Krill meal as attractant in plant-based for Nile tilapia

JOYDEB PAUL & Qiaona Yan

Norwegian University of Life Sciences
Department of Animal and Aquacultural Science
Master Thesis 30 credits 2012

Krill meal as attractant in plant-based diets for Nile tilapia

Master thesis (30 credits)

Joydeb Paul and Qiaona Yan

Department of Animal and Aquacultural Sciences
Norwegian University of Life Science

Ås 2012

Abstract

Three diets for juvenile Nile tilapia *Oreochromis niloticus* were prepared substituting 5% of plant protein with products from Antarctic krill (*Euphausia superba*). Plant material was used as the sole source of protein in the control (Diet 1). Diets 2 and 3 had plant protein with different levels of krill meal (2.5% KM+2.5%KFC and 5% KM, Diets 2 and 3 respectively). The diets provided 384 g crude protein kg^{-1}, 275 g pre-extruded starch kg^{-1} and 111 g lipid kg^{-1}. The diets were fed approximately 10% in excess of satiation to triplicate groups of Nile tilapia four times daily for 28 days. Each diet contained 80 mg Y_2O_3 kg^{-1} diet for digestibility determination. Fish of an initial average weight 25 ± 2g were stocked in nine tanks (70×50×50 cm), with 20 fish per tank, supplied with 27 ± 1^{o}C water. The final mean weight of the tilapias was 90-104 g (28 days of feeding). Fish fed 5% krill meal supplemented diets had no significant difference in growth performance ($P > 0.05$) compared with fish fed plant control diet. While fish fed the 2.5% KM+2.5% KFC supplemented diet had significantly inferior growth ($P < 0.05$) compared with control and 5% KM diet. Feed intake and feed conversion ratio did not differ significantly for fish fed 5% KM diets compared with the plant control. But fish fed the 2.5% KM+2.5% KFC diet had the significantly more efficient feed conversion compared to other two diets. Fatty acids composition was significantly changed in fish body by feeding the different diets and fish fed by KM significant higher omega-3 fatty acids content in whole body. There were no significant differences found from chemical compositions of fish body (except ash content), digestibility of nitrogen and phosphorus, and utilization of the nutrients, such as nitrogen retention, energy retention and phosphorus retention.

Contents

Acknowledgements

The practical part of the study presented in this thesis was carried out in the Feed Ingredients and Processing (FIP) section of the Aquaculture Protein Centre (APC) as a part of our Master of Science degree at the Department of Animal and Aquaculture sciences, Norwegian University of Life Sciences.

This thesis was accomplished under the supervision of Prof. Trond Storebakken. We hereby presenting our great gratefulness to our dear supervisor, for his meticulous teaching and correcting during the experiment as well as writing work. In addition, thanks for trusting a lot and guiding us to become a good researcher, thinker and hard worker with honesty.

Secondly we would like to thank Dilip Kumar Chowdhury for his initial support during our feed processing. A special appreciation goes to Frank Sundby with professional and skilful contribution to the sample analysis.

Furthermore, lots of love and thanks to parents for encouraging and supporting us in taking this education abroad.

We express respect to all teachers, Department of Animal and Aquaculture Sciences, UMB for their kind co-operation during the study period.

