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Full Paper

Enhancement of oxidative defence and growth performance of Nile tilapia by omega-9-rich freshwater fish oil

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Abstract: Freshwater fish oil (FFO) was extracted from adipose tissue of freshwater hybrid catfish and evaluated for its physical and chemical properties. The clear yellow oil showed properties similar to those of commercial fish oil. Interestingly, the omega-9 monounsaturated fatty acid content of the FFO is four times that found in marine fish oil. Fish feed was formulated with the FFO and fed to Nile tilapia in cages for four months. Growth performance including body weight, weight gain and average daily gain of the Nile tilapia treated with feed supplemented with 1% and 1.5% FFO dramatically increased ($p < 0.05$) and the feed conversion rate decreased significantly. Moreover, the malondialdehyde level also significantly decreased in the plasma, liver and kidney of the fish fed with 0.5%, 1% and 1.5% FFO. In addition, glutathione levels increased significantly in erythrocytes of Nile tilapia fed with 0.5% and 1.0% FFO. The findings indicate that FFO can improve both the growth performance and oxidative defence in Nile tilapia. The oil enhances oxidative defence by decreasing lipid peroxidation and increasing endogenous antioxidants. Therefore, FFO can be supplemented in fish feed, replacing marine fish oil, to improve growth and decrease the cost of aquaculture.

Keywords: freshwater fish oil, omega-9 fatty acid, oxidative defence, Nile tilapia

INTRODUCTION

The freshwater-fish processing industry in Thailand has notably expanded recently, especially the frozen fish-fillet industry. In the production of frozen fish fillets, up to 50% of the whole fish is usually discarded as waste. This waste can be utilised for fish oils, enzymes, vitamins

or flavourants and in the production of gelatine for animal feed products [1]. However, waste adipose tissue (5-10% of fish total body weight) has not yet been properly utilised.

Fat is a vital nutrient for growth and development of fish. It is a source of energy and plays a role in the transportation of fat-soluble vitamins. Marine fish oil (MFO) contains omega-3, -6 and -9 fatty acids and has been utilised as aquaculture feed worldwide [2, 3]. Increases in bodyweight, survival rate, food conversion efficiency, immune system strength, and resistance to disease among fish have been demonstrated by research into marine fish oil supplementation [4].

Farming of tilapia is one of the most popular types of aquaculture [5]. The global production of tilapia was about 2.8 million metric tons in 2008 [6] and is estimated to increase to 8.89 million metric tons by the year 2020 [3]. This rapidly increasing global production of tilapia is associated with increasing usage of commercial pelleted feeds and the introduction of improved strains of Nile tilapia (*Oreochromis niloticus*), which is the major farmed tilapia species. Tilapia feeding costs account for more than 60% of the production cost in intensive aquaculture systems. Also, the nutrient requirements of high-performing tilapia strains are different from those of non-improved strains. This information is important for feed formulators and fish farmers in optimising feed formulation and managing maximum cost competitiveness [7].

Accordingly, fish oil is the major source of dietary lipids in commercial fish feeds including tilapia feeds. However, global fish oil production is stagnating, and there is a corresponding increase in demand and fish oil prices, necessitating the use of alternative lipid sources for aquafeeds [8]. This study aims to determine the fatty acid composition as well as some physical and chemical properties of freshwater fish oil (FFO) extracted from the visceral adipose tissue of catfish. It also aims to evaluate the effect of FFO supplementation on the growth performance and oxidative defence of Nile tilapia.

MATERIALS AND METHODS

Preparation of Freshwater Fish Oil

FFO was extracted from the adipose tissue of freshwater hybrid catfish (*Pangasius* sp.) obtained from Thai Panga Farm, Kalasin, Thailand. Briefly, the adipose tissue from the visceral abdomen was steamed at 90°C for 30 min. The liquid oil was filtered with a filtering sack, which was squeezed by a screw compressor. To separate the solid particles, the obtained liquid was subsequently centrifuged at 4500 rpm at 25°C for 10 min. The supernatant FFO was separated and analysed for its fatty acid composition at the Central Laboratory (Thailand) Co. Ltd., Chiang Mai Branch, following an in-house method based on AOAC 996.06 [9]. In addition, the properties of the FFO, namely the acid value, iodine value and free fatty acid content, were determined [10] and compared to a commercial MFO which was purchased at the pharmacy.

