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## Effects of oil enriched diets on growth, feed conversion ratio and fatty acid content of Nile tilapia (*Oreochromis niloticus*) in biofloc system

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**Abstract** The effect of oil enriched diets to increase omega-3 fatty acids in the flesh of Nile tilapia (*Oreochromis niloticus*) by biofloc technology (BFT) system cultivation was investigated. Six feed diets containing 30% protein were added with different types of oil; control diet added with soybean oil, feed diet added with fish oil (FO), feed diet added with fish oil and soybean oil 1:1 (FO:SO), feed diet added with lard oil (LO), feed diet added with lard oil and soybean oil 1:1 (LO:SO) and feed diet added with fish oil and lard oil 1:1 (FO:LO). Each treatment was done in triplicate. Nile tilapia (initial weight  $30 \pm 1.20$  g) larvae were cultured in glass tanks and reared for 8 weeks. Tilapia fed with FO diet showed highest growth and weight gain ( $62.7 \pm 0.18$  g) ( $p > 0.05$ ). However, there were no differences in the survival rate (95-97%) and feed conversion ratio in every treatment (1.4-1.5) ( $p > 0.05$ ). The fatty acids profile of flesh fed with 6 oil supplemented diets was studied. Fatty acid composition of FO was highest in total omega-3 (21.49%), followed by LO:SO (11.28%). FO treatment had the highest total omega-6 (52.78%), followed by control treatment (46.94%). This study demonstrated the efficacy of omega-3 fatty acids supplementation of fish fed in biofloc system that could be meet commercially valuable in the future.

**Keywords:** Biofloc, Nile tilapia, Omega-3 fatty acid

### Introduction

At present, the popularity of health care is increasing and demand of healthy food is high because most consumers focus on the nutrition and health. So, many kinds of healthy food have been developed and produced which focus on the functional food such as fishery products from salmon, tuna, and tilapia (Jongyotha *et al.*, 2016). In 2016, the export of fishery products for Thailand was 1,660,432 tons and value up to 220,997 million baht (International Fisheries Trade Analysis Group, 2016). Although, Thailand has exported many kinds of fishery products but still imported many fishery products from foreign countries. In 2016, Thailand imported 318.8 tons of tilapia products (37.7 million baht) from India (Nurit, 2016)

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and still consume a large number of freshwater fish which increases consistently because of increasing population. Currently, most of the aquaculture is intensive culture. Fish farming is often affected by the blooming of phytoplankton due to high appropriate nutrients in the environment. This directly affects the water quality of the ponds. Aggregation and sedimentation of phytoplankton can be used to feed tilapia. The ideal concept of aquaculture waste was to use biofloc technology (BFT) in the aquaculture system (Avnimelech, 2015). Fish can use biofloc as an additional feed source. The BFT in aquaculture has been proven to be an important source of nutrition for aquatic animals, to maintain water quality and prevent diseases (Ballester *et al.*, 2010). This technology can remove metabolic or nitrogenous wastes (ammonia and nitrite) from aquatic production system (Azim and Little, 2008). The heterotrophic bacteria that convert ammonia to nitrite and nitrate are cultured in the rearing tank. The bacteria form aggregates or colonies (bioflocs) suspended in the water column. The biofloc becomes an additional feed source, which decrease the feed conversion ratio of commercial feeds and reduce costs (Avnimelech, 1999). Biofloc technology is considered as a sustainable and environmentally friendly aquaculture system (Widanarni and Siti, 2012), and has been applied in the laboratory and commercial scale for various aquacultures such as tilapia (Avnimelech, 2007; Azim and Little, 2008; Crab *et al.*, 2009), shrimp (Burford, 2004; Hari *et al.*, 2004; Taw, 2010), sturgeon and snook (Serfling, 2006). This system is cost effective for long-term investment. The concentration of protein in the artificial diet may be reduced if there is abundant natural food such as the bioflocs in the farming system (Jatoba *et al.*, 2014). Therefore, the feeding costs can be reduced by adoption of the BFT system, since the expense with commercial diets is more than 50% of the operating costs (Chamberlain *et al.*, 2001).

