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Enrichment of plant-protein based diets for Nile tilapia (*Oreochromis niloticus*) with krill protein hydrolysate with high concentration of phospholipids rich in n-3 fatty acids

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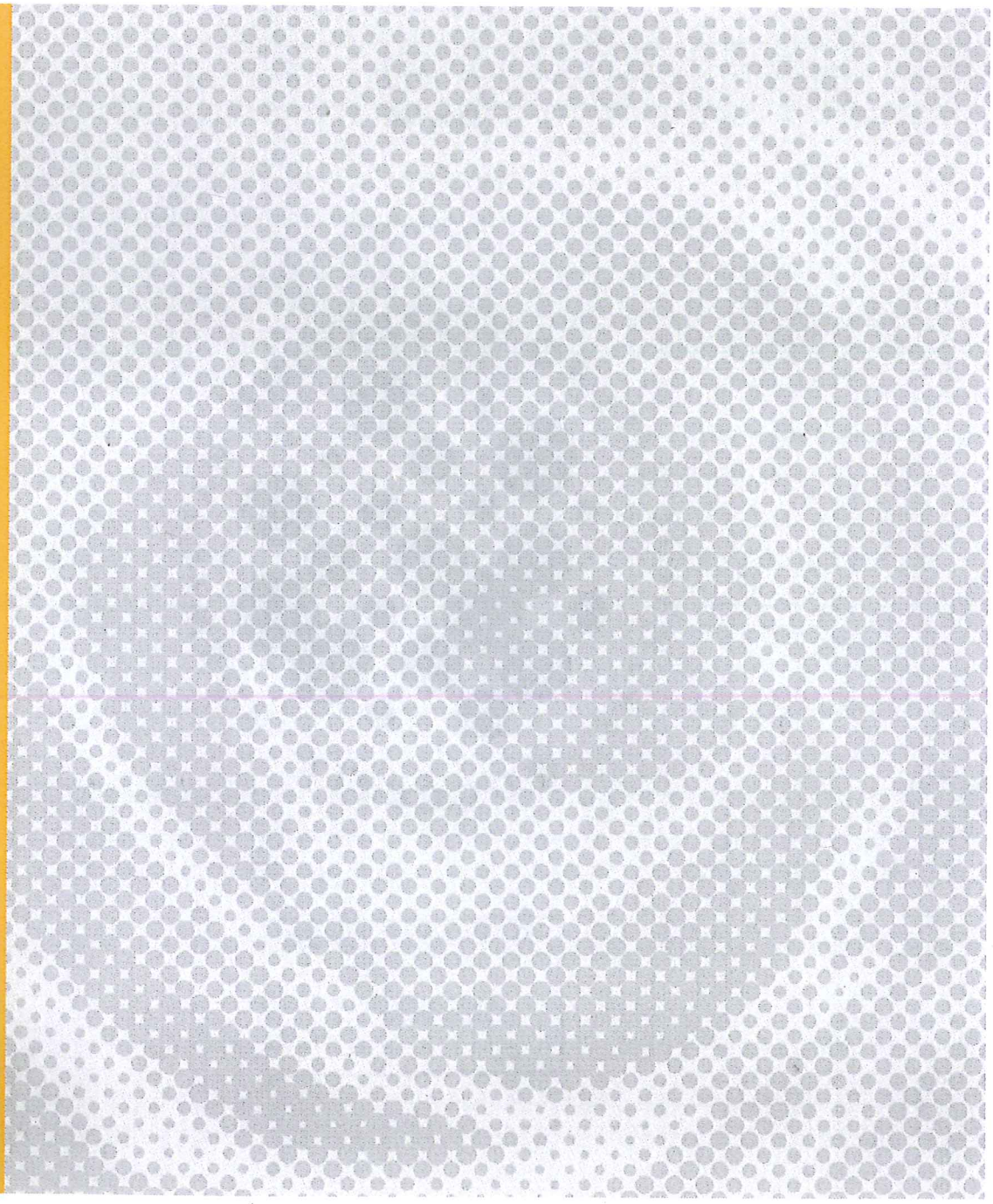

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Hao Zhang and Haibo Wang

Abstract

a 28-day experiment was conducted to investigate the effects of dietary krill (*Euphausia superba*) hydrolysate with high concentration of phospholipids rich in *n-3* fatty acids (KHPL) on growth performance, body composition, fillet fatty acid profile and sensory characteristics of Nile tilapia (*Oreochromis niloticus*). Three diets were produced with pre-gelatinized starch and plant protein and lipid sources in a pasta machine. Plant diet was formulated with no krill product. Two diets were formulated with 5 and 10% KHPL, respectively. Each diet was fed to triplicate groups of 25 fish of 43.2±0.9 g (mean ± SE.) initial body weight. The Tilapias were fed for 28 days at 26.7±0.5 °C in 9 100-L cylinder tanks with a re-circulated system whose water flow rate of about 5.6 l min^{-1}. The tilapias were thereafter kept in clean water to reduce muddy off-flavor, and after 6 days of purging they were served to a consumer taste panel consisting of 20 untrained no-smoking judges (10 Chinese, 10 Caucasian, 5 females and 5 males within each ethnic group). The judges were served pieces of baked unsalted Tilapia fillet and were asked to judge fishy taste/smell, muddy taste/smell and other taste/smell, and to rank the fish on a scale from 1 to 3, where 1 represented least attractive and 3 represented the most preferred.

Dietary inclusion of KHPL had no significant effect (P>0.05) on growth rates, feed conversion or body composition of the tilapia. The mean final body weight of fish fed by each diet was 123.93±10.92-128.37±2.67 g. The mean feed conversion rate (FCR) was 1.30±0.06 kg ingested per kg gain. The contents of whole body lipid were from 12.6% to 13.7%, whole body protein ranged from 14.3% to 14.8%, and the ash levels were between 9.9% and 10.4%. The contents of fillet protein were from 18.9% to 19.7%, lipid levels of fillet varied from 1.6% to 2.0%. Significant differences were found in the content fatty acid in the fillet. Increasing dietary KHPL resulted in decreasing concentration of monoenic fatty acids, while polyenes, EPA, DHA and total *n-3* fatty acids, as well as the of *n-3*/*n-6* ratio increased with increasing KHPL. The sum of *n-3* fatty acids ranged from 5 to 10 mg (kg fillet)$^{-1}$.

Tilapia fed the diet with most KHPL tended to be preferred by the judges, followed by the fish fed the diet with 2% krill product and the fish fed plant materials only. A significant gender difference was found in smell (P=0.032). Females seemed more sensitive than male in detecting differences in smell, while males seemed more perceptive to taste than females. The Chinese judges discriminated fishy taste more efficient than the Caucasians (P< 0.0001), while the Caucasians seemed more sensitive than the Chinese both differentiating muddy taste (P=0.012) and “other taste” (P=0.0065).

Contents

1. Introduction

Polyunsaturated fatty acid (PUFA) has been highly paid attention to for its use on human cardiovascular disease prevention and infant brain development (Olsen et al., 1990; Castella et al., 1994; Steffens, 1997; Enser et al., 2000; Chou et al., 2001; Caballero et al., 2002). Numerous studies have been done on the main resource of PUFA (Sargent et al., 1999; Steffens, 1997; Hatlen et al., 2012): fish oil (Al-Souti et al., 2012; Teoh et al., 2011), and its vegetable substitutions (Nasopoulou and Zabetakis, 2012; Karapanagiotidis et al., 2007b; Bahurmiz and Ng, 2007).