Fish Experiment and Diet

Four hundred Nile tilapia (*Oreochromis niloticus*) fingerlings (50 ± 3 g each) were obtained from the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. All fish were acclimated to the experimental system for two weeks before beginning the experimental FFO feeding trial. The fish were fed with an equal amount of their normal diet which contained similar protein and energy content in all treatments. After acclimation was finished, the fish were divided into four groups of 100 fish, and each group was further subdivided into two subgroups, resulting in 8 subgroups of 50 fish each. The fish were fed with diets containing four different levels of FFO: 0%, 0.5%, 1% and 1.5%. The ingredients in the fish feeds are shown in

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Table 1. The fish were provided with 3% of their body weight in feed daily, in twice daily feedings (09:00 a.m. and 04:00 p.m.) for four months. They were kept in 1.2 m × 1.2 m × 1.2 m net cages. The animal facilities and protocols were approved by Maejo University Animal Care and Use Committee.

Table 1. Composition of Nile tilapia feeds

Ingredient	Composition (%)			
	0% FFO	0.5% FFO	1% FFO	1.5% FFO
Fish meal	15	15	15	15
Soybean meal	37	39	37	37
Broken rice	26	31	26	23
Rice bran	20	13	20	22
Freshwater fish oil	0	0.5	1	1.5
Soybean oil	1	0	0	0
Multi-Vitamin (Premix)	1	1	1	1
Energy (kcal/100 g)	400	400	400	400
Protein (%)	30	30	30	30

Growth Performance

The growth rate of all fish was measured every month for four months. Body weight (BW), weight gain (WG), average daily gain (ADG) and feed conversion rate (FCR) were determined as follows: BW = final weight − initial weight; WG = (final weight − initial weight)/duration (month); ADG = weight gain / duration (day); and FCR = weight of diet / weight gain in fish.

Sampling Procedures

Four fish from each group were randomly selected and anesthetized with 1% clove oil. Blood samples (3 mL) were collected from the caudal vein of each fish with a sterile syringe. The plasma was prepared by centrifugation of the blood at 1300 rpm, 4°C for 10 min., and kept frozen at −80°C. The erythrocytes were lysed by adding deionised water with shaking for 5 min. and then centrifuging at 13000 rpm, 4°C for 10 min. The supernatant was precipitated with meta-phosphoric acid and kept frozen at −20°C. Liver and kidney tissues were quickly dissected and kept in an ice box for a few min. They were then cut into small pieces of 40 mg and suspended in a lytic buffer containing a protease inhibitor. The tissues were homogenised and centrifuged at 2500 rpm, 4°C for 10 min. The supernatant was collected and stored at −80°C for lipid peroxidation assay.

Lipid Peroxidation Assay and Glutathione Assay

The liver and kidney tissues and plasma were analysed for the highly reactive and unstable lipid hydro-peroxides resulting from the formation of malondialdehyde (MDA), which can be quantified colourimetrically following a controlled reaction with thiobarbituric acid. Plasma and tissue homogenates were measured via a thiobarbituric acid reactive substances (TBARS) assay kit (Cayman, USA) according to plate-based colourimetric measurements (530–540 nm). The glutathione (GSH) level of erythrocytes was determined using a GSH assay kit (Cayman, USA).

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The GSH content was estimated for total GSH disulphide (GSSG) and/or the reduced sulphydryl (GSH) in erythrocyte lysates by plate-based colourimetric measurement (405–414 nm).

Statistical Analysis

The data were expressed as mean ± standard error (S.E.) and analysed via one-way analysis of variance (ANOVA). In cases of significant differences ($p < 0.05$) the Tukey honest significant difference post hoc test was applied.

RESULTS

Yield of FFO

Clear yellow FFO extracted from the visceral fat of catfish was obtained at a yield of 270 mL per kg. The measured acid value, iodine value and free fatty acids are given in Table 2. The fatty acid composition of FFO and MFO was compared (Table 3). The quantities of saturated and unsaturated fatty acids in FFO were similar to those in the commercial MFO. However, the quantity of polyunsaturated fatty acids in the FFO was lower than that in MFO, especially omega-3 fatty acid. Interestingly, the omega-9 acid (oleic acid) content in FFO was four times higher than that found in MFO.