Biofloc Technology can be applied in the organic aquaculture, which currently has become a modern trend in value adding of aquaculture development for human food and safety consumption. Essential fatty acids supplementation in biofloc system will be beneficial to the fish cultivation. Omega-3 ( $\omega$ -3) fatty acid supplement in feed diet increases the value added in tilapia (Jinkan, 2008). Omega- 3 fatty acids is classified as polyunsaturated fatty acid, mostly found in marine fish and plankton. In freshwater fish, the amount of omega- 3 fatty acid ranged from 62 to 1,052 mg/100 g body weight, except in *Pangasianodon hypophthalmus* the level is up to 2,111 mg/100 g. Omega-3 fatty acid is an essential fatty acid, that can reduce the risk of obstruction of the arteries, prevent memory degeneration in the elderly and stimulate brain development and mood in childhood. Based on a research by the Department of Fisheries of Thailand, fish oil in 100g tilapia flesh contains 42.86 mg of omega- 3 (EPA and DHA) (Prompong, 2017). Omega-3 fatty acids in flesh of Channel catfish, Rainbow trout and hybrid red tilapia fed with supplemented diets with

omega-3 fish oil are increased (Ng *et al.*, 2003; Manning *et al.*, 2006; Chen *et al.*, 2008). Furthermore, add various kinds of oil in the diets to increase omega-3 fatty acid content in tilapia flesh by using biofloc technology had been conducted by the present research group. The fish product will be suitable for health in the development of food valley in Thailand.

## **Materials and methods**

### ***Fish stocking and tank management***

Stock of mixed sex Nile tilapia, was supplied by King Fish Group Co. Ltd., Chiang Mai, Thailand, and kept in a 200 L fiberglass tank. After three days, the 5 fish were randomly and equally stocked in glass aquaria for rearing 18 aquaria in total and fed with the experimental diets. The initial weight of Nile tilapia larvae were  $30 \pm 1.20$  g. A pump system was installed in each aquarium to maintain the solids in suspension using aeration blower. Biofloc was already developed in the treatment tanks during the previous experiment (modified from Avnimelech, 1999; 2007). Molass was added to each aquarium every day to adjust C:N ratio to 15:1. The fish were cultured for 8 weeks during January to March 2018.

### ***Diet preparations***

Six practical diets contained 30% crude protein and 9% crude lipid (Table 1). Different types of oil were added; control diet added with soybean oil, feed diet added with tuna fish oil (FO), feed diet added with fish oil and soybean oil 1:1 (FO:SO), feed diet added with lard oil (LO), feed diet added with lard oil and soybean oil 1:1 (LO:SO) and feed diet added with fish oil and lard oil 1:1 (FO:LO). Each experiment was done in triplicate. Fish oil supplement diet was fed to fish for 2 weeks before the end of this experiment (modified from NSTDA, 2017). Feeding rates were based on fish weight and feeding behaviour of fish in the BFT treatments during the first seven days and fixed at 5% of the weight, and adjusted feed two weeks after weighing a fish sample. The same amount of feed was applied to all treatments and control tanks. Daily feed rations were split into two equal amounts given at 09:00 and 17:00 h to all tanks.

### ***Assessment of water quality parameters***

Water samples were collected weekly for 8 weeks around 12:00 am from each glass aquarium. Water and air temperatures, dissolved oxygen (DO meter HI9147), pH (pH meter HI98127), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ), orthophosphate phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ), total phosphorus concentrations were measured

according to standard methods (APHA, 1998). Total suspended solids (TSS), total settable solid, biochemical oxygen demand (BOD) and chlorophyll-a (APHA, 2005) were also measured.

**Table 1.** Ingredients of the experimental diets

	Diet (g)					
	Control	FO	FO:SO	LO	LO:SO	FO:LO
<b>Diet ingredients (g kg<sup>-1</sup> dry diet)</b>						
Soybean meal	450	450	450	450	450	450
Fish meal	100	100	100	100	100	100
Rice bran	100	100	100	100	100	100
Broken rice	250	250	250	250	250	250
Vitamin (premix)	10	10	10	10	10	10
Soybean oil	90	-	45	-	45	-
Fish oil	-	90	45	-	-	45
Lard	-	-	-	90	45	45
<b>Biochemical composition (%DM)</b>						
Crude protein	30.4±0.00	30.5±0.01	30.5±0.01	30.5±0.01	30.5±0.01	30.4±0.01
Crude lipid	9.3±0.00	9.3±0.00	9.3±0.00	9.2±0.01	9.3±0.01	9.3±0.01
Ash	7.5±0.01	7.6±0.01	7.6±0.01	7.6±0.01	7.6±0.01	7.6±0.00
Fiber	8.6±0.01	8.6±0.01	8.6±0.01	8.5±0.00	8.5±0.00	8.6±0.01
NFE	38.9±0.01	38.8±0.01	38.8±0.01	38.8±0.01	38.9±0.01	38.8±0.00