Ås, December, 2012

Abbreviations

ADC = Apparent digestibility coefficient

ANF = Anti-nutritional factors

BWI = Body weight increase

DE = Digestible energy

DM = Dry matter

FCR = Feed conversion ratio

FI = Feed intake

MCP=Mono calcium phosphate

IAA = Indespensible amino acid

KFC = Krill flavour concentrate

KM = Krill meal

P = Phosphorus

SGR = Specific growth rate

WG = Weight gain

1. Introduction

1.1 Protein resources for aquaculture

Aquaculture has been rapidly on the rise in the world during the last few decades (FAO, 2002). The higher growth rate of the aquaculture, 8.9% per year since 1970, compares favorably with the 2.8% per year exhibited by other animal food-production sector (FAO, 2004). In intensive aquaculture production, the elevated demand of feed has been observed in recent years to parallel amplified total production volumes (FAO, 2004). The requirement of a high quality protein sources are also increasing as intensive aquaculture continues to expand. In aquaculture diets fish meal (FM) is considered to be the most enviable animal protein ingredient because of its high protein content, balanced amino acid profiles, high digestibility and palatability. It is also preferred as a basis of essential *n-3* polyenoic fatty acids (Muzinic et al., 2006). Only 5-7 million tons of fish meal are available from wild fish per year (Chamberlain, 2011). This raises the demand on traditional marine ingredients (Olsen et al., 2006), causing higher prices (Hansen, 2011). Aquaculture nutritionists have approached this demand by partial or total substitution of fish meal having less expensive animal and/or plant protein sources (Muzinic et al., 2006).

In contrast to carnivorous fish species, like the salmonids, tilapias can grow well on diets virtually without fishmeal or concentrated plant sources. In European fish feed, terrestrial animal by-products are banned (EC1774/2002 and 93/2005) because of probable transmit of animal infectious agents such as Bovine Spongiform Encephalopathy (BSE) and avian influenza. This has lead to increased consumer consciousness of feed and food safety, particularly in Asia (Scahaws, 2003). Availability of other sources such as single cell ingredients limits the availability in market due to high prices. Other possibilities for new feed materials may include widespread marine macroalgae or fresh water weed hyacinth (El-Sayed and Tacon, 1997), as well as marine by-products, that is, crustacean products such as krill and

shrimp (Suontama, 2006). Locally, there is some scope for their incorporating them into fish feeds, particularly for tilapia and mullets. Some studies of the high inclusion of plant proteins show a slowing down of feed intake due to poor palatability, resulting in reduced growth performance (Mundheim et al., 2004). However, the use of attractants and processed ingredients may improve the palatability of feed (Deng et al., 2006).

1.2 Importance of tilapia production

Nile tilapia (*Oreochromis niloticus*) is omnivorous fish which possess morphological and physiological adaptations for utilization of diets high in fibre content. Most formulated feeds for tilapia resembles those for omnivorous fish in that they contain significant levels of animal proteins (Hughes and Handwerker, 1993). Tilapias are commercially the second most vital cluster of wild-captured freshwater fish after the carp with a worldwide capture (harvest reaching) 769,936 tonnes (metric tons) in 2007 (FAO, 2009). Nile tilapia was ranked fifth among the most cultured species in the world in 2008, involving a total aquaculture production of 2.3 million tons (FAO, 2009). Approximately 84% of total global tilapia production has been represented by Nile tilapia (FAO, 2009). Traditionally Asian and African people were consumed tilapia earlier. However, in current years tilapia has been peddled as the “new white fish” to reinstate the dwindling ocean stocks of cod and hake, leading to a worldwide demand for tilapia (Yue and Zhou, 2008). China is by far the largest producer and consumer (About 50% of the global production) of tilapia with a production of 3 million tons in august 2011 (FAO, 2011). Other main producing countries of farmed tilapia (2003-2004 data) are Egypt (290,000 tonnes), Indonesia (206,000 tonnes), Philippines (241,000 tonnes), Thailand (180,000 tonnes) and Brazil (100,000 tonnes) (FAO, 2010).

The U.S is the single largest importer of tilapia in the world (ERS, 2010). The U.S imported 183,295 tons of tilapia product in 2009, valued around 695.1 million dollar (ERS, 2010).

Fairly distinctive place grasps by tilapia amongst the foremost aquaculture fishes as a key product in international trade produced in huge perpendicularly integrated farming operations, at the same time as still being produced in large amounts as a continuation crop by some of the world's poorest farmers (Fitzsimmons, 2010).