Table 2. Acid value, iodine value and free fatty acids content of FFO and MFO

Oil	Acid value (mg/g)	Iodine value	Free fatty acids %
FFO	4.48 ± 0.00	4.49 ± 0.24	2.25 ± 0.65
MFO	2.61 ± 0.64	0.93 ± 0.22	1.31 ± 0.64

Note: Values expressed as mean ± S.E.

Table 3. Fatty acid composition of FFO and MFO

Fatty acid composition	Amount (g/100 g)	
	FFO	MFO
Saturated fatty acids	37.70	35.27
Unsaturated fatty acids	62.30	64.75
Monounsaturated fatty acids	49.67	23.78
- Oleic acid (C18:1 n-9) (omega-9)	44.57	11.24
Polyunsaturated fatty acids (PUFA)	12.63	40.97
- Linoleic acid (C18:2 n-6)	10.11	4.47
- α-Linolenic acid (C18:3 n-3)	0.62	0.83
- Ecosapentaenoic acid (C20:5 n-3)	0.05	20.98
- Docosahexaenoic acid (C22:6 n-3)	0.44	12.25
Omega-3	7.25	34.15
Omega-6	11.15	6.24
Omega-9	44.74	11.94

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Effects of FFO on Growth Performance and Feed Efficiency

The growth performance of Nile tilapia treated with feed supplemented with 0% (control), 0.5%, 1% and 1.5% FFO is shown in Figure 1. The growth performance (BW, WG and ADG) of Nile tilapia treated with 1% FFO was statistically different compared with the control group ($p < 0.05$) at the second month (Figures 1A-C). Furthermore, there was a statistical difference in the growth performance of Nile tilapia treated with 1% and 1.5% FFO in the first, third and fourth months. There was also a statistical difference in FCR compared with the control group (Figure 1D).