### *Fish sampling*

Growth parameters were determined after the experiment. The observed body weight and food intake data were calculated using the following indices:

$$\text{Weight gain (\%)} = 100 \times \frac{(\text{Final body weight} - \text{Initial body weight})}{\text{Initial body weight}}$$

$$\text{Survival rate (\%)} = 100 \times (\text{Final fish count} / \text{Initial fish count})$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry weight of feed supply}}{\text{Total fish wet biomass increase}}$$

$$\text{Average daily growth (g/day)} = \frac{(\text{Final body weight} - \text{Initial body weight})}{\text{days}}$$

### ***Fatty acid composition and proximate composition***

After 8 weeks, fatty acid analysis were carried out as described by Bligh and Dyer (1959) and Holub and Skeaff (1987). Proximate composition of the experimental diets and fish flesh was determined using standard method (AOAC, 1990). The crude protein content was determined by measuring nitrogen using the Kjeldahl method; the crude lipid content was measured by ether extraction using Soxhlet extractor; Ash contents, a known amount of dry sample was burnt in the muffle furnace at 550 °C for 4 h; crude fiber, an amount of sample after digestion in H<sub>2</sub>SO<sub>4</sub> and NaOH solution and the residue calcined.

### ***Statistical analysis***

All data were subjected to one-way ANOVA. All percentage data were arcsine transformed prior to analysis. Significant difference, the group means were analysed with Tukey's multiple range tests. All statistical analyses were carried out using SPSS/PC for windows ver. 15.1.

## **Results**

### ***Water quality parameters***

Water quality parameters monitored are shown in Table 2. Some water quality parameters in all the experimental groups remained within recommended levels for fish culture throughout the 8 weeks of experimental period. Although, nutrients levels (N and P compounds) were slightly higher than the recommended levels, but they did not affect the fish growth. The air and water temperature in the tanks of bioflocs treatments were sometimes slightly below the range considered to be optimal because fish were cultured in winter. There was no significant difference ( $p>0.05$ ) in water quality among all the treatments.

### ***Growth performance of Nile tilapia***

As presented in Table 3, There is no difference between initial weight of tilapia among the control, FO, FO:SO, LO, LO:SO and FO:LO ( $p>0.05$ ). After 8 weeks, growth performance was evaluated through weight gain percentage, food conversion ratio, average daily growth and survival rate. There was no significantly difference ( $p>0.05$ ) in the growth of the tilapia in all bioflocs treatments. The FO treatment was the best treatment, it increased weight gain  $107.0\pm 0.35$  g/fish, although there was no difference among all treatments.

**Table 2.** Water quality parameters in glass tanks of *O. niloticus* test diet containing oil enriched diets. (Mean  $\pm$  SE)

Parameters	Standard water quality <sup>a</sup>	Treatment					
		Control	FO	FO:SO	LO	LO:SO	FO:LO
Air temperature (°C)		28.0 $\pm$ 0.63- 33.8 $\pm$ 0.10	28.0 $\pm$ 0.63- 33.8 $\pm$ 0.10				
Water temperature (°C)	26-32 °C	22.0 $\pm$ 0.52- 25.3 $\pm$ 0.13	21.1 $\pm$ 0.74- 25.1 $\pm$ 0.03	22.0 $\pm$ 0.68- 25.0 $\pm$ 0.03	22.0 $\pm$ 0.51- 25.2 $\pm$ 0.20	21.5 $\pm$ 0.61- 25.1 $\pm$ 0.06	21.4 $\pm$ 0.72- 25.2 $\pm$ 0.03
pH	6.5-9.0	6.8 $\pm$ 0.02- 7.1 $\pm$ 0.03	6.8 $\pm$ 0.02- 7.1 $\pm$ 0.04	6.8 $\pm$ 0.01- 7.1 $\pm$ 0.07	6.9 $\pm$ 0.03- 7.2 $\pm$ 0.11	6.9 $\pm$ 0.03- 7.1 $\pm$ 0.04	6.9 $\pm$ 0.01- 7.2 $\pm$ 0.06
Dissolved oxygen (mg/l)	>4.0	5.4 $\pm$ 0.09- 8.8 $\pm$ 0.15	5.3 $\pm$ 0.07- 8.8 $\pm$ 0.12	5.4 $\pm$ 0.01- 8.5 $\pm$ 0.20	5.4 $\pm$ 0.04- 8.5 $\pm$ 0.22	5.3 $\pm$ 0.07- 8.4 $\pm$ 0.07	5.3 $\pm$ 0.03- 8.3 $\pm$ 0.08
Biochemical oxygen demand (BOD) (mg/l)	1-2	1.0 $\pm$ 0.23 - 1.8 $\pm$ 0.21	1.0 $\pm$ 0.26 - 2.0 $\pm$ 0.61	1.3 $\pm$ 0.08 - 2.0 $\pm$ 0.20	1.1 $\pm$ 0.21 - 2.1 $\pm$ 0.06	1.4 $\pm$ 0.25 - 2.2 $\pm$ 0.11	1.1 $\pm$ 0.07 - 2.2 $\pm$ 0.09
NH <sub>3</sub> -N (mg/l)	<0.1	0.1 $\pm$ 0.01 - 0.2 $\pm$ 0.07	0.1 $\pm$ 0.01 - 0.2 $\pm$ 0.05	0.1 $\pm$ 0.01 - 0.3 $\pm$ 0.05	0.1 $\pm$ 0.01 - 0.2 $\pm$ 0.06	0.1 $\pm$ 0.01 - 0.2 $\pm$ 0.07	0.1 $\pm$ 0.01 - 0.2 $\pm$ 0.07
NO <sub>2</sub> <sup>-</sup> -N (mg/l)	<8	2.0 $\pm$ 0.02 - 4.0 $\pm$ 0.48	2.0 $\pm$ 0.05 - 4.3 $\pm$ 0.57	2.0 $\pm$ 0.03 - 3.5 $\pm$ 0.71	2.0 $\pm$ 0.04 - 4.1 $\pm$ 0.54	2.0 $\pm$ 0.04 - 4.2 $\pm$ 0.54	2.0 $\pm$ 0.04 - 4.0 $\pm$ 0.34
NO <sub>3</sub> <sup>-</sup> -N (mg/l)	<1000	0.5 $\pm$ 0.20 - 1.7 $\pm$ 0.12	0.5 $\pm$ 0.28 - 1.7 $\pm$ 0.06	0.7 $\pm$ 0.34 - 1.9 $\pm$ 0.06	0.7 $\pm$ 0.34 - 1.8 $\pm$ 0.07	0.7 $\pm$ 0.36 - 1.7 $\pm$ 0.10	0.7 $\pm$ 0.39 - 1.7 $\pm$ 0.07
PO <sub>4</sub> <sup>3-</sup> -P (mg/l)	<1000	0.1 $\pm$ 0.03 - 1.0 $\pm$ 0.20	0.1 $\pm$ 0.01 - 0.9 $\pm$ 0.20	0.1 $\pm$ 0.01 - 1.0 $\pm$ 0.14	0.1 $\pm$ 0.01 - 0.9 $\pm$ 0.10	0.1 $\pm$ 0.01 - 0.9 $\pm$ 0.19	0.1 $\pm$ 0.01 - 0.8 $\pm$ 0.22
Total phosphorus (mg/l)		1.0 $\pm$ 0.09 - 3.0 $\pm$ 0.36	1.3 $\pm$ 0.20 - 3.7 $\pm$ 0.27	1.3 $\pm$ 0.15 - 3.7 $\pm$ 0.23	1.5 $\pm$ 0.11 - 3.9 $\pm$ 0.37	1.6 $\pm$ 0.19 - 3.7 $\pm$ 0.02	1.4 $\pm$ 0.10 - 3.6 $\pm$ 0.12
Chlorophyll-a ( $\mu$ g L <sup>-1</sup> )		1.6 $\pm$ 0.24 - 2.5 $\pm$ 0.04	1.6 $\pm$ 0.26 - 2.8 $\pm$ 0.14	1.6 $\pm$ 0.24 - 2.6 $\pm$ 0.10	1.6 $\pm$ 0.29 - 2.8 $\pm$ 0.10	1.6 $\pm$ 0.23 - 2.8 $\pm$ 0.11	1.6 $\pm$ 0.24 - 2.6 $\pm$ 0.15
Total suspended solid (mg/l)		200. $\pm$ 0.50 - 400 $\pm$ 0.60	200 $\pm$ 0.50 - 400 $\pm$ 0.54	200 $\pm$ 0.50 - 500 $\pm$ 0.56	100 $\pm$ 0.20 - 400 $\pm$ 0.54	100 $\pm$ 0.50 - 400 $\pm$ 0.49	200 $\pm$ 0.50 - 400 $\pm$ 0.46
Total settable solid (ml/l)		22.4 $\pm$ 0.26-44.3 $\pm$ 0.51	23.9 $\pm$ 0.20- 50.4 $\pm$ 0.28	25.6 $\pm$ 0.11- 43.4 $\pm$ 0.10	24.2 $\pm$ 0.16- 39.5 $\pm$ 0.92	20.8 $\pm$ 0.56- 44.9 $\pm$ 0.94	20.8 $\pm$ 0.58- 41.4 $\pm$ 0.56

<sup>a</sup>The optimal ranges for Tilapia culture (Boyd and Tucker, 1998 and El-Sayed, 2006)

**Table 3.** Growth and feed utilisation in Nile tilapia test diet containing oil enriched diets. (Mean  $\pm$  SE)

	Control	FO	FO:SO	LO	LO:SO	FO:LO
Initial fish weight (g fish <sup>-1</sup> )	30.3 $\pm$ 0.27	30.2 $\pm$ 0.05	30.5 $\pm$ 0.15	30.3 $\pm$ 0.15	30.2 $\pm$ 0.13	30.0 $\pm$ 0.39
Final fish weight (g fish <sup>-1</sup> )	61.6 $\pm$ 0.06	62.7 $\pm$ 0.12	62.1 $\pm$ 0.20	61.6 $\pm$ 0.34	61.9 $\pm$ 0.17	61.8 $\pm$ 0.13
Weight gain (%)	103.3 $\pm$ 0.42	107.0 $\pm$ 0.35	103.6 $\pm$ 0.29	103.3 $\pm$ 0.15	104.9 $\pm$ 0.45	106.0 $\pm$ 0.32
Food conversion ratio	1.5 $\pm$ 0.07	1.4 $\pm$ 0.13	1.4 $\pm$ 0.14	1.5 $\pm$ 0.04	1.4 $\pm$ 0.09	1.5 $\pm$ 0.17
Average Daily Growth (g/day)	0.5 $\pm$ 0.01	0.5 $\pm$ 0.13	0.5 $\pm$ 0.03	0.5 $\pm$ 0.04	0.5 $\pm$ 0.05	0.5 $\pm$ 0.10
Survival rate (%)	87 $\pm$ 6.67	93 $\pm$ 6.67	93 $\pm$ 6.67	93 $\pm$ 6.67	87 $\pm$ 6.67	87 $\pm$ 6.67

### *Proximate composition analysis*

The proximate composition of the oil enriched diet collected from all the 6 treatments is presented in Table 1. There were no significant differences in any nutritional parameters among all treatments. The levels of the protein control at the start of the experiment was 30% and 9% of crude lipid. Table 4 shows the proximate analysis of the fish flesh fed by different oil supplemented diets. The protein content of all treatments was significantly different ( $p < 0.05$ ). The flesh of FO: LO treatment had the highest protein content ( $53.8 \pm 0.60$ ). High percentage of crude lipid was found in FO treatment ( $10.4 \pm 0.16$ ), which may indicate the accumulation of fatty acids in the flesh. Highest ash content was found in the LO and LO: SO diets ( $6.3 \pm 0.03$ ), which may indicate that lard oil component has much of inorganic elements, there are remained residues in fish. Control diet contained the highest crude fiber and NFE content ( $15.8 \pm 0.37$  and  $17.2 \pm 1.03$ ).