1.3 Genetically improved farmed tilapia

The Genetic Improvement of Farmed Tilapia (GIFT) project was instigated in 1988 as a breakthrough for tropical finfish genetic improvement around the world (Acosta and Gupta, 2010). The GIFT project succeeded in producing tilapia was based on the selective breeding of Nile tilapia with faster growth rates, higher survival rates, and a shorter harvest time, resulting in a noticeable increase in fish yield. The GIFT project gained genetic improvements of 7.1 percent genetic change over nine generations of fish, or 64 percent tilapia overall growth over the base population. This was based on the successful selective breeding of *O. niloticus* (Ponzoni et al., 2010). Eknath and Acosta (1998) figure out higher improvement (12-17 percent) over five generations of fish, 60-85 percent collectively increased. The inherent physiological potential might not have been promoted, due to concern with energy metabolism and digestion efficiencies connected with the genetic improvement or modification in the GIFT or GMNT (Mamun et al., 2007). In order to reduce the major disease agent (*Streptococcus agalactiae*), it was determined that resistance should be genetically improved to lessen the exercise of pharmaceuticals (Thodesen et al., 2011). Thodesen et al. (2011) also illustrated that after six generations of multi-trait selection, genetic (Eknath and Acosta, 1998) improvement of growth (60–90% higher body weight at

harvest) was achieved significantly by the ongoing selective breeding of Nile tilapia in China. Minimal dependency on marine derived raw materials for aquafeed production was required when farming with improved strains of Nile tilapia (Teoh et al., 2011).

1.4 Diets for tilapia, common practice and optimum formulation

The optimum protein requirement of Nile tilapia depends on size, age and water temperature. Several studies have estimated that the protein requirement for juvenile tilapia varies from 32 to 50 % and for larger tilapia 25 to 30% (Nguyen et al., 2009). The optimum lipid requirement for tilapia is 5 to 12% (Lim et al., 2011). Significant superior growth has been observed by increasing dietary lipids from 55-85g per kg diet (Han et al., 2011). However, tilapia require essential fatty acids from the linoleic acid series *(n-6)* (18:2 or 20:4) and it can enhance better growth than the *n-3* series (18:3, 20:5 or 22:6) (Lim et al., 2011). Tilapia is efficient to utilize starch from 22 to 46% dietary starch, while 22% is considered the optimum level for juvenile tilapia (Wang et al., 2005).The growth of tilapia can therefore be improved by using optimum proteins, lipids, carbohydrates, and other nutrients have similar influence on their growth performance. Moreover, a number of studies have shown that feeding frequency can manipulate the production performance of tilapia. Increased growth performance, protein and lipid contents can be obtained with increasing feeding rate. Increased meal frequency provided superior carbohydrate utilization for hybrid tilapia (Tung and Shiau, 1991).

Polyculture farming of tilapia, common carp and silver carp shows increased growth, body fat and gross energy gain as feeding rate (0 to 5% and to apparent satiation) increased(Abdelghany and Ahmad, 2002). Photoperiod also influences the growth of

tilapia and longer photoperiod stimulated the growth of tilapia showed by (El-Sayed and Kawanna, 2004).

1.5 Tilapia production for alleviation of poverty

The utmost significant query for a farmer or a government policy-maker interested in promoting aquaculture is what species will be cultured. To encounter the upcoming demand for animal protein for negligible populations, tilapia has to be the prospective tropical fish of choice (Hishamunda and Ridler, 2006). Consequently, if poverty alleviation is concerned in aquaculture expansion, rather than using the traditional criteria, supplementary comparative factors such as growth in availability of protein for the rustic poor and relative growth in income of the rural poor should be used (Ahmed and Lorica, 2002). Apart from increasing income and improving household food security, tilapia aquaculture in smallholding ponds will enhance women's participation (Chowdhury et al., 2007). Chowdhury et al. (2007) also stated that tilapia farming can also generate income generation, employment and fulfill protein requirement to the poor farmers.

1. 6 Krill products, characteristics and nutritional value

Krill is the familiar term for euphausiids, a wide family of pelagic marine crustaceