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Effects of Vegetable Oil Source and Dietary Vegetable-fish Oil Ratio on the Histological Alterations of Liver and Intestine of Juvenile Malaysian Mahseer, *Tor tambroides*

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ABSTRACT

For many years, fish oil has been used as the major source of lipid in aquafeed production. However, the replacement of this marine origin ingredient with a sustainable resource such as vegetable oils is critically important. On the other hand, most of the vegetable oils are high in omega-6 polyunsaturated fatty acids while a diet rich in these fatty acids can increase liver lipid deposition in some fish. This study was conducted to investigate the effects of partial or total replacement of fish oil with different vegetable oil sources (sunflower and palm) on the histological alterations in the liver and intestine of juvenile Malaysian mahseer, *T. tambroides*. Five isonitrogenous and isocaloric diets were formulated in order to either completely or partially (50%) substitute fish oil with sunflower oil or palm oil. A diet without substitution was also used as a control. The effects of these diets on the histological alterations of liver and intestine of fish after a nine week feeding period were investigated. The results showed few inflammatory cells and lipid vacuoles in the liver of fish fed all the vegetable oil diets indicating minor hepatic steatosis. Lipid vacuoles were also observed in the intestinal wall of fish fed all the vegetable oil diets.

Key words: Mahseer, *Tor tambroides*, vegetable oil, liver, histology, hepatic steatosis

INTRODUCTION

The mahseers (*Tor* spp.) are one of the large cyprinids occurred in Southeast Asia (Bhatt *et al.*, 2000). These fish are distributed in many rivers, streams and lakes of mild hills of the Himalayan belt from Afghanistan to Indo-China and Myanmar and form a significant stock of indigenous fish in India, Nepal, Bangladesh and Pakistan (Bista *et al.*, 2002; Islam, 2002). *Tor tambroides* and *Tor douronensis*, are two species of mahseers living in most major river systems of Malaysia, in particular Perak, Terengganu, Pahang and Kelantan states. These two sought-after species have a cultural and economic importance and are considered as a high-valued fish in Sarawak, Malaysia (Ingram *et al.*, 2005). Similar to other mahseers in many countries, the natural stock of this species is rapidly declining in recent years (Ingram *et al.*, 2007; Ramezani-Fard *et al.*, 2011a) and the government plans to develop its culture. Formulating a diet which meets the nutritional requirements of Malaysian mahseer is an important factor for launching the commercial aquaculture of this fish.

For many years, fish oil has been used as the major source of oil in aquafeed production. Although fish oil is a rich source of n-3 long chain polyunsaturated fatty acids (LC-PUFA) and prevents Essential Fatty Acid (EFA) deficiency in farmed fish, the dependence of aquaculture on fishery-derived products is a vicious circle and may not be sustainable in the future. Therefore, the replacement of marine origin resources such as fishmeal and fish oil is at the core of a heated global debate (Miller *et al*., 2007) and the only practical substitute for fish oil is still vegetable oils. Most of vegetable oils used in aquafeed industry are high in C18 Polyunsaturated Fatty Acids (PUFA), particularly n-6 PUFA and low in LC-PUFA. A diet rich in n-6 PUFA can increase liver lipid deposition in some fish (Robaina *et al*., 1998). In these cases, histopathology provides information to detect effects of irritants in various organs (Chezhian *et al*., 2012). Development of large lipid vacuoles in the liver leads to hepatocytes disruption and pyknotic nuclei occurrence. The current study was carried out to investigate the effects of partial or total replacement of fish oil with different vegetable oil sources (sunflower and palm) on the histological alterations in the liver and intestine of juvenile Malaysian mahseer, *T. tambroides*.

MATERIALS AND METHODS

Diet preparation: Five isonitrogenous and isocaloric diets with a lipid content of 5% were formulated in order to either completely or partially (50%) substitute fish oil with sunflower oil (diet S100 and S50) or palm oil (diet P100 and P50). A diet without substitution (containing 100% cod liver oil; CLO) was also used as a control. The compositions of the diets are shown in Table 1.

Table 1: Ingredients and proximate composition of the experimental diets

	Experimental diet				
Item	CLO	S100	S50	P100	P50
Ingredient (g kg^{-1} as fed basis)					
Fishmeal[a]	195	195	195	195	195
Soy meal	500	500	500	500	500
Corn meal	162	162	162	162	162
Corn starch	35	35	35	35	35
Rice bran	40	40	40	40	40
Cod liver oil[b]	38		19		19
Sunflower oil		38	19		
Linseed oil[c]					
Palm oil[d]				38	19
Vitamin premix[e]	20	20	20	20	20
Mineral premix[f]	10	10	10	10	10
Proximate composition (g kg^{-1} as fed basis)					
Crude Protein	390	390	394	394	394
Crude lipid	62	61	60	63	62
Ash	103	102	100	99	100
Carbohydrate[g]	331	330	330	332	335
Gross energy (kJ g^{-1})	18.0	18.0	17.8	18.0	18.1
Moisture	114	117	116	112	109

[a]Defatted Malaysian fishmeal (690 g kg^{-1} crnde protein), [b]Seven Seas, [c] Sigma-Aldrich, [d]Refined, bleached and deodorized palm olein, [e]Vitamin premix (g kg^{-1} premix): Ascorbic acid: 45, Myo-inositol: 5, Choline chloride: 75, Niacin: 4.5, Riboflavin: 1, Pyridoxine: 1, Thiamin mononitrate: 0.9, Ca-pantothenate: 3, Retinyl acetate: 0.6, Cholecalciferol: 0.08, Vitamin K menadione: 1.7, α-tocopherol acetate (500 IU g^{-1}): 8, Biotin: 0.02, Folic acid: 0.1, Vitamin B_{12}: 0.001, Cellulose: 845.1, [f]Mineral premix (g kg^{-1} premix): KCl: 90, KI: 0.04, $CaHPO_4.2H_2O$: 500, NaCl: 40, $CuSO_4.5H_2O$: 3, $ZnSO_4.7H_2O$: 4, $CoSO_4$: 0.02, $FeSO_4.7H_2O$: 20, $MnSO_4.H_2O$: 3, $CaCo_3$: 215, MgOH: 124, Na_2SeO_3: 0.03, NaF: 1, [g]Carbohydrates = Dry matter - [protein + lipid + ash]

Prior to mixing of the basal ingredients, fishmeal was defatted by soaking in a mixture of chloroform and methanol (2:1, v/v) for 24 h in order to eliminate any effect of residual LC-PUFAs originated from fishmeal (Kim and Lee, 2004). Defatted fishmeal was then oven dried and mixed with the other dry ingredients according to method explained by Kamarudin *et al.* (2011).

Rearing and sampling: Fish obtaining and rearing methods for this experiment are similar to those explained by Kamarudin *et al.* (2011). Briefly, wild-caught *T. tambroides* juveniles of an initial weight of 5.0±0.4 g (Mean±SD) were randomly distributed into 21 rectangular-shaped glass aquaria with the stocking density of 10 fish per aquarium. Each aquarium was supplied with 65 L dechlorinated public utility water and equipped with a recirculating system. The experiment was conducted for 9 weeks and fish were fed twice per day close to visual satiety. At the end of experiment, three fish per aquarium were sacrificed and dissected and their visceral organs including liver were extracted. The extracted tissues were fixed in Bouin's solution at room temperature for 24 h, then washed and stored in 70% ethanol until the wax embedding (Ramezani-Fard *et al.*, 2011a). Serial 5 μm sections were prepared, stained with haematoxylin-eosin and slides were examined under a light microscopy (Zeiss Primo Star) fitted with a digital camera (Canon A640).