**Table 4.** Proximate composition (%) of the flesh of 6 experimental fish fresh containing oil enriched diets. (Air dry basis) (Mean  $\pm$  SE)

	Control	FO	FO:SO	LO	LO:SO	FO:LO
Protein	51.8 $\pm$ 0.04 <sup>a</sup>	52.7 $\pm$ 0.33 <sup>a</sup>	52.7 $\pm$ 0.04 <sup>a</sup>	53.7 $\pm$ 0.60 <sup>b</sup>	52.5 $\pm$ 0.03 <sup>a</sup>	53.8 $\pm$ 0.60 <sup>b</sup>
Lipid	6.5 $\pm$ 0.05 <sup>b</sup>	10.4 $\pm$ 0.16 <sup>c</sup>	6.0 $\pm$ 0.09 <sup>b</sup>	4.7 $\pm$ 0.07 <sup>a</sup>	7.8 $\pm$ 0.09 <sup>c</sup>	8.6 $\pm$ 0.03 <sup>d</sup>
Ash	4.7 $\pm$ 0.04 <sup>a</sup>	5.4 $\pm$ 0.10 <sup>b</sup>	5.6 $\pm$ 0.00 <sup>b</sup>	6.3 $\pm$ 0.03 <sup>c</sup>	6.3 $\pm$ 0.03 <sup>c</sup>	6.2 $\pm$ 0.00 <sup>c</sup>
Fiber	15.8 $\pm$ 0.37 <sup>b</sup>	15.1 $\pm$ 0.27 <sup>ab</sup>	14.8 $\pm$ 0.33 <sup>a</sup>	14.6 $\pm$ 0.04 <sup>a</sup>	15.5 $\pm$ 0.11 <sup>ab</sup>	14.4 $\pm$ 0.12 <sup>a</sup>
NFE	17.2 $\pm$ 1.03 <sup>b</sup>	11.2 $\pm$ 0.59 <sup>a</sup>	15.3 $\pm$ 0.10 <sup>b</sup>	15.6 $\pm$ 0.61 <sup>b</sup>	11.7 $\pm$ 0.05 <sup>a</sup>	11.3 $\pm$ 0.64 <sup>a</sup>

Each value represents mean  $\pm$  SE. Values in the same row with different superscript letters are significantly different ( $p < 0.05$ ).

### *Fatty acid composition*

Fatty acid composition in every diets and fish flesh was determined after 8 weeks. The percentage fatty acid composition is shown in Table 5.

**Table 5.** Fatty acid composition (% of total fatty acid) of experimental diets and fish flesh with supplemented diets

% Fatty acid	Diet						Fish flesh					
	Con trol	FO	FO: SO	LO	LO: SO	FO: LO	Con trol	FO	FO: SO	LO	LO: SO	FO: LO
C14:0	2.80	14.71	10.39	2.20	1.53	4.35	2.60	6.57	3.12	5.92	7.84	4.91
C16:0	89.99	64.54	43.03	41.61	41.76	50.96	48.23	93.87	47.11	38.35	131.39	77.63
C18:0	12.14	10.46	7.32	10.89	8.75	8.15	Tr	10.95	8.03	18.87	28.62	21.88
C18:1	Tr	23.37	50.73	50.01	49.56	43.79	60.80	35.82	12.25	10.28	Tr	Tr
C18:2 (n-6)	Tr	Tr	Tr	Tr	Tr	Tr	43.92	52.78	33.00	28.27	Tr	Tr
$\alpha$ C18:3 (n-3)	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
$\gamma$ C18:3 (n-6)	26.95	6.28	4.19	2.48	8.69	4.04	3.02	Tr	1.90	2.40	4.75	3.08
C22:6 (DHA) (n-3)	1.88	7.61	1.56	0.45	0.44	1.11	3.87	21.49	4.55	2.58	11.28	10.02
Total saturated FA	104.93	89.71	60.74	54.70	52.04	63.46	50.83	111.39	58.26	63.14	167.85	104.42
Total unsaturated FA	28.83	37.26	56.48	52.94	58.69	48.94	111.61	110.09	51.70	43.53	16.03	13.1
Total n-3	1.88	7.61	1.56	0.45	0.44	1.11	3.87	21.49	4.55	2.58	11.28	10.02
Total n-6	26.95	6.28	4.19	2.48	8.69	4.04	46.92	52.78	34.9	30.67	4.75	3.08

Tr = trace (<0.1 g 100g<sup>-1</sup> fatty acid).