Krill is a shrimp-like crustacean. Antarctic Krill (*Euphausia superba* and *E. crystallorophias*) are the main krill species. Lipid of Antarctic krill is 20-50 %, phospholipids mainly, and is higher than that of *E. pacifica* and *Thysanoessa spinifera* (5%-20%, mainly glycerol), and krill in the North Pacific (Ju.S.-J. et al., 2009). Pierce et al showed the lipid content of krill (36%-48%) outnumbered that of red crab (*Gecarcoidea natalis*) (Pierce et al., 1969). Antarctic krill has higher lipid content (11.3%) than oriental river prawn (*Macrobrachium nipponense*), greasy-back shrimp (*Metapenaeus ensis*), and Chinese white prawn (*Penaeus chinensis*) (4.9%~6.7%) (Li et al., 2011). As a newly-developed feed resource, krill is of great abundance and is a regenerative resource with additional benefits (Colombo-Hixson et al., 2011). The yield of krill kept at 120 thousand tons from 1992 to 2009 and increased to more than 200 thousand tons in 2010 (Nicol et al., 2012).

Recently, krill hydrolysate with high content of phospholipids (KHPL) has become available, targeting human health products. In 1988, T. Storebakken reviewed the potential of krill as feed for salmonid (Storebakken, 1988). Krill meal was used as natural astaxanthin pigment before synthetic astaxanthin was introduced (Yoshitomi et al., 2006) and both krill hydrolysate and meal have been claimed useful as feed attractant (Kolkovski et al., 2000; Olsen et al., 2006). Allahpichay and Shimizu have determined that non-muscle krill meal was more efficient than whole-krill meal in feeding (Allahpichay and Shimizu, 1984). In

2005, Gaber determined the replacement of krill meal for soybean meal as protein source for juvenile Nile tilapia (Gaber, 2005). The utilization of three different crustacean meals, from *T. inermis*, *E. superba* and *Themisto libellula*, has been evaluated on fillet quality of Atlantic salmon (*Salmo salar*) ( ). Beeancor et al. evaluated the use of three different krill by-products in feed of sea bream (*Sparus aurata*) lavae (Betancor et al., 2012). The substitution percent of krill meal (*E. superba* meal) for fish meal should be lower than 20% (Yoshitomi et al., 2006). Zhang also mentioned using krill meal or water-soluble krill by-product in plant-based feed to increase feed intake (Zhang, 2011). Yet, the effect of KHPL on tilapia hasn't been investigated.

Tilapia, a group of cichlids, is cultured mainly in Asia and South America, making it one of the fastest growing aquaculture species, and in most countries where tilapia is produced, they are a main species for consumption (Norman-López and Bjørndal, 2009). Most species of tilapia are omnivorous and can reproduce and grow rapidly. Considerable genetic research and selective breeding on tilapia have been done (Thodesen et al., 2012; Yan et al., 2012; Bentsen et al., 2012; Pierce et al., 2012; Sarker et al., 2012; Eknath et al., 1993).

Consumers are willing to buy food with high PUFA for the benefits to human health (Foxall et al., 1998). The nutrient composition and requirement of tilapia have been investigated (Gonzales and Brown, 2006; El-Sayed and Teshima, 1992; Garduño-Lugo et al., 2007a). Tilapia has a potentiality of being a *n-3* enriched functional food (Al-Souti et al., 2012). To date, a variety of feed resources have been proved that they can be utilized in tilapia feeding (Hussein et al., 2012; Al-Souti et al., 2012; Tonial et al., 2012; Fall et al., 2012). Normally, whole-body lipid content of tilapia is lower than 8% (Clement and Lovell, 1994). The fillet is lean, and by being fed with commercial diet, fillet of red hybrid tilapia contained lipid even lower than 1% (Garduño-Lugo et al., 2003). Tonial et al. (2012) tested increasing dietary amounts of a flaxseed oil- and sunflower-enriched feed, and achieved increased fillet total lipid from 2.4% to 10% (Tonial et al., 2012). Fitzsimmons suggested energy need of tilapia could be met by carbohydrate resources in order to decrease level of dietary lipid in tilapia feed (Fitzsimmons et al., 1997). Concentrations of *n-3* PUFA in tilapia fillet will descend by using vegetable oils in feed (Ng and Chong, 2004).

Feed will influence the body composition of an animal (Visentainer, 2005), and also alter the sensory characteristics of animal products. Sensory analysis is used to evaluate the quality in several food (Garduño-Lugo et al., 2007b; Palmeri et al., 2008; Dhanapal et al., 2010) and diet habit (Munoz and Chambers, 1993; Bryhni, 2002; Ryffel et al., 2008; Ng and Bahurmiz, 2009) experiments. Sensory analysis can obtain provide detailed information on product attributes (Rødbotten et al., 1997).

There are multiple examples of significant gender difference in the results of sensory analysis. For example, women showed a clearer preference than men for fruits and vegetables (Blanck et al., 2008). This was also found in a study with senior citizens (Baker and Wardle, 2003). Another example showed that Swiss men described sheepy and goaty cheese more animalitic than women (Ryffel et al., 2008). Doty et al demonstrated that women performed better than men in odor identification ability (Doty et al., 1985), and males preferred goat yoghurt than females regardless of flavor (Young et al., 2012). Guinard et al. found gender difference in strong taste beer among American population (Guinard et al., 2000). In another beverage study, it was proved that men were more sensitive than women in retro nasal correct response rate (Hollis and Halpern, 2012). Yet in some food flavor sensory analysis, gender difference was not significant: no sexual difference on flavor of flax seed-added bagels or muffins (Aliani et al., 2012; Ramcharitar et al., 2005). Gender difference also influence other analysis based on human beings. In 2005, Komiyama et al. found that females couldn't take pain which was stimulated by electricity as much as males could (Komiyama et al., 2005). In his book, Schulkin pointed out that women have higher calcium preference than men do (Schulkin, 2001). Females are also more sensitive than males on odor familiarity (Severiano-Perez et al., 2012).

Ethnic differences may also cause major effects in the sensory analysis. Ethnic difference may be caused by diet habit, culture/tradition or/and genetic differences. Doty et al also reported that in an odor identification ability experiment, Korean American ranked the first, followed by black and white American and native Japanese (Doty et al., 1985). In a children and snack experiment, Boyer et al has determined that energy of Caucasian children intake

from snack is higher than that of Asian kids (Boyer et al., 2012). In Americans, ethnic difference appeared in dietary intake in African American and Hispanic families. Also the ethnicity plays a vital role on the choice of food: not only on food itself, also on the ideals and identities (Devine et al., 1999). Researches in Oslo showed that food from the host country lacked attraction to the female immigrants from non-Europe countries or the immigrants who changed their diet after immigration (Garnweidner et al., 2012; Wandel et al., 2008). Anyway, ethnic difference on food existing in the immigrants will fade away generation by generation (Jamal, 1998). But in a kiwifruit experiment, ethnic difference showed no significant differences (Wismer et al., 2005).

The first aim of this experiment was to find out if KHPL-enrichment of a plant-protein based diet affected feed intake, growth and utilization of the feed in Nile tilapia. The second aim was to quantify how efficient the *n-3* fatty acid rich KHPL was in modifying the fatty acid profile of the edible parts of Nile tilapia, and to find out how efficient fillets from tilapias fed KHPL-enriched feed could be to satisfy the requirement for *n-3* fatty acids in humans. The third aim was to find out if KHPL enrichment of the feed influenced the sensory characteristics of tilapia, and if differences in sensory characteristics can be ascribed to gender or ethnicity.

2. Materials and methods

2.1 Fish population

Tilapia from the 12th generation of selection in the GIFT (Genetically Improved Farmed Tilapias) program (Eknath et al., 1993) was hatched and fed to adequate size at UMB. During the 28 days of feeding, 225 fish were kept in 9 100-L PVC tanks in which the water was circulated and maintained at 27℃.

2.2 Krill hydrolysate with high concentration of phospholipids (KHPL)

Krill hydrolysate with high concentration of phospholipids rich in *n-3* fatty acids (OlyMeg®, KHPL) used in this experiment was produced on board the vessel in Antarctica by Emerald fishery Co., Ålesund, Norway. The hydrolysate was processed immediately after catch by rinsing with freshwater, enzymatic hydrolysis, shell removal by decanting, heating, repeated decanting, and vacuum drying (Storebakken, pers. comm.). The nutrient composition, as specified by the producer, is shown in Table 1.

Table 1. Nutrient composition of the KHPL

Total dry matter, %	92.5
Crude protein, %	44.9
Crude fat, %	48.1
Ash, %	4.9

2.3 Feed formulation and processing

Three diets were produced. In the experiment, the gradients of KHPL were set as 0% (plant diet, common group), 5% (KHPL 5% diet) and 10% (KHPL 10% diet). Main protein sources in all 3 diets were soybean meal, sunflower seed meal, rapeseed meal, and pea protein and corn gluten. Starch was provided from wheat and lipid was offered by KHPL and rapeseed in different ratios. MCP, vitamin C and several essential amino acids were supplemented to fulfill tilapia requirements. Y_2O_3 was applied as inert indicator for digestibility assessment, and sodium alginate was added as binder. Table 3 shows the composition of diets while Table 4 shows nutrients composition.

The dry ingredients were ground (1 mm) on a laboratory mill (MCP was ground by pestle and mortar), mixed, added 30% hot (80^oC) water, passed 3 times through a pasta machine, and dried to a target dry matter content of 90% in a hot air drying cabinet set at 65^oC.

Table 2. Formulation of diets

	Plant diet	**KHPL 5% diet**	**KHPL 10% diet**
KHPL, g kg^{-1}	0	50	100
Soybean meal, g kg^{-1}	140.3	132.9	125.8
Sunflower seed meal, g kg^{-1}	115.5	109.4	103.6
Rapeseed meal, g kg^{-1}	64.9	61.5	58.2
Pea protein, g kg^{-1}	45.1	42.7	40.4
Corn gluten, g kg^{-1}	150.2	142.2	134.6
Pre-extruded wheat, g kg^{-1}	327.4	329.3	330.3
Rapeseed oil, g kg^{-1}	70	47	23.8
Mono calcium phosphate, g kg^{-1}	20	20	20
Lysine, g kg^{-1}	11.2	10.7	10.2
Threonine, g kg^{-1}	7.7	7.3	6.9
Methionine, g kg^{-1}	12.7	12.0	11.3
Phenylalanine, g kg^{-1}	0.00	0.00	0.00
Taurine, g kg^{-1}	3.9	3.7	3.5
Tryptophan, g kg^{-1}	1.2	1.2	1.2
Vitamin premix, g kg^{-1}	10	10	10
Y_2O_3, g kg^{-1}	0.1	0.1	0.1
Vit-C 35%, g kg^{-1}	0.10	0.10	0.10
Sodium alginate, g kg^{-1}	20	20	20
Total	1000	1000	1000

Table 3. Nutrient composition of the diets

	Plant diet	**KHPL 5% diet**	**KHPL 10% diet**
Moisture, g kg^{-1}	88.7	89.8	90.8
Dry matter, g kg^{-1}	911.3	910.3	909.2
Protein, g kg^{-1}	345.2	352.9	361.1
Lipid, g kg^{-1}	97.9	98.1	98.0
Starch, g kg^{-1}	251.1	251.0	250.2
Ash, g kg^{-1}	57.2	58.3	59.4

2.4 Feeding trial

During the 28-day feeding trial, feed was delivered by automatic band feeders. Feeders ran for 30 min, 4 times per day, at 0900, 1300, 1700 and 2100, respectively. Feeding rate was set at 0.04 initially and was altered every 3 days to keep uneaten feed up to 10% body weight. Uneaten feed were recorded by collection of uneaten feed as described by Choudhury (Chowdhury, 2011). Fish was weighed at the beginning, middle and the end of experiment.

2.5 Body composition and fatty acid analysis:

2.5.1 Sample preparation

Fifteen fish (3×5) were sampled as initial samples before experiment and killed by being frozen. Fish samples (10 fish per tanks) were taken from each tank at the end the feeding trial and were terminated by a sharp blow to the head and subsequent freezing. Five fish per tank were filleted for fatty acid profile. The rest of samples were ground whole used for analysis of whole-body composition.

2.5.2 Preparation of fatty acid profile analysis

The fatty acid profile was analyzed by IHA, UMB. The samples were extracted by chloroform-methanol method (Folch et al., 1957). Semi-frozen samples were for fatty acid profile, and muscle was separated from skin. Fillets from each tank were squashed on the cutting board following homogenization with a fork. Before extraction, samples were freeze-dried for 6 days. Afterwards, samples were weighed into flasks, and 20 ml chloroform-methanol (1:1) was added into each flask, and the flasks were left in the fridge overnight. The next day, the extract was separated from dry matter by running samples from a funnel

with filter. A 4 ml NaCl solution (0.9%) was added after filtering: at first 1 ml was added and mixed for 5 sec, then another 3 ml was added and mixed. The solution stood on bench overnight until the extract was clear. The upper layers (aqua layer) were removed. A 5 ml aliquot from the organic layer was evaporated in water bath (50℃) under a nitrogen flux, and weighed. Meanwhile, 2 ml heptane was added to each sample to dissolve the lipids. To start methylation, each sample was added 25µl 2M sodium metoxid ($NaOCH_3$). Samples were mixed 10 sec and left for 30 sec and mixed for another 10 sec. Then, 0.4 ml 3 N methanolic-HCl was added to each sample. Samples were put into 85℃ water bath for 15 min, and the samples were shaken twice during the time in the water bath. The samples were cooled under running tap water. After 5 min with 3,000 RPM centrifugation, 1ml samples were transferred into GC tube and subject to GC analysis.

2.5.3 Whole body and fillet nutrition analysis

Fish samples were half-thawed, cut into pieces and weighed. Then, samples were smashed by a meat grinder. The ground meat was freeze-dried for 6 days. Freeze-dried meat was homogenized in a blender with dry ice.

Energy (diet and whole body of Tilapia) was determined in an Automatic Isoperibol Calorimeter (Parr Instrument Company, USA). The whole body and fillet composition were analyzed by the AOAC official methods (Horwitz and latimer, 2005). The contents of dry matter were measured by drying the samples of whole body, fillets and diets in 105℃ overnight. The contents of ash were measured by muffle furnace, at 550℃ overnight. Lipid was quantified by the Accelerated solvent extraction method. Protein was measured by automatic Kjeldahl-N method; and crude protein was calculated by multiplying total N with 6.25.

