The regression equations were achieved by y = –0.0038x2 + 0.1329x + 24.736, R ²= 0.9063 (for average body weight) and y = –0.0011x2 + 0.0377x + 15.789, R^2 = 0.9402 (for specific growth rate). The optimum dietary MSM replacement in red hybrid tilapia was estimated to be 17.46 and 17.14% to gain the maximum growth performance (Figure 2A, B).

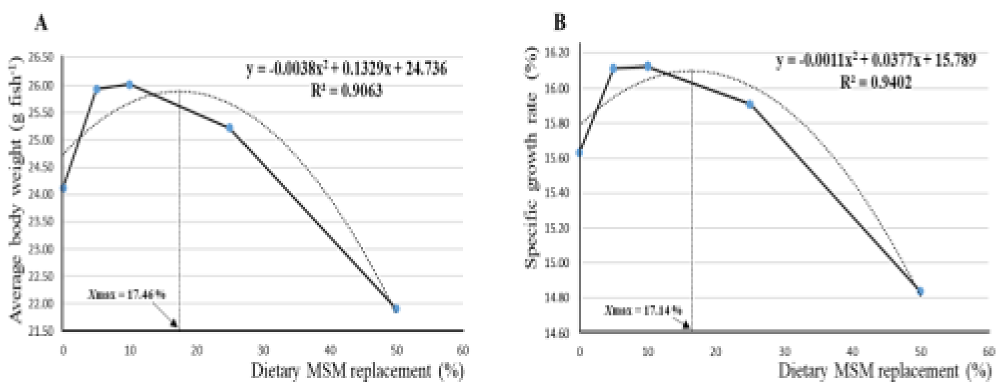

Figure 2. Polynomial regression analysis for red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) fed with diets containing graded levels of mango seed meal for 8 weeks. (A) Relationship between dietary MSM replacement and average body weight (y = $-0.0038x^2$ + 0.1329x + 24.736, R ² = 0.9063, when x = dietary MSM replacement, y = average body weight)

The results showed that average body weigh reached the maximum when dietary MSM was 17.46%. (B) Relationship between dietary MSM replacement and SGR ($y = -0.0011x^2 + 0.0377x + 15.789$, $R^2 = 0.9402$, when x = dietary MSM replacement, y = specific growth rate). The results showed that SGR reached the maximum when dietary MSM was 17.14%.

Feed utilization

The red hybrid tilapia fishes fed with diet containing 10% MSM showed the highest feed utilization in terms of FE and FER (Table 3) but this level was not significantly different from the fish fed of the control diet, 5% MSM, and 25% MSM replacement ($P > 0.05$). FCR was lowest in the fishes fed with diet containing 10% MSM but no significant differences ($P > 0.05$) were found between the control diet, 5% MSM, and 25% MSM levels (Table 3).

Protein utilization

The effects of different dietary MSM replacement levels on protein utilization of red hybrid tilapia are shown in Table 4. PER and PPV increased gradually with increasing dietary MSM level up to 10%, but gradually decreased thereafter. However, the PER of fishes fed with diets containing MSM in all treatments was not significantly different ($P > 0.05$). The PPV of fishes fed with 10% MSM diet was significantly higher ($P > 0.05$) than the other groups except for fishes fed with 25% MSM. The fishes fed with diet containing 50% MSM had the lowest PPV (Table 4).

Economic parameters

The economic analysis was evaluated and shown in Table 5. The feeding cost shows a progressive decrease with increasing inclusion levels of MSM. The control diet showed higher feeding costs than those of the experimental diets. Overall, the highest ECR was observed in 10% MSM diet, but it was not significantly different from the control, 5% MSM, and 25% MSM diets ($P > 0.05$) while the diet containing 10% MSM showed the lowest value ($P < 0.05$). The economic performance indicated that economic performance and cost-effectiveness could be improved with MSM supplementation in the diet (Table 5).