From table 5, fish oil supplement diet (FO) has high omega-3 content. So the fish flesh contained the highest value of omega-3 (7.61%), while other types of oil supplemented diets (soybean and lard oil) gave low omega-3 in the flesh. However, the proportion of fish oil suitable for increasing the amount of omega-3 in the flesh was better with other types of oil.

## Discussion

### *Water quality parameters*

In this study, some of the parameters of water quality were found to be the optimal criteria for culturing Tilapia (Table 2). Boyd and Tucker (1998) and Ruangnuppakun (2012) reported the following water qualities suitable for growth of Tilapia: dissolved oxygen concentration should not lower than 4 mg/l, ammonia-N, nitrite-N and nitrate-N should not exceed 0.1, 8.0 and 1,000 mg/l, respectively. According to El-Sherif and El-Feky (2009), the different water temperature levels along this experiment would affect the growth performance of the tilapia. During this experiment, the optimal growth performance of the tilapia was observed at 25-30 °C. Tran-Duy *et al.* (2007) reported that at two different levels of oxygen i.e. 3.0 and 5.6 mg/l, different growth performance of the tilapia was observed. The average dissolved oxygen level above 5.0 mg/l, tilapia would grow better.

In this study, the pH was found to be within the optimal standard that the aquatic animals could live. The value is between 6.5-9.0 (Wurt and Durborow, 1992); Bhatnager *et al.*, 2004). The ideal pH is between 7.5 and 8.5 if it is higher or lower than this, it would be stressful to the fish. The suitable pH for fish cultivation is 6.7 - 9.5 (Santhosh and Singh, 2007; Bhatnager and Devi, 2013).

Dissolved inorganic nitrogen ( $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ ) concentration among all treatments was no significant difference throughout the experimental period (Table 2). At the start of the experiment,  $\text{NH}_3\text{-N}$  levels in BFT treatments remained elevated in all the treatment aquaria. After Day 7,  $\text{NH}_3\text{-N}$  concentration in all the treatments began to decrease. It was reported to be commonly occurred in BFT system. This confirms that in the case of only wet floc as feed, microbial assimilation of nitrogen and nitrification reduced the total ammonia nitrogen (TAN),  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  (Mahanand *et al.*, 2012). Azim and Little (2008) reported a high degree of fluctuation in TAN and  $\text{NO}_2\text{-N}$  levels in biofloc treatments during the entire experimental period. TAN concentrations varied significantly between treatments and sampling dates with higher mean values in the tanks fed with 35% CP and biofloc compared to the control tanks without biofloc. The  $\text{NO}_2\text{-N}$  concentrations in biofloc treatments were significantly higher than that in the control. In the tanks, the ammonia and nitrite were quite

unstable. Due to a significant reduction in the nitrification process, ammonia concentrations increased in the first day, and it was necessary to add molasses to control ammonia by its conversion into heterotrophic bacterial biomass (Avnimelech, 1999). Ortho phosphate phosphorus and total phosphorus ranged from 0.1-1.0 and 1.0-3.9 mg/l, respectively with no significant difference ( $p>0.05$ ). Bhatnager and Devi (2013) reported the orthophosphate phosphorus and total phosphorus were 0.01-3 mg/l in pond fish culture. The lower orthophosphate concentration in biofloc treatment was likely associated with increased bacterial biomass production (because of the high molass input) and removal of bacteria by the settling chambers. Schneider *et al.* (2006) found that the increase in C:N ratio from 3.4 to 16.5 allowed higher conversion of orthophosphate which is assimilated into heterotrophic bacterial biomass. Total phosphate phosphorus in this experiment (1.1-14.4 mg/l) was suitable for the fish to live and did not affect tilapia fed in biofloc systems (Schveitzer *et al.*, 2013).

Chlorophyll-a concentration was influenced by light (Brito *et al.*, 2014). The decrease in the concentration of chlorophyll-a during the study is probably associated with the use of molasses to control ammonia content. The use of carbon sources in intensive systems promotes succession and dominance of bacteria over microalgae (González *et al.*, 2007; Ju *et al.*, 2008). Chlorophyll-a concentration in the present study was lower than those reported by Burford *et al.* (2003, 2004); Decamp *et al.* (2003).