2.6 Sensory analysis

The remaining fish were purged to reduce off flavor components before sensory analysis. During the 6-day purging period, tilapia were pooled by dietary treatment, into 3 tanks

containing tap water which was pre-heated to 25^{o}C and aerated with air stones. The purging water was totally replaced every day.

A consumer taste panel consisting of 20 non-smoking untrained judges was established. Both ethnicity ratio (Chinese: Caucasian) and gender ratio within each ethnic group were 1:1. Judge No 1-10 were Chinese, while No 11-20 were Caucasian.

Before the taste test, all purged fish were killed by a sharp blow to the head and bled by cutting gill arches on one side of the head. Afterwards visceras were removed and fish were put into 10-L boxes. A total of 12 fish per dietary treatment were subject so sensory analyses. Then each fish was weighed and wrapped into aluminum-foil. In preparation for the sensory analysis, the tilapias were baked in at 200 ℃ for 10 min. All samples were baked unsalted.

Tilapias were baked and served to the panelists in three subsequent rounds within the same day. Each panelist received three samples of fish from each dietary treatment (front, middle, and back piece of fillets from different tilapias). Chinese panelists got left side from samples while Caucasian panelists got right side from samples. In each round, one panelist was served with the same position from 3 different fish, one from each dietary treatment.

Immediately after tasting, judges answered a questionnaire. The questions were as follows:

1. Do you smell or taste any difference among the 3 pieces?
2. If yes, can the difference be characterized by differences in smell, taste or both?
3. Is the difference due to intensity of fishy smell/taste, intensity of muddy smell/taste or intensity of other smell/taste?

The judges answered question 1 and 2 by grading 1(yes) and 0 (no) and question 3 by giving grade (1/least preferred, 2/medium or 3/most preferred) to all samples according to their mouth feel.

2.7 Statistic analysis

The data of growth, whole body and fillet composition and fatty acid profile were analyzed by one-way ANOVA. Duncan's new multiple test was used to analyze the comparisons between treatments. The level of significance was set at $P<0.05$. One-way ANOVA was conducted by Microsoft Excel, while SPSS 16.0 was used to analyze Duncan's new multiple test. The results of sensory analysis were checked factorial ANOVA by SAS 2.0.

3. Results

3.1 Growth and survival

During the 28 days experiment, all tilapia survived.

Table 4 shows the average growth and feed conversion of tilapia fed with each diet. No statistically significant differences were seen

Table 4. Initial and final weight (mean±SE), weight gain (mean±SE) and FCR (mean±SE) of tilapia in the 28-days feeding trial.

	Plant diet	KHPL 5% diet	KHPL 10% diet	P-value
Survival rate	100%	100%	100%	
Initial weight, g fish^{-1}	42±2.2	43±1.0	42±1.1	0.93
Final weight, g fish^{-1}	125±6.4	128±2.7	124±3.3	0.47
Weight gain, g fish^{-1}	83±7.4	85±1.7	81±4.1	0.62
FCR[1]	1.30±0.01	1.23±0.001	1.35±0.01	0.28

[1]Feed conversion ratio, dry matter intake (g) / Weight gain (g)

3.2 Body composition

The body compositions of tilapia before and after feeding trial are shown in Table 5.

Table 5. Initial and final whole body and fillet composition (% in wet weight) (mean±S.E)

	Initial	Final			
		Plant diet	KHPL 5% diet	KHPL 10% diet	P-value
Whole body, %					
Moisture	71.5	67.1±0.3	67.5±0.3	67.7±0.5	0.58
Protein	14.6	14.4±0.1	14.3±0.3	14.8±0.3	0.34
Lipid	8.2	13.7±0.2	13.0±0.2	12.6±0.5	0.096
Ash	11.9	9.9±0.1	10.3±0.2	10.4±0.3	0.58
Fillet, %					
Moisture	21.4	22.9±0.6	22.1±0.2	22.1±0.4	0.10
Protein	18.7	19.2±0.3	19.1±0.5	19.5±0.2	0.18
Lipid	1.5	2.0±0.4	1.7±0.2	1.6±0.0	0.32

Apart a tendency for whole body lipid content ($p<0.10$), KHPL had no effect on the whole body composition of tilapia ($p>0.05$). Fish fed with plant diet tended to have the highest content of lipid, 13.7±0.2%, the second was fish with KHPL 5% diet, 13.0±0.2%; Fish fed with KHPL 10% diet contained the lowest lipid, 12.6±0.5%. The fillet compositions of tilapia fed with the different diets were at the same levels.

3.3 Fatty acid profile of Tilapia fillet and KHPL

The fatty acid profile was expressed as the weight percentage of each fatty acid relative to the total fatty acids (%). The fatty acid profile of the KHPL is described in Table 6, while that of the fish is described in Table 7.

In tilapia fillet, oleic acid (C18:1n-9) and palmitic acid (C16:0) were the most abundant fatty acids in all 3 diets group. The proportions of oleic acid in groups were 22.0%-32.0% while the quantities of palmitic acid were 20.7%-26.1%.

In overall view, diets impacted fatty acid profile greatly. Saturated fatty acids in fish fed the different diets were significantly different ($p<0.0001$), and contents were 30.1±0.3 % (plant diet), 33.0±0.2% (KHPL 5% diet) and 36.2±0.2% (KHPL 10% diet). Monounsaturated fatty acids also decreased greatly ($p<0.01$), and contents from plant diet to KHPL 10% diet were 42.3±2.0%, 37.4±0.5% and 32.0±0.5%, respectively. Content of C18:2- and 3*n-6* fatty acids also decreased significantly ($p<0.001$): 11.1±0.1% (plant diet), 10.5±0.2% (KHPL 5% diet) and 9.4±0.0% (KHPL 10% diet). Contents of C20:1/C18:3*n-3* from plant diet to KHPL 10% diet presented a declined trend: contents in plant diet (3.0±0.1%) and 2 (2.9±0.1%) showed no difference ($p>0.05$) while content in KHPL 10% diet (2.3±0.0%) showed a significant difference against plant diet and KHPL 5% diet ($p<0.01$). both of EPA and DHA contents had a clearly increasing trend: difference was significant ($p<0.0001$) in EPA, contents from plant diet to KHPL 10% diet were 0.3±0.0 3%,0.5±0.02% and 0.8± 0.1%, respectively. DHA contents in plant diet and KHPL 5% diet wasn't significantly different ($p>0.05$), while content in KHPL 10% diet differed drastically from plant diet ($p<0.001$) and KHPL 5% diet ($p=0.01$). DHA contents in each diet (from plant diet to KHPL 10% diet) were 1.9±0.3%, 3.0±0.4% and 4.6±0.2%. The total amount of *n-3* HUFA varied greatly ($p<0.001$) and contents were, in turn of plant diet to KHPL 10% diet, 2.8± 0.4%, 4.3±0.4% and 6.7±0.1%. However, contents of sum of *n-6* HUFA were 1.5±0.2%, 1.1±0.1% and 1.2±0.1%, showing no difference ($p>0.05$). Ratio of *n-3* HUFA /*n-6* HUFA was significantly different ($p<0.01$).