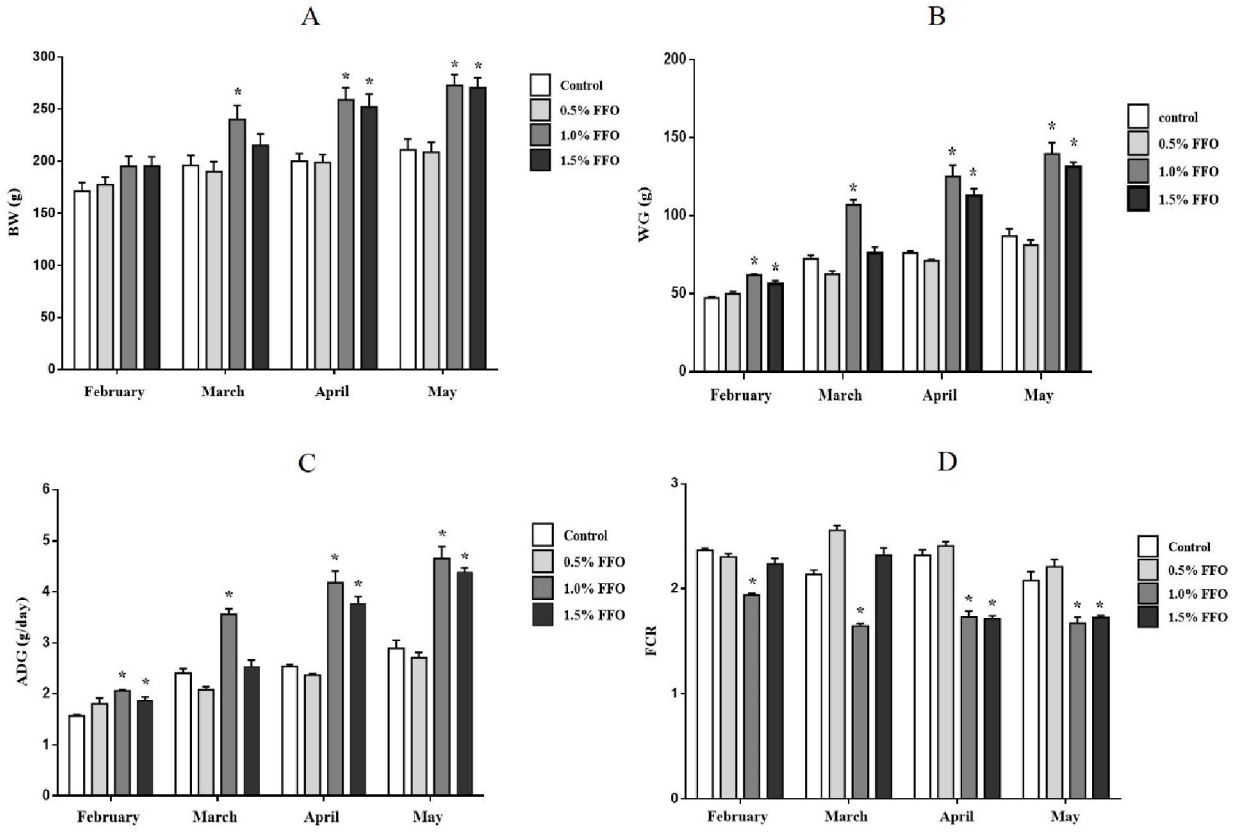

Figure 1. Effects of FFO on growth performance over a period of four months: (A) BW, (B) WG, (C) ADG and (D) FCR. Values are expressed as mean ± S.E. * = statistically different from control ($p < 0.05$).

Effects of FFO on MDA Level in Liver, Kidney and Plasma

An assay for TBARS was conducted to determine oxidative stress conditions using the total level of MDA, which is the end product of lipid peroxidation. The group treated with 1.5% FFO showed a lower MDA level in the kidney compared to the control group ($p < 0.05$) in the second month. The MDA level in all treatment groups was dramatically lower than that in the control group ($p < 0.05$) in the fourth month (Figure 2A). In the plasma the MDA level in the group treated with 1.5% FFO was significantly lower ($p < 0.05$) compared to that in the control group in the second month through to the fourth month. The group treated with 1% FFO showed notably lower MDA levels ($p < 0.05$) compared with the control group only in the fourth month (Figure 2B). In the liver

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there was a statistically significant difference in the MDA level in the group treated with 0.5% FFO compared to the control group in the second and fourth months (Figure 2C).