The different TSS concentrations in the aquarial culture were 100 - 500 mg/l. Lower TSS in the treatment indicates a lower concentration of biofloc versus the heterotrophic treatments. (Ray *et al.*, 2010). However, Schveitzer *et al.* (2013) reported fish can tolerated the high level of TSS (400-1,000 mg/l). At the same time, the reduced TSS may result from fish being pulled back into food. The natural bioflocs are always available to the aquatic organisms as a supplemental food supply (Avnimelech, 1999). They can be ingested and digested by the aquatic animals, replacing a significant fraction of the artificial feed (Crab *et al.*, 2010; Xu and Pan, 2012; Anand *et al.*, 2014).

### ***Growth performance of Nile tilapia***

The results from Table 3 showed that the weight gain percentage of the Nile tilapia in the FO treatment (receiving fish oil 100%) was higher than other experimental groups because of short cultivation period. This shows that the use of fish oil affect the fish growth and food utilization. Sargen *et al.* (1999) reported that omega-3 fatty acids are essential fatty acids that are important for cell membrane formation, balance of water and osmoregulation, stimulating effects, also the immune system. In addition, omega-3 fatty acids have resulted in better growth and prevent abnormalities such as delayed appetite and high mortality rates (Watanabe

*et al.*, 1974). However, the final weight of all treatments was not significant difference ( $p > 0.05$ ). Previous study by Sagne *et al.*, (2013) reported the growth rate and feed conversion ratio of red tilapia fingerlings fed with cod liver oil diet, substituted corn oil, showed no significant differences ( $p > 0.05$ ). Fish were fed with all types of oil supplemented diets had no significant effects on the growth, although slightly reduced weight gain was observed. Yoojam *et al.* (2014) reported the weight gain of hybrid catfish fed with fish oil supplemented diet throughout 5 month culture period. Hybrid catfish in all experimental units were not significantly different.

Feed conversion ratio (FCR) of all treatments was 1.4-1.5 (Table 3) with no significant difference ( $p > 0.05$ ). Tilapias reared in the biofloc system have lower FCR compared with the fish fed with basal diet group. Caldini *et al.* (2015) reviewed the effect of feeding Nile tilapia with artificial diets and dried bioflocs on the growth. FCR level of fish was 0.78-1.72, because biofloc is utilized by tilapia as food (Azim and Little, 2008). This research supported by Yuangsoi *et al.* (2013) who studied tilapia cultivation fed with tuna oil at 0, 3, 6 and 9 percent and FCR was from 1.75-2.55. It can be concluded that conventional fish cultivation has higher FCR in BFT system.

The average daily growth (ADG) value was approximately 0.5 g / day, similar to the research of Day *et al.* (2016) who demonstrated that Nile tilapia cultured in BFT system had the highest growth rate (an average daily gain of  $0.693 \pm 0.018$  g/day).

### ***Proximate composition and Fatty acid***

Proximate composition of all types of oil supplemented diet was not significant difference (Table 1). Crude protein content of diets was approximately 30%, whereas crude lipid was 9%. Jauncey (1998) reported that the suitable protein levels for fish size 0.5, 0.5 to 10, 11-30, and  $> 30$  g were approximately 40-45, 30-35, 25-30 and 25-30%, respectively, while essential amino acid requirement was 5-12% of lipid content. This fatty acid level stimulated protein utilization in the fish. Feed diet supplemented with fish oil (FO) has highest omega-3 fatty acid level (7.61%) (Table5). Bawonsupakidkul (2008) estimated the dietary supplemented with different types of fatty acid; lard oil, Tuna oil, coconut oil, corn oil and soybean oil. Fish fed with fish oil (Tuna oil) supplemented diet for 12 weeks was able to accumulate fatty acid. High level of DHA and EPA was found. Highest crude lipid and omega-3 content in the flesh was detected in FO treatment;  $10.4 \pm 0.16$  and 21.49% (Table 4 and 5). Manning *et al.* (2006) reported Channel catfish size 50-60 g fed with 1.5% of fish oil supplemented diet for 6 weeks and the research of Chen *et al.* (2008) on adult Rainbow trout (240 g) fed with 8.5 and 15% omega-3 fatty acid for 120 days, the omega-3 content was more accumulated in the muscle.

This study demonstrated that the fish oil enriched diets provided high growth rate, lowest feed conversion ratio, highest fatty acid and omega-3 content of Nile tilapia in BFT system. BFT system cultivation can reduce the capital cost of feed and zero-exchanged water. Omega-3 fatty acids supplementation in fish can upgrade flesh product in the fish product market.

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