Table 6. Fatty acid profile of KHPL (mean percentage in samples) used in diets fed for tilapia for a 28-day feeding trial

	Saturates	Monoenes	18:2-and 3n6	20:1	Sum of *n-6* HUFA	Sum of *n-3* HUFA	EPA	DHA
KHPL	44.975	16.215	1.635	1.215	0.445	16.485	11.65	4.44

Table 7. Content of fatty acid profile in fillet (percentage in total fat) of tilapia (Mean ±SE)

	Plant diet	KHPL 5% diet	KHPL 10% diet	P-value
Saturates	30.1±0.5^{a}	33.0±0.3^{b}	36.2±0.4^{c}	<0.0001
Monoenes	42.3±3.5^{c}	37.4±0.8^{b}	32.0±0.9^{a}	0.0032
C18:2-&3*n-6*	11.1±0.2^{c}	10.5±0.4^{b}	9.4±0.02^{a}	0.0005
C20:5*n-3*	0.3±0.03^{a}	0.5±0.02^{b}	0.8±0.1^{c}	<0.0001
C22:6*n-3*	1.9±0.5^{a}	3.0±0.7^{a}	4.6±0.4^{b}	0.0025
C20:1/C18:3*n-3*	3.0±0.3^{b}	2.9±0.3^{b}	2.3±0.1^{a}	0.0098
Sum *n-3* HUFA	2. 8±0.8^{a}	4.3±0.7^{b}	6.7±0.2^{c}	0.0008
Sum *n-6* HUFA	1.5±0.3	1.1±0.1	1.2±0.1	0.25
Ratio *n-3*/*n-6* PUFA	1.9±0.1^{a}	3.8±0.4^{b}	5.5±0.4^{c}	0.0002

Difference level was set at $P<0.05$
Mean±SE with the same superscripts in line were not significantly different at 5% significance level

Contents of lipid and fatty acid profile in fillet were also calculated (Table 8). Total fillet lipid content did not show significant differences: 20.3±2.1 g/kg from fish fed with plant diet, 16.6±1.3 g/kg fish fed with KHPL 5% diet and 15.9±0.3 g/kg fish fed with KHPL 10% diet. Fatty acid contents, however, were significantly affected by KHPL inclusion. Differences in monounsaturated, C18:2-and 3n-6 and total amount of *n-6* HUFA were significant ($p<0.05$): contents of monounsaturated fatty acid, from plant diet to KHPL 10% diet, were 86.6±11.7mg/kg, 62.1±5.5 mg/kg and 50.9±1.6 mg/kg, among which fish in plant diet differed from fish in KHPL 5% diet and KHPL 10% diet ($p<0.01$) while there were no difference between KHPL 5% diet and KHPL 10% diet ($p>0.05$). Contents of C18:2-and 3 *n-6* in fish fed with plant diet to KHPL 10% diet were 22.5±2.3 mg/kg, 17. 5±1.5 mg/kg and 15.0±0.3 mg/kg, respectively, and also fish fed with KHPL 10% diet showed difference from fish in plant diet ($p<0.05$) but neither did fish fed with plant diet and KHPL 5% diet,

nor fish fed with KHPL 5% diet and KHPL 10% diet showed difference (p>0.05). Contents of *n-6* HUFA from fish fed with each diet differed from each other greatly (p<0.01): 2.9±0.3 mg/kg (plant diet), 1.9±0.1 mg/kg (KHPL 5% diet) and 1.9±0.1 mg/kg (KHPL 10% diet), among which fish in plant diet differed from fish in KHPL 5% diet and KHPL 10% diet greatly (p<0.01) whereas fish in KHPL 5% diet and KHPL 10% diet had no difference (p>0.05). Contents of *n-3* HUFA, DHA and EPA have great significance (p<0.01). Contents of *n-3* HUFA in tilapia fed with different diets had significantly increasing trend (p<0.0001): 5.5±0.5 mg/kg (fish in plant diet), 7.0±0.1 mg/kg (fish in KHPL 5% diet) and 10.6±0.1 mg/kg (fish in KHPL 10% diet). Fish fed with different diets contained DHA and EPA showed highly significant difference (p<0.01): contents of EPA in fish in plant diet, KHPL 5% diet and KHPL 10% diet were 0.7±0.1 mg/kg, 0.7±0.1 mg/kg and 1.3±0.1 mg/kg, respectively. Fish in KHPL 10% diet showed a significant difference from fish in plant diet and KHPL 10% diet (p<0.01) whilst fish fed with plant diet and KHPL 10% diet showed no difference (p>0.05). Moreover, contents of DHA in fish fed with different diets showed a clearly increasing tendency: 3.8±0.3 mg/kg (fish in plant diet), 4.9±0.2 mg/kg (fish in KHPL 5% diet) and 7.3±0.3 mg/kg (fish in KHPL 10% diet). However, contents of saturated fatty acid and C18:3n-3/C20:1 had no difference in fish fillet (p>0.05): fish from plant diet to KHPL 10% diet contained 61.3±7.1 mg/kg, 54.7±4.1 mg/kg and 57.6±0.7 mg/kg, respectively. Contents of C18:3n-3/C20:1 in fish fed with each diet were 6.1±0.9 mg/kg, 4.9±0.6 mg/kg and 3.6±0.1 mg/kg, respectively. However, content of C18:3n-3/C20:1 in fish in KHPL 10% diet was significantly difference from that of fish in plant diet (p<0.05) while contents in fish plant diet and KHPL 5% diet as well was fish in KHPL 5% diet and KHPL 10% diet were not different (p>0.05).

Table 8. Content of lipid and fatty acid profile in fillet of tilapia (in wet weight, mean ±SE)

	Plant diet	KHPL 5% diet	KHPL 10% diet	P-value
Lipid in fillet, g kg^{-1}	20.3±2.1	16.6±1.3	15.9±0.3	0.15
Fatty acids, mg kg^{-1}				
Saturated fatty acids	61.3±7.1	54.7±4.1	57.6±0.7	0.63
Monounsaturated	86.6±11.7^{b}	62.1±5.5^{ab}	50.9±1.6^{a}	0.039
C18:2-&3 *n-6*	22.5±2.3^{a}	17.5±1.5^{ab}	15.0±0.3^{b}	0.040
C20:5 *n-3*	0.7±0.1^{a}	0.7±0.1^{a}	1.3±0.1^{b}	0.0006
C22:6 *n-3*	3.8±0.3^{a}	4.9±0.2^{b}	7.3±0.3^{c}	0.0003
C20:1/C18:3 *n-3*	6.1±0.9^{b}	4.9±0.6^{ab}	3.6±0.1^{a}	0.082
Sum *n-3* HUFA	5.5±0.5^{a}	7.0±0.1^{b}	10.6±0.1^{c}	<0.0001
Sum *n-6* HUFA	86.6±11.7^{c}	62.1±5.5^{b}	50.9±1.6^{b}	0.012

Difference level was set at $P<0.05$
Mean±SE with the same superscripts in line were not significantly different at 5% significance level

3.4 Sensory analysis

In the sensory analysis, tilapia fed with KHPL 10% diets was the most preferred by the panelists (n=28), followed by plant diet (n=14) and with fish fed with KHPL 5% diet (n=12).

Four questionnaires (1 Chinese female, 1 Chinese male, 2 Caucasian females) were not suitable for further processing, because they were not able to identify differences among the samples. Ethnicity was balanced while gender was unbalanced. The gender difference was found in smell (P=0.032) but not in taste (P=0.052). The female is more sensitive than male in smell (♀=0.67, ♂=0.48). Ethnic difference was shown by Chinese being more sensitive than Caucasians towards fishy taste (Chinese=2.39, Caucasian =0.49) (P< 0.0001). On the other hand, Caucasians are sensitive than Chinese both in muddy taste (Chinese=1.65.Caucasian =1.19) (P=0.012) and other taste (Chinese=0.47.Caucasian =0.097) (P=0.0065).