Biochemical analysis: Analytical methods for the determination of crude protein, crude lipid and ash contents as well as gross energy of experimental diets have been already explained (Kamarudin *et al.*, 2011). Lipid of the experimental diets were extracted with a chloroform:methanol (2:1, v:v) mixture (Folch *et al.*, 1957; Ramezani-Fard *et al.*, 2011b) and transesterified with methanolic boron trifluoride according to Kamarudin *et al.* (2011). Fatty acid methyl esters were then separated on a fused silica capillary column (Supelco SP-2330: 30 m×0.25 mm, film thickness 0.20 μm) in a gas chromatograph (Agilent 7890N) according to the method described by Ramezani-Fard *et al.* (2012).

RESULTS

Fatty acid composition of all the experimental diets is shown in Table 2. Substitution of fish oil with sunflower or palm oil decreased total n-3 PUFA content of experimental diets. The highest content of 20:5 n-3 and 22:6 n-3 were observed in control diet. Palm oil substitution for fish oil increased total Saturated Fatty Acid (SFA) content of diets.

The liver of fish fed control group were composed of basophilic polygonal cells with a spherical nucleus in the center and showed no histopathological changes in the tissues tested by light microscope (Fig. 1a). The intestinal wall of this group consisted of mucosa, lamina propria, muscularis and serosa did not reveal any histopathological changes (Fig. 1b). Few inflammatory cells, lipid vacuoles and migrated nuclei were observed in the liver of fish fed sunflower oil diets (Fig. 2a, c). Degenerated hepatocytes and minor hepatic steatosis were also observed in fish fed palm oil diets (Fig. 2d). However, regenerated liver cells that tend to exhibit a resistance to the fatty change were also observed in fish fed these diets (Fig. 2b). Lipid vacuoles also existed in the intestinal wall of fish fed all the vegetable oil diets (Fig. 3).

Table 2: Fatty acid composition (% of total fatty acid) of the experimental diets

	Experimental diet				
Fatty acid	S100	S50	P100	P50	CLO
14:0	0.3	2.2	1.0	2.5	3.9
16:0	10.4	13.4	33.0	26.3	16.9
16:1 n-7	0.5	2.6	0.5	2.6	5.0
18:0	4.5	3.8	4.4	4.0	3.2
18:1 n-9	28.9	25.5	40.8	33.1	22.9
18:2 n-6	54.4	36.6	19.1	16.6	13.7
18:3 n-3	0.2		0.5	0.1	
20:0	0.8	1.3	0.9	1.2	1.8
20:1 n-9		3.3		3.1	7.2
20:4 n-6		0.5		0.4	0.9
22:1 n-11		3.7		3.5	8.1
20:5 n-3		3.0		2.7	6.8
22:5 n-3		0.8		0.8	1.9
22:6 n-3		3.3		3.1	7.7
ΣSFA	16.1	20.6	39.2	33.9	25.9
ΣMUFA	29.4	35.1	41.3	42.4	43.2
ΣPUFA n-3	0.2	7.2	0.5	6.7	16.5
n-3/n-6	0.0	0.2	0.0	0.4	1.1

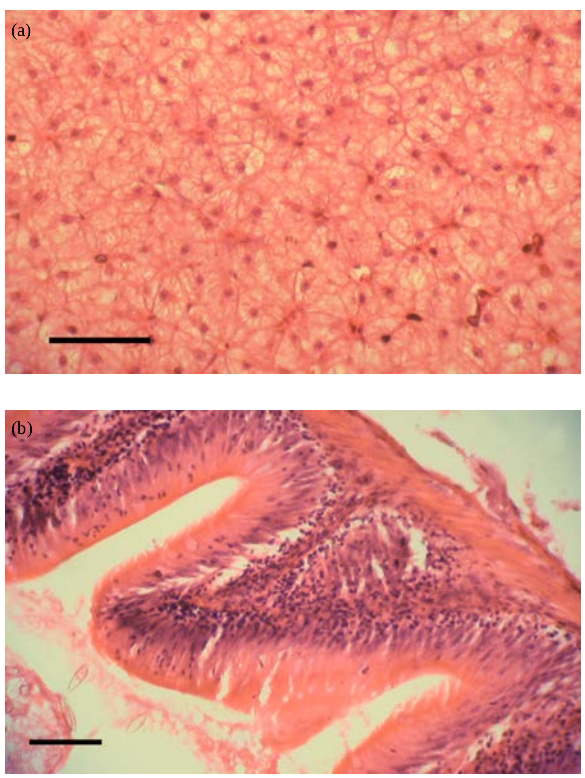

Fig. 1(a-b): Histology of (a) Liver and (b) Intestine of fish fed with control diet, Scale bar = 50 μm