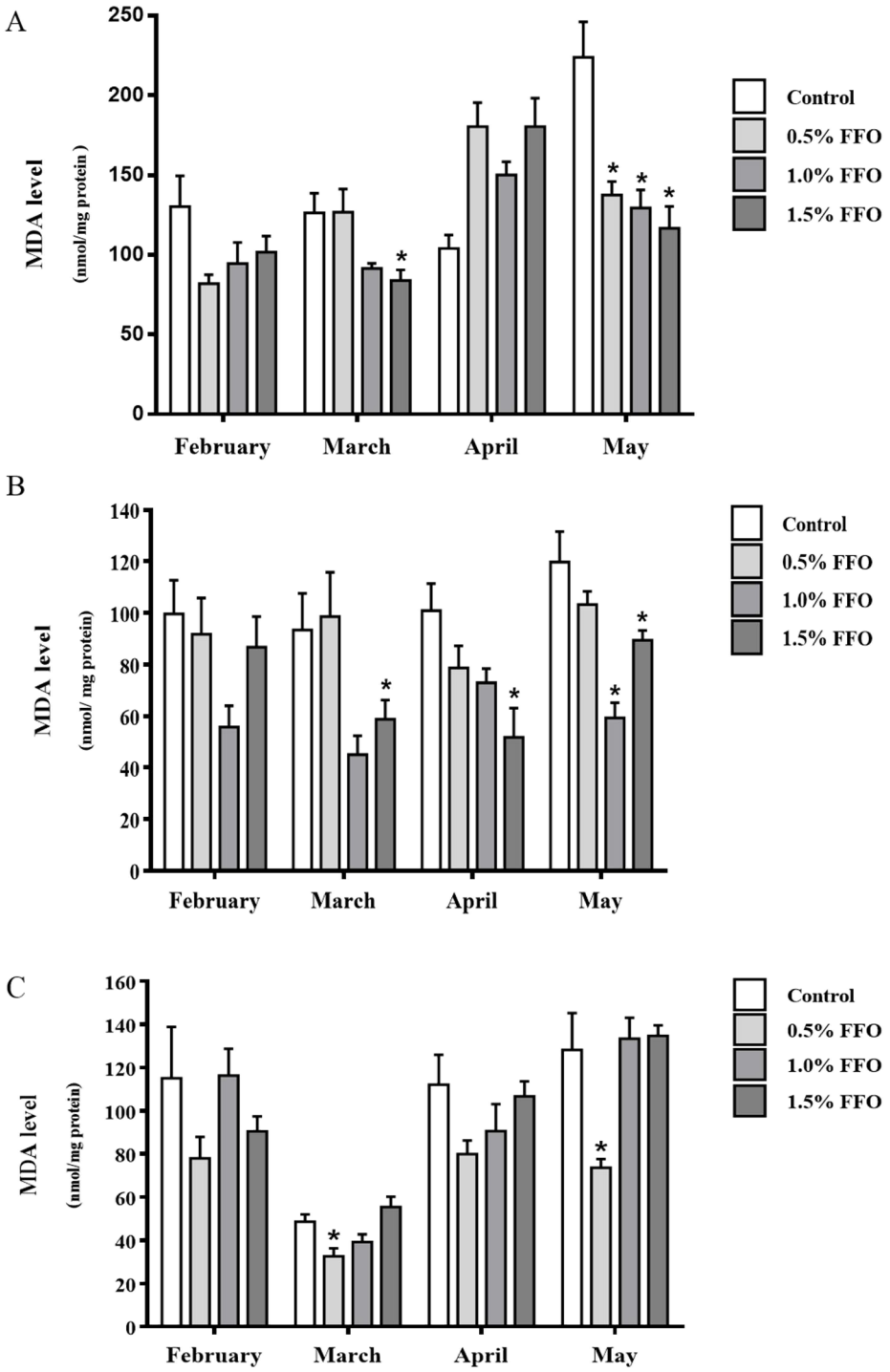

Figure 2. Effects of FFO on MDA level over four months: levels of MDA in kidney (A), plasma (B) and liver (C). Values are expressed as mean ± S.E. * = statistically significant from control ($p < 0.05$)

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Effects of FFO on GSH Level in Erythrocytes

GSH levels in tilapia erythrocytes after treatment with FFO diets for four months are shown in Figure 3. The increase in GSH levels was statistically significant in Nile tilapia fed with 0.5% and 1.0% FFO compared to the initial treatment.

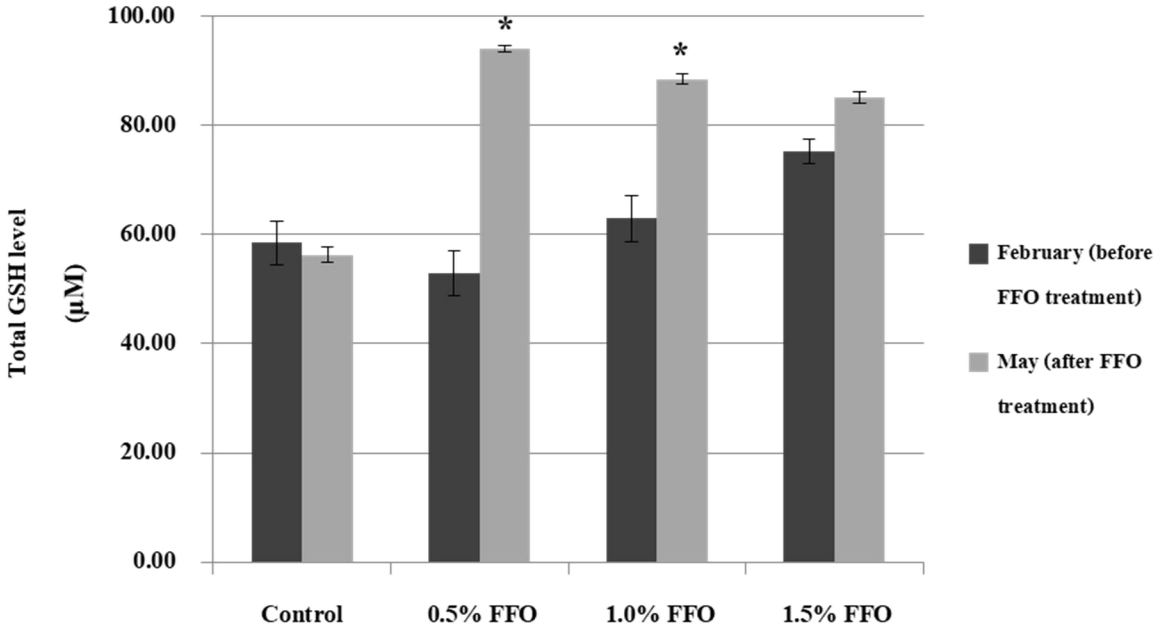

Figure 3. Effect of FFO on GSH level in erythrocytes. Values are expressed as mean ± S.E. * = statistically significant from control ($p < 0.05$)