Table 9. Result of tilapia sensory analysis

	Diet			
	Plant diet	KHPL 5%	KHPL 10%	P-value
Prefference	0.33[b]	0.25[a]	0.52[c]	0.018
	Ethnicity			
	Caucasian	Chinese		
Smell	0.67	0.61		0.47
Taste	0.89	0.79		0.28
Fishy taste/smell	0.49[a]	2.39[b]		<0.0001
Muddy taste/smell	1.19[a]	1.65[b]		0.012
Other taste/smell	0.097[a]	0.47[b]		0.0065
	Gender			
	Female	Male		
Smell	0.67[b]	0.48[a]		0.023
Taste	0.85	0.95		0.052
Fishy taste/smell	1.01	1.12		0.63
Muddy taste/smell	1.18	1.40		0.52
Other taste/smell	0.40	0.49		0.20

Difference level was set at $P<0.05$
Mean±SE with the same superscripts in line were not significantly different at 5% significance level.

4. Discussion

4.1 growth trial

4.1.1 Effects of feed ingredient on growth

Various studies have been demonstrated diet formulation influences weight gain and FCR of tilapia (Clement and Lovell, 1994; Hussein et al., 2012; Fall et al., 2012; Al-Souti et al., 2012). In present study, dietary KHPL did not significantly affect tilapia feed intake, growth, survival, or feed conversion. This can be concluded as the increasing content of *n-3* PUFA and decreasing content of *n-6* fatty acids in KHPL cannot promote faster growth of tilapia, when the essential fatty acid requirement already is met from plant oild. Freshwater fish, such as tilapia, has a greater need of *n-6* lipid compared to that of *n-3* lipid (NRC, 1993). The *n-6* fatty acids are claimed as the only fatty acids that were needed by tilapia according to Kanazawa et al. (Kanazawa et al., 1980a; Kanazawa et al., 1980b). High content of *n-3* lipids has previously been demonstrated not to enhance growth in tilapia (Takeuchi et al., 1983), or even negatively affects growth (Huang et al., 1998). This conclusion is also supported by Ng et al., they reported growth of tilapia was significantly depressed by cod liver oil diet of which the FER was 0.82 g wet gain /g dry weight of feed while FERs of other lipid resources were from 0.97 to 1.12 wet weight gain/g dry feed weight (Ng et al., 2001). Also, Hanley confirmed 5%-12% dietary lipid with up to 7.2% yellow grease additive had no effect on tilapia growth: neither weight gain nor FCR showed statistical difference (Hanley, 1991). However, the evidences of the beneficial effect of *n-3* PUFA on growth are reported by several other experiments. Chou et al. stated that both of *n-3* and *n-6* PUFA were needed to get the maximum growth rate of hybrid tilapia (Chou and Shiau, 1999). Later, they reported up to 5% cod liver oil which is rich in *n-3* PUFA doesn't slow down growth of hybrid tilapia (Chou et al., 2001). Santiago and Reyes found that cod liver oil improved growth but reduced rehydrolysation of broodstock of tilapia, compared to other lipid resources, e.g. corn oil and soybean oil (Santiago and Reyes, 1993).

4.1.2 Effect of feeding strategy on growth

Growth rate in this experiment is higher and FCR more efficient than those in earlier experiments. Jackson et al. (1982) verified a lower weight gain (15 g $fish^{-1}$) and higher FCR on tilapia fed with fish oil-enriched feed (Jackson et al., 1982). In another juvenile Nile tilapia growth trial (Kumar et al., 2012), FCR was from 3.1-4.9, while FCR observed at this experient was 1.3. Apart from feed ingredients (KHPL versus fish oil/plant oil), genetic fish material, and feeding strategy (frequent feeding, feeding to satiation without stressing the fish), and farming environment (resirated and aerated water) may have contributed to the high feed intake, rapid growth, and efficient feed conversion in our experiment.

In present test, feeding rate was adjusted once every 3 days and rate of overfeeding was kept at 10% total weight. In 2012, Al-Souti et al. conducted an experiment of fish oil-enriched feed on tilapia (Al-Souti et al., 2012). In this feeding trial, gradients of fish oil were from 0%, 4%, 8%, and 12%. The weight gain varied from 42.5 g $fish^{-1}$ to 46.1 g $fish^{-1}$ and the FCR were from 1.56 to 1.70. The fish was raised from proximate 15.5g to about 60g. Compared with the Al-Souti et al test, the present results showed much higher weight gain and lower FCR. Firstly, the flexibility of feeding rate in KHPL trial contributed to these results. In the current experiment, the feeding rate was adjusted to keep uneaten feed up to 10%. The flexible feeding rate was supported by Chowdhury (2011), who conducted a series of experiment to investigate the best feeding rate for tilapia. In one of those experiments, 4 declining feeding rates (16%-, 14%-, 12%-, and 10%- to 8%) and 1 fixed feeding rate were set. It turned out the fixed feeding rate had the lowest weight gain (300%) meanwhile the highest gain was obtained by feeding rate 14-8% (377%), the rest were from 330.8% to 364.2%. the difference was significant (Chowdhury, 2011). However, too high feeding rate may increase weight gain but survival rate may drop dramatically (Santiago et al., 1987).

Secondly, the difference of weight gain and FCR compared to other experiments was also from feeding frequency. Generally, a 4-time-feeding system (0900, 1300, 1700 and 2100) was applied in the present study so fish had sufficient time to absorb nutrients. Growth will ascend when fish is fed in a higher feeding frequency because of the higher feed intake (Grayton and Beamish, 1977). In fish oil-enriched test, fish was fed 2 times a day (0700 and

1800) and feed volume was determined by fish appetite. This caused an inefficient digestion because the diet habit may affect enzymes to digest nutrients, such as protein, carbohydrate (Tung and Shiau, 1991). Tung and Shiau found a significant difference on weight gain when fed more frequently (6 times) than feeding 2 times (Tung and Shiau, 1991). Another feeding experiment on 0-year-old hybrid sun fish (*Lepomis macrochirus*) also accord with the current experiment: SGRs in 4-time feeding regime was significantly higher than that of 1-time feeding (Wang et al., 1998). On another hand, Estuary Grouper (*Epinephelus akaara*) was found that have higher growth and food conversion rate at one feeding every two days, rather than one or two feedings per day (Thia-Eng and Seng-Keh, 1978).

4.2 Effect of KHPL on body composition

In present study, KHPL showed no significant effects on body composition, except for lipid composition. This was in keeping with previous observations. But crude palm oil, linseed oil and soybean oil, regardless of sole or their blends, are showed no significant difference on the body composition of tilapia as partial replacement of fish oil (Ng et al., 2012). In 2001, Ng et al. also has proved that lipid sources made no difference on tilapia body composition. Fish fed with various oils have nutrients at the same level: protein at 15%, ash at 4% and lipid at 5.5% (Ng et al., 2001).

Contents of fillet protein and lipid were similar to those in other experiments. A similar body composition was demonstrated by Puwastien et al in tilapia (Puwastien et al., 1999). Justi et al reported a lower fillet lipid composition (around 1.08%) in tilapia when fish was fed with flaxseed feed enriched with *n-3* fatty acid for 10-30 days (Justi et al., 2003) while a much higher lipid content, 2.26%, was reported by Izquierdo et al (Izquierdo et al., 2000).