Table 2. Growth performance of red hybrid tilapia fed diets containing graded levels of mango seed meal for 8 weeks*

Parameters	**Level of MSM in diets (%)**					*p* – value
	Control	5% MSM	10% MSM	25% MSM	50% MSM	
Initial body weight	2.71 ±0.05	2.72 ±0.03	2.72 ±0.03	2.72 ±0.06	2.73 ±0.03	0.937
Final body weight	24.12 ±1.39^{bc}	25.94 ±0.66^{ab}	26.02 ±2.13^{a}	25.23 ±1.45^{ab}	21.63 ±2.12^{c}	0.009
Weight gain	763.20 ±43.06^{a}	823.57 ±57.29^{a}	827.30 ±109.70^{a}	796.90 ±33.76^{a}	635.42 ±93.31^{b}	0.009
Average daily gain	1.53 ±0.10^{b}	1.66 ±0.05^{ab}	1.72 ±0.05^{a}	1.61 ±0.10^{ab}	1.35 ±0.15^{c}	0.007
Specific growth rate	15.63 ±0.36^{a}	16.11 ±0.23^{a}	16.36 ±0.11^{a}	15.91 ±0.31^{a}	14.75 ±0.66^{b}	0.004
Survival rate	96.67 ±2.89	96.67 ±2.89	96.67 ±5.77	96.67 ±2.89	96.67 ±2.89	1.000

*Values are means ± S.D. of three replicates and values within the same row with different letters are significant difference ($P < 0.05$, n = 3).

Table 3. Feed utilization of red hybrid tilapia fed diets containing graded levels of mango seed meal for 8 weeks*

Parameters	**Level of MSM in diets (%)**					*p* – value
	Control	5% MSM	10% MSM	25% MSM	50% MSM	
Feed intake	49.97 ±2.82	53.55 ±0.97	52.99 ±3.27	52.83 ±1.72	52.49 ±1.97	0.067
Feed conversion ratio	2.34 ±0.24^{b}	2.31 ±0.11^{b}	2.20 ±0.20^{b}	2.35 ±0.17^{b}	2.79 ±0.20^{a}	0.024
Rate of feed intake	26.21 ±2.24^{b}	26.25 ±087^{b}	25.16 ±1.48^{b}	26.60 ±1.68^{b}	30.35 ±1.75^{a}	0.026
Feed efficiency	0.79 ±0.06^{a}	0.79 ±0.04^{a}	0.84 ±0.05^{a}	0.78 ±0.04^{a}	0.62 ±0.07^{b}	0.009
Feed efficiency ratio	44.36 ±4.25^{a}	44.74 ±2.14^{a}	46.85 ±4.13^{a}	43.93 ±3.13^{a}	36.89 ±2.70^{b}	0.040

*Values are means ± S.D. of three replicates and values within the same row with different letters are significant difference ($P < 0.05$, n = 3).

Table 4. Protein utilization in red hybrid tilapia fed diets containing graded levels of mango seed meal for 8 weeks*

Parameters	Level of MSM in diets (%)					*p* – value
	Control	5% MSM	10% MSM	25% MSM	50% MSM	
Protein efficiency ratio	3.02 ±0.10	2.37 ±0.21	3.41 ±0.18	3.09 ±0.02	3.00 ±0.16	0.067
Protein productive value	27.56 ±4.49^{b}	36.24 ±1.63^{b}	43.17 ±6.23^{a}	42.74 ±1.63^{a}	14.57 ±0.80^{c}	0.034

*Values are means ± S.D. of three replicates and values within the same row with different letters are significant differences (P < 0.05, n = 3).

Table 5. Economic analysis of Nile tilapia fed different levels of mango seed meal for 8 weeks*

Parameters	Level of MSM in diets (%)					*p* – value
	Control	5% MSM	10% MSM	25% MSM	50% MSM	
Diet cost	4.03	4.02	4.00	3.96	3.88	-
Economic conversion ratio	9.45 ±0.86^{b}	9.28 ±0.39^{b}	8.82 ±0.72^{b}	9.32 ±0.61^{b}	10.85 ±0.68^{a}	0.063
Economic profit index	0.55 ±0.03^{b}	0.60 ±0.02^{ab}	0.63 ±0.02^{a}	0.58 ±0.03^{ab}	0.46 ±0.05^{c}	0.001

*Values are means ± S.D. of three replicates and values within the same row with different letters are significant differences (P < 0.05, n = 3).