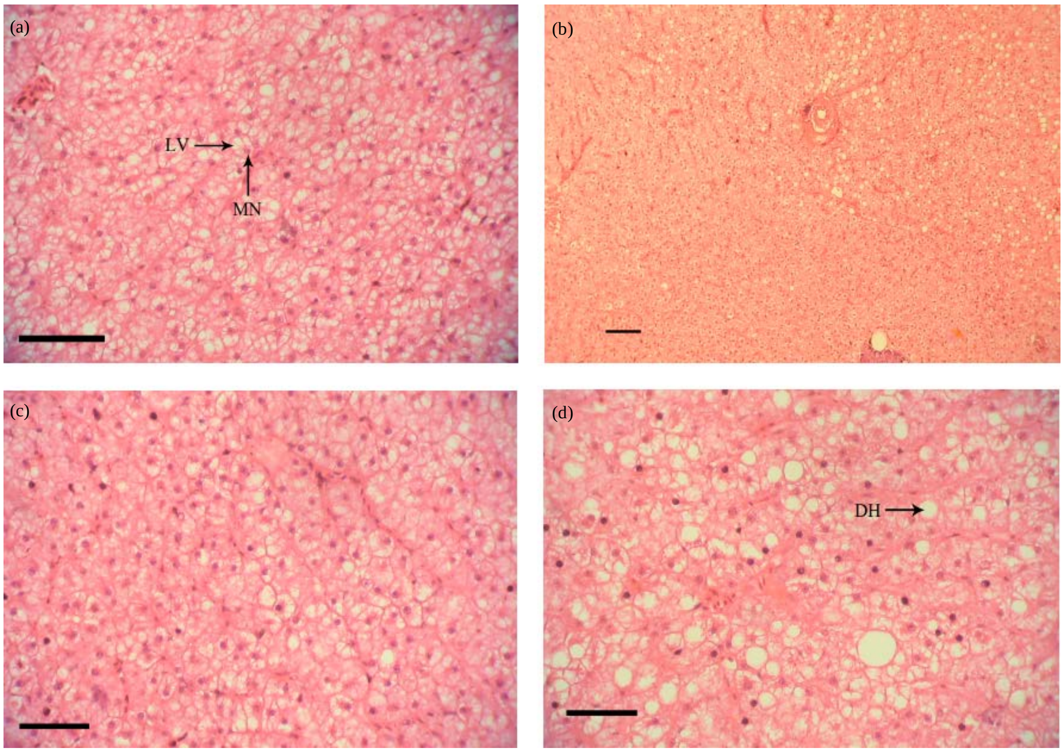

Fig. 2(a-d): Histology of the liver of fish fed with vegetable oil diets, (a) Lipid vacuoles (LV) and migration of nuclei (MN) in the liver of fish fed with S50 diet, Scale bar = 50 µm, (b) Minor hepatic steatosis in the liver of fish fed with P50 diet, Liver cells with minimal steatosis in the bottom half of this photograph represent regenerated liver hepatocytes that tend to exhibit a resistance to the fatty change, Scale bar = 200 µm, (c) Minor hepatic steatosis in the liver of fish fed with S100 diet, Scale bar = 50 µm and (d) Degenerated Hepatocytes (DH) in the liver of fish fed P100 diet, Scale bar = 50 µm

Fig. 3: Lipid vacuoles (LV) in the intestinal wall of fish fed with S50 diet, Scale bar = 50 µm