4.3 Fatty acid profile

4.3.1 Effect of KHPL on fatty acid profile

Fatty acid profile represents to what extent a feed ingredient, KHPL in this case, impacts fish lipid composition. In this trial, effect of KHPL on fillet fatty acid was great.

The result of fatty acid profile in current test is agreed with de Souza et al: the major lipids were oleic acid (18:1n-9), palmitic acid (16:0) and linoleic acid (18:2*n-6*). In his experiment, flaxseed oil was used as treatment and sunflower seed as common group. After 5-month feeding trial, fish was slaughtered and the fatty acid composition was analyzed by FAME, the contents of oleic acid, palmitic acid and linoleic acid were 20.0-21.1%, 14.3-15.0 and 20.7-29.2%, respectively (de Souza et al., 2007). Huang et al also proved content of linoleic acid of tilapia fed with soybean oil-included feed was more than 50% (Huang et al., 1998).

KHPL contained a high content of *n-3* PUFA. Compared to the control group, content of *n-3* PUFA increased as content of KHPL increased. The dietary lipid of fish body is affected by dietary lipid of feed (Huang et al., 1998). In the current test, KHPL has a similar effect on tilapia fatty acid composition as fish oil does. This is totally agreed with by Al-Souti: tilapia fed with 12% fish oil has the same FA profile as KHPL-enriched tilapia (Al-Souti et al., 2012), e.g., palmitic acid, oleic acid and DHA are three the most abundant fatty acids in fillet, and contents of DHA (0.4-0.9%) and EPA (5.8-16.1%) amplified as fish oil increased while contents of *n-6* PUFAs decreased. In addition, Karapanagiotidis et al. found the same trend: they investigated effect of fish oil, plant oil, and their blend. The composition of DHA and EPA in fish oil feed was significantly higher than other lipid resources, (Karapanagiotidis et al., 2007a). Increased *n-3* PUFA and decreased *n-6* PUFA were also found in sea bream and sea bass when fed with fish oil (Mourente et al., 2005).

Tilapia need a moderate amount of LNA (18:2*n-6*) and a lesser amount of LA (18:3*n-3*) as they are precursors for all PUFAs in freshwater fish. In this experiment, content of C18:3*n-6* and C18:2*n-6* was significantly higher in the control group which was made of plant resources, while it decreased in KHPL 5% diet and KHPL 10% diet as content of KHPL increased. The relatively high content of C18:3*n-6* and C18:2 *n-6* was resulted in assimilation and esterification of dietary lipid (Henderson, 1996), especially plant lipid. This was indicated that body lipid composition is affected by dietary lipid (Ferreira et al., 2011).

In the resulting fatty acid profile of the fish, the C18:3*n-3*/C20:1 is of ambiguity. This can be explained as C18:3*n-3* and C20:1(C20:1 (n-7) + (n-9)) couldn't be differentiated by GC machines due to uncertain reasons. Generally, C18:3*n-3* constantly exists in plant resource while C20:1 is abundant in marine resource. In current experiment, plant diet had no marine resource so the content of C18:3*n-3*/C20:1 should be mostly contributed to C18:3*n-3*, which was 3.0%. In KHPL 5% diet, ratio of rapeseed oil and KHPL was 1:1 so content of C18:3*n-3* outnumbered C 20:1. In KHPL 10% diet, KHPL content increased significantly, so C 20:1 took major proportion of C18:3*n-3*/C20:1. This indicated lack of C18:3*n-3* in KHPL. The fact that content of C18:3*n-3*/C20:1 decreased greatly from 2.9% to 2.3% was observed in KHPL 5% diet and KHPL 10% diet. Meanwhile, contents of EPA and DHA increased significantly (0.5-0.8% and 1.94-4.60 %, respectively). The reason should be the increasing contents of KHPL in diets lead to decreasing content of C18:3*n-3*, meanwhile C18:3*n-3* is the precursor to EPA and DHA. As the accumulation of EPA and DHA, content of C18:3*n-3* kept dropping. This was agreed with by Cleland et al.: they found a inverse relationship between linolenic acid and EPA/DHA, meaning higher linolenic acid content results in a lower EPA and DHA contents (Cleland et al., 1992). The same trend was reported by several other research groups (Garg et al., 1990; McMurchie et al., 1990; James et al., l991; Karapanagiotidis et al., 2007a; Karapanagiotidis et al., 2007b).

In addition, variation of contents of saturated fatty acid and monounsaturated fatty acid was different from the results of other experiments. Content of saturated fatty acid increased while monounsaturated fatty acid decreased due to different content of these two groups in KHPL. In Ng et al test, tilapia fed with palm oil showed a stable content of monounsaturated

fatty acid as palm oil increase (Ng et al., 2006). The increased saturated fatty acid with increased KHPL was agreed with tilapia fed with fish oil (Al-Souti et al., 2012).

However, KHPL-enriched tilapia can contribute a small amount to human dietary intake of *n-3 and n-6* fatty acids. Simopoulos (2000) reported a recommendation on daily intake of DHA and EPA where 650 mg/d was the minimum for an adult while either of DHA and DPA should be more than 220 mg/d (Simopoulos, 2000). However, the maximum of DHA+EPA in KHPL-enriched tilapia is less than 9 mg/kg (fish fed with KHPL 10% diet). This indicates consumers must consume other foods to fulfill daily need. In another research on relation of *n-3* fatty acid intake and occurrence of Alzheimer disease (AD), higher daily intake of *n-3* fatty acid decrease incidence of AD to 20-30% (Morris et al., 2003). Compared to PUFA intake in Alzheimer disease research, tilapia in current experiment can hardly offer sufficient PUFA to the aged.

4.3.2 Effect of KHPL on *n-3*/*n-6* ratio

The use of marine resource modified PUFA of tilapia fillet: *n-3* increased while *n-6* decreased. *N-3* /*n-6* ratio is a critical index of PUFA composition for human health (Mourente et al., 2005; Nasopoulou and Zabetakis, 2012; Tonial et al., 2012; Mourente and Bell, 2006). In the present study, *N-6*/*n-3* ratio depresses when KHPL increases.

In human dietary history, plant is a new choice on menu since 10000 years ago. Before vegetarian diet, human beings ate meat and wild fruit which figured our dietary habit genetically (Simopoulos, 2002). Now, 17% plant offers 90% food supply (Karger, 1995). But cereal grains are rich in *n-6* fatty acid and low in *n-3* fatty acid. In Caucasian diet, ratio of *n-6*/*n-3* is 10-20:1 while the recommended ratio is 1-2:1 (Simopoulos, 1999). A investigation reported high ratio of *n-6* and *n-3* leads to a high frequency of thrombus and atheromas (Simopoulos, 2002). In feeding trials, plant resources give fish more *n-6* fatty acid while marine resources give more *n-3* PUFA. Karapanagiotidis et al reported fish oil-fed tilapia was 2.0, ranking the first on *n-3*/*n-6* ratio, followed by linseed oil-fed Tilapia (1.5), while mixture of palm oil and fish oil/linseed oil (1.2 and 0.9, repectively) and coconut oil has significantly adverse effect on *n-3*/*n-6* ratio (0.3) (Karapanagiotidis et al.,

2007b). Compared with the result above, 10% KHPL has similar effect on *n-3*/*n-6* ratio as linseed oil and weaker than fish oil. In another human-related experiment, linseed oil decreased *n-6*/*n-3* ration in plasma of carnivorous consumers down to a relative higher value, 3.8 (Weill et al., 2002).