Discussion

In the last two decades, a significant amount of research has been carried out to investigate the replacement of high cost feed ingredients by searching for several potential by-products. The successful replacement in terms of growth performance with no effect on fish physiology has been found to be variable among fish species and environmental conditions (Kaushik *et al.*, 2004; Pereira and Oliva-Teles, 2003; Sitjà-Bobadilla *et al.*, 2005). MSM is a by-product that has been considered as potential alternative feed ingredient for animals. MSM has been investigated in diets in some vertebrate species such as chicken (Mbunwen *et al.*, 2015; Odunsi, 2005), sheep (Sanon *et al.*, 2013), and rabbits (Oluremi and Musa, 2004). However, there is only one study on MSM supplementation in the minnow carp (*Labeo senegalensis*) diet (Omoregie, 2001).

In the present study, red hybrid tilapia fed with 10% MSM in diet yielded the highest growth performance, feed utilization, protein utilization, and cost benefit compared to the control group. This results are within the range as previously shown where MSM successfully replaced diets for herbivorous fish that supplemented 10% of MSM in diets without compromising growth performance in minnow carp, *Labeo senegalensis* (Omoregie, 2001). Although the observation of 25% MSM inclusion in the diet was higher in fishes fed with the control diet, no significant differences were observed between the control and 25% MSM inclusion level. Recent studies showed that MSM could replace maize for 20% and 60% without any negative growth performance in rabbits and broiler chicks (Diarra *et al*., 2010; Oluremi and Musa, 2004).

All experimental feeds were well-accepted; however, fishes fed with diet containing MSM higher than 25% supplementation significantly reduced growth performance, feed utilization, and protein utilization. These results may be attributed to the presence of tannin that known as the anti-nutritional factor. However, little information is known about the effects of MSM replacement in diets on growth performance in fishes. The growth reduction and poor palatability observed in fishes fed with a high inclusion level could be related to an inappropriate amino acid composition in the diets, such as deficiency of essential amino acids or a reduction in digestibility of high plant inclusion level in the experimental diets (García-Ortega *et al.*, 2016). It has been reported that the dietary methionine level decreases with increasing plant ingredient replacement (García-Ortega *et al.*, 2016; Luo *et al.*, 2004; Wu *et al.*, 2017). Although the essential amino acid content in MSM and the requirement in red hybrid tilapia remain largely unknown, it is likely that dietary requirements are similar to other fish species wherein methionine is a limiting factor (Belghit *et* *al.*, 2014; Wu *et al.*, 2017). In addition, recent studies have reported that mango seeds contain phenolic compounds, such as phytate, oxalate, saponin and alkaloids (Abdalla *et al.*, 2007; Arogba, 2000). Moreover, reduced growth performance and feed utilization in red hybrid tilapia fed with MSM higher than 25% could possibly be a result of the presence of trypsin inhibitory activities. A recent study reported that MSM contains trypsin-inhibitor with approximately 18.42 TIU/mg protein (Fowomola, 2010). Generally, the trypsin inhibitor may interrupt digestive enzyme activities and cause deformity of microvilli in the intestine, however, further studies need to be investigated in the future.

In conclusion, the results of this study indicated that 10% MSM supplementation in the diet for red hybrid tilapia does not compromise growth performance, feed utilization and protein utilization. Moreover, this level slightly improved the feeding economic value. However, supplementation of MSM in the fish diet higher than 25% significantly decreased growth performance, palatability and protein utilization. These results further support the potential nutritional use of MSM as an ingredient for red hybrid tilapia feeds, highlighting the need to be aware of when high levels of MSM are included in the diet.

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