DISCUSSION

Fish oil derived from industrial fisheries has been the only practical source of EFA in the aquafeed production since many decades ago. Global fisheries are stagnating now while the demand for fish oil is increasing along with the development of aquaculture industry (Sargent *et al.*, 2002). This paradox can limit the development of aquaculture production in the future. Therefore, finding a feasible alternative for fish oil is urgently needed in aquafeed industry. It is now completely clear that fish oil can be partially or fully substituted by vegetable oil in the diet of some freshwater species without any adverse effect on their growth performance (Ng *et al.*, 2003). It has been also shown that substitution of fish oil with palm oil in the diet of Malaysian mahseer improves the growth performance of juveniles (Kamarudin *et al.*, 2011).

On the other hand, fish oil replacement usually decreases the n-3 LC-PUFA content of muscle and reduces the nutritional value of fish for human consumption. Therefore, the most important limiting factor in replacing fish oil in the diet of freshwater teleost is reduction of n-3 LC-PUFA in the muscle of fish fed fish oil free diets. Although freshwater species are theoretically able to produce n-3 LC-PUFA from the 18:3 n-3, most of them cannot desaturate and elongate large quantity of 18:3 n-3. As an illustration, rainbow trout fed a 22% linseed oil diet can only convert 12.4% of the dietary 18:3 n-3 to longer and more unsaturated homologues, while 58.1% of this fatty acid is accumulated and 29.5% is oxidized (Turchini and Francis, 2009). Therefore, maximum efforts have to be made to retain the n-3 LC-PUFA in the body of farmed fish and to prevent its oxidation. Ramezani-Fard *et al.* (2012) suggested that only 2.5% n-3 PUFA in the diet of *T. tambroides* with adequate amount of saturated fatty acid provide the best growth performance and retain the n-3 content of tissues.

It should be taken into account that high inclusion of vegetable oil in the diet of fish in order to provide sufficient amounts of saturated fatty acids may have negative effects on the fish liver. Minor hepatic steatosis was observed in this study when fish fed a diet with partial fish oil substitution. Most of vegetable oils, sunflower oil in particular, contain high levels of 18:2 n-6. This fatty acid has a lipogenic effect and it has been shown that its high percentage causes intense steatosis in the most fish hepatocytes (Caballero *et al.*, 2004). However, the high level of 18:3 n-3, which is similar to n-3 LC-PUFAs in term of hypolipidemic effect, does not histologically change the hepatocytes cells (Caballero *et al.*, 2004).

The pathogenic effects of steatosis in fish have not been fully understood. Mosconi-Bac (1990) emphasized the pathogenic alteration made to liver by steatosis while (Caballero *et al.*, 2004) considered steatosis as a physiological adaptation to the diet which can be reversed after fish are fed a balanced diet.

CONCLUSION

In conclusion, this study showed that replacement of fish oil with vegetable oil increases lipid vacuoles in the epithelial cells of intestine and in the hepatocytes of Malaysian mahseer. Moreover, such replacement causes minor steatosis in this fish. The effects of these alterations on the growth performance and survival rate of this fish in a long time rearing period should be further investigated.

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REFERENCES

Bhatt, J.P., P. Nautiyal and H.R. Singh, 2000. Population structure of Himalayan mahseer, a large cyprinid fish in the regulated foothill section of the river Ganga. Fish. Res., 44: 267-271.

Bista, J., B.R. Pradhan, A.K. Rai, R.K. Shrestha and T.B. Gurung, 2002. Nutrition, Feed and Feeding of Gold Mahseer (*Tor putitora*) for Domestication and Production in Nepal. In: Cold Water Fisheries in the Trans-Himalayan Countries, Petr, T. and S.B. Swar (Eds.). Food and Agriculture Organization of the United Nations, Rome, pp: 107-117.

Caballero, M.J., M.S. Izquierdo, E. Kjorsvik, A.J. Fernandez and G. Rosenlund, 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils recovery of normal morphology after feeding fish oil as the sole lipid source. J. Fish Dis., 27: 531-541.