4.4 Tilapia sensory analysis

The effect of KHPL on sensory analysis of tilapia is focused on in this experiment. The sensory panel could identify difference in smell and taste.

4.4.1 Effect of KHPL on sensory analysis

KHPL used in this experiment was rich in phospholipids. KHPL gave tilapia a special olfactory response compared to feed with plant resource. Phospholipids contain much more unsaturated fatty acids than triglyceride. In cooking process, high level of unsaturated fatty acids presents a desirable smell by being oxidized and increases total amount of volatile compounds (Salter et al., 1988). The phospholipids in tilapia is believed put forth a quenching effect on the sum of heterocyclic Maillard compounds during food preparing (Whitfield et al., 1988). Olfactory difference is the result of diverse lipid composition and polar extent, also lipid interaction in the Maillard reaction plays a vital role in flavor formulation (Whitfield et al., 1988). Deficiency of phospholipids affected aroma of roast beef greatly (Mottram and Edwards, 1983; Mottram, 1998). Also, a hypothesis that lipid optimized level of sulfur compounds which gave food sulfurous odors affirmed the role of phospholipids in cooked meat (Mottram, 1998).

In respect of taste modification, up to 1.5 mg/g long-chain polyunsaturated fatty acid worsened the salmon flavor with metal taste and bitterness according to Refsgaard et al. (Refsgaard et al., 2000). Also, total phospholipids as well as fatty acid content among phospholipids were highly linked to flavor uniqueness of beef (Larick and Turner, 1990). Igene and Pearson demonstrated that phospholipids donated much more than triglyceride did

on warmed-over flavor (Igene and Pearson, 1979). In current research, content of long-chain polyunsaturated fatty acid in phospholipids is up to 0.01mg/g fillet, so the adverse effect could be neglected.

4.4.2 Effect of gender on sensory analysis

In aspect of smell, female has a gift of detecting odor, compared to male. This may be attributed to hormones, memory to smells and other causes. The gonad hormones are one of the reasons that lead to difference between odors detective ability of genders. They works directly on relevant organs or affect stimulations of central nerve system (Gandelman, 1983). The rehydrolysateive hormones impact electronic connection between brain and olfactory cues (Simerly, 1990). For example, the excretion of estrogen increased performance of odor identification during ovulation and pregnancy (Good et al., 1976; Caruso et al., 2004). Date back to 1899, the perception of female to camphor solution was 0.001 ‰, higher than that of male to the same solution, 0.009‰ (Toulouse and Vaschide, 1899). In a later test, woman proved more sensitive than man on artificial chemical (Le. Magen, 1952). The perceptivity of women's olfactory is 8 times accurate than that of men's (Doty and Cameron, 2009). . Females also have advantage of naming and remembering odors. According to Lehrner, women performed outweighed men on smell identification test which had up-to-21-day retention interval (Lehrner, 1993). Women has better performance on smell verbal processing, i.e. memory for naming memorable odor and classification. However, man and woman perform equally on primarily sensory acuity (Öberg et al., 2002). But in some studies, sexual variation was found in childhood when gonad hormones were not secreted in high level (Koelega and Köster, 1974). In addition, Larsson et al. claimed that based on result of an experiment in Sweden, gender has nothing to deal with odor detection nor identification (Larsson et al., 2000). In current sensory analysis, the gender ratio was balanced. In female group, no one was pregnant, so odor identification ability was not affected by estrogen.

4.4.3 Effect of ethnicity on sensory analysis

More than an identification of one's birth, the word "ethnicity" presents a group of people having the same ancestry. Apart from anthropology, ethnicity includes the culture they were raised up and lifestyle they had, to be specific, food they got used to eat. In the current sensory analysis, ethnicity plays a role of great vitality in smell/taste distinguish. The sensory panelists were divided into 2 groups: Caucasian and Chinese.

Chinese have a 3000-year history of freshwater aquaculture since Yin dynasty. Freshwater fish, such as common carp, silver carp and so on, has become an unchangeable part in Chinese diet, so Chinese can bear high level of the smell/taste of mud and other smell/taste of which most were generated from on living environment of freshwater fish or the diet they have. In Chinese sensory panelists, 6 of them were from south coastal area of China, by whom the mean level of fishy taste/smell tolerance was increased. The reason was that marine fish take a higher portion on diet of Chinese living in the coastal area, compared to inland Chinese. A prove is the usage of marine algae: due to the availability and price, coastal dwellers consume higher portion of algae as vegetable or its substitution than inland dwellers (Bangmei and Abbott, 1987). However, fishy taste/smell endurance of Chinese was still lower than that of Caucasian. In contrast to Chinese, Caucasians are familiar with oil-rich marine fish, so they are not sensitive to fishy smell/taste. But due to unpopularity of freshwater fish in Europe, Caucasians have a strong response to freshwater fish, mainly on muddy smell/taste. It was the first time that 10 Caucasian judges eat Tilapia, so they would experience and classify odors from samples into olfactory memory that they have had.

In general, judgments of consumers to food and the originality of consumers are connected (Guerrero et al., 2009). It was believed that odor judgment associated with mother's diet started can begin at even in pregnancy period (Hudson and Distel, 1999). Like language learning, odor/taste adaption depends on environments and ethnicity strongly: people will accept food with odors/tastes they are familiar and attempt to conclude all odors into their sensory memory. In Italy, children has developed a habit of drinking hot coffee which has become a part of their family and social life (Rozin and Cines, 1982). Children intake of

vegetable and fruit was also in the same habit formation: amount of vegetable and fruit parents ate, traditional family mealtime and intake-start age matters in a positive correlation (Cooke et al., 2003). A consumer-acceptance research showed that ethnicity had a great influence on snack choice of Americans and Asians: significant difference on fish snack choice while no difference on peanut snack. Asian consumers had a higher evaluations on fish snack than Americans did, due to the fish-eating habit of Asian (Suknark et al., 1998). Familiarity is one of key roles in taste acceptance and can explain the alterations in cross-cultural food evaluation (Chung et al., 2012).

5. Conclusions

The series of experiments proved the applicability of KHPL in tilapia and enhanced effect of KHPL on fillet sensory characteristics.

In feeding trial, KHPL was utilized as main lipid resource in tilapia feed at the first time and it proved has no effect on growth. The effect of KHPL on body composition was detected on whole body lipid content only, and had no effect on other nutrients. However, the modification of fatty acid profile, especially *n-3* and *n-6* fatty acids, was significant. Compared to plant diets, diets applying KHPL alters contents of DHA, EPA and linoleic acid. Moreover, with help of KHPL, *n-3*/*n-6* ratio of tilapia becomes higher than that of fish fed with whole-plant feed, which was much more beneficial to human being's health. But eventually, tilapia is a lean fish (fillet lipid less than 2%), so it can hardly satisfy dietary requirement of *n-3* PUFA in human.

In sensory analysis, higher content of KHPL proved a favorite of assumed consumers, both Caucasian and Chinese. Through this test, we also find the difference can be attributed to gender and ethnicity: due to nurture and learning, Chinese has a higher endure of earthy and other smell/taste, while cannot tolerate fishy smell/taste; *au contraire*, Caucasian have a higher fishy tolerance but was weak at bearing muddy smell/taste.

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