DISCUSSION

The effects of FFO supplementation on the growth performance and oxidative defensive mechanism in Nile tilapia were determined. FFO was extracted from adipose tissue by-product from the fisheries industry and its properties were evaluated. It was a clear yellow liquid exhibiting acid value, iodine value and fatty acid content in accordance with the Food and Drug Administration Standard, Thailand [11]. Interestingly, the quantity of monounsaturated fatty acid (omega- 9) in the FFO was four times higher than that in the MFO.

The results of the experiments on fish treated with FFO were analysed. The fish fed with 1% and 1.5% FFO feed displayed significant increases in growth performance and decreases in FCR. Similar results have been found in many other studies such as that of Ng et al. [12], who reported high levels of growth performance and decrease in FCR in red hybrid tilapia fed with 10% cod liver oil. Moreover, supplementation with high levels of PUFA in fish feed caused a decrease in FCR in *Tilapia zillii* and hybrid tilapia (*Oreochromis niloticus* X *O. aureus*) [13-15].

With regard to oxidative defence, a decrease in MDA levels in kidney and plasma was demonstrated in the groups supplemented with 1% and 1.5% FFO as well as in liver of the group supplemented with 0.5% FFO. Lipid peroxidation is a well-established mechanism of cellular injury in animals and is used as an indicator of oxidative stress in cells and tissues [15, 16]. The TBARS method is commonly used to determine MDA in biological samples [17]. A decrease in MDA level is directly associated with the level of free radicals in lipid peroxidation. However, this reaction is relatively nonspecific: both free and protein-bound MDA can react. Decreasing free radicals can improve the health and immunity in fish as well as the healthy condition of biological membranes

that are rich in PUFA [16]. It was found that PUFA supplementation in rainbow trout (*Oncorhynchus mykiss*) causes a decrease in MDA levels in blood and tissues [18].

Ng et al. [19] found that Nile tilapia fed with a diet with 8% MFO gave the highest fillet TBARS among all dietary treatments and their control group. In a previous study the crude oil of freshwater hybrid catfish showed high antioxidant activities when the scavenging activity of ABTS radicals was assayed, whereas a dose of the crude oil at 5000 mg/kg administered to albino rats did not show any signs of acute toxicity nor cause any adverse symptoms and mortality [20]. The relationship between lipid peroxidation and the process of aging has been extensively investigated. The results suggest that lipid peroxidation is caused by a reduction of antioxidant activity with aging and that the associated changes depend on species, strain and sex, and are specific to tissue type. Interestingly, the decrease in MDA levels in this study was shown at FFO concentrations of 0.5% – 1.5%.

GSH is a tripeptide and a major endogenous antioxidant produced by cells and helps to protect cells from reactive oxygen species such as free radicals and peroxides [21]. It is the most abundant intracellular non-protein thiol and one of the most important intracellular antioxidants. It has numerous intracellular functions including reduction of hydrogen peroxide and lipid peroxides (as a co-factor for glutathione peroxidises), detoxification of electrophilic toxicants (as a conjugant for glutathione transferases), regulation of protein function, and regulation of nucleotide metabolism [22]. This indicates that the enhancement of GSH enzyme may be used to prevent free radical damage. One report showed that rainbow trout fed with 2% n-3 PUFA exhibited increased GSH levels in the blood, gonad, liver and kidney [18]. In our study high omega-9 FFO supplementation in fish feed with 0.5% and 1% FFO exhibited an increase in GSH level in the erythrocytes of Nile tilapia. The findings indicate that FFO might be involved in the anti-oxidative activity and might increase endogenous antioxidants, leading to high growth performance in Nile tilapia.

CONCLUSIONS

Growth performance and oxidative defence in Nile tilapia have been shown to be enhanced by the supplementation of fish feed with FFO. The oil, a by-product of the freshwater-fish processing industry, therefore seems capable substituting for MFO as a feed supplement in order to enhance the oxidative defence and growth performance of Nile tilapia in aquaculture.

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