Chezhian, A., D. Senthamilselvan and N. Kabilan, 2012. Histological changes induced by ammonia and ph on the gills of fresh water fish *Cyprinus carpio* var. communis (Linnaeus). Asian J. Anim. Vet. Adv., 7: 588-596.

Folch, J., M. Lees and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Ingram, B., S. Sungan, G. Gooley, S.Y. Sim, D. Tinggi and S.S. De Silva, 2005. Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, *Tor tambroides* and *T. douronensis*. Aquacult. Res., 36: 983-995.

Ingram, B.A., S. Sungan, D. Tinggi, S.Y. Sim, G.J. Gooley and S.S. De Silva, 2007. Development in the spawning of *Tor tambroides* and T. *Douronensis* in captivity. Proceeding of the International Symposium on the Mahseer, March 29-30, 2006, Malaysian Fisheries Society, Kuala Lumpur, Malaysia, pp: 123-126.

Islam, M.S., 2002. Evaluation of supplementary feeds for semi-intensive pond culture of mahseer, *Tor putitora* (Hamilton). Aquaculture, 212: 263-276.

Kamarudin, M.S., E. Ramezani-Fard, C.R. Saad and S.A. Harmin, 2011. Effects of dietary fish oil replacement by various vegetable oils on growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer, *Tor tambroides*. Aquacult. Nutr. 10.1111/j.1365-2095.2011.00907.x

Kim, K.D. and S.M. Lee, 2004. Requirement of dietary n-3 highly unsaturated fatty acids for juvenile flounder (*Paralichthys olivaceus*). Aquaculture, 229: 315-323.

Miller, M.R., P.D. Nichols and C.G. Carter, 2007. Replacement of fish oil with *Thraustochytrid schizochytrium* sp. L oil in Atlantic salmon parr (*Salmo salar* L.) diets. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 148: 382-392.

Mosconi-Bac, N., 1990. Reversibility of artificial feed-induced hepatocyte disturbances in cultured juvenile sea bass (*Dicentrarchus labrax*): An ultrastructural study. Aquaculture, 88: 363-370.

Ng, W.K., P.K. Lim and P.L. Boey, 2003. Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. Aquaculture., 215: 229-243.

Ramezani-Fard, E., M.S. Kamarudin, S.A. Harmin, C.R. Saad, M.K. Abd Satar and S.K. Daud, 2011a. Ontogenic development of the mouth and digestive tract in larval Malaysian mahseer, *Tor tambroides* Bleeker. J. Applied Ichthyol., 27: 920-927.

Ramezani-Fard, E., M.S. Kamarudin, C.R. Saad and S.A. Harmin, 2011b. Changes over time in muscle fatty acid composition of Malaysian mahseer, *Tor tambroides*, fed different dietary lipid percentage. Afr. J. Biotechnol., 10: 18256-18265.

Ramezani-Fard, E., M.S. Kamarudin, S.A. Harmin and C.R. Saad, 2012. Dietary saturated and omega-3 fatty acids affect growth and fatty acid profiles of Malaysian mahseer. Eur. J. Lipid Sci. Tech., 114: 185-193.

Robaina, L., M.S. Izquierdo, F.J. Moyano, J. Socorro, J.M. Vergara and D. Montero, 1998. Increase of the dietary n-3/n-6 fatty acid ratio and addition of phosphorus improves liver histological alterations induced by feeding diets containing soybean meal to gilthead seabream, *Sparus aurata*. Aquaculture, 161: 281-293.

Sargent, J.R., D.R. Tocher and G.J. Bell, 2002. The Lipids. In: Fish Nutrition, Halver, J.E. and R. Hardy (Eds.). 3rd Edn. Academic Press, San Diego, CA., USA., ISBN: 9780123196521, pp: 181-257.

Turchini, G.M. and D.S. Francis, 2009. Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. Br. J. Nutr., 108: 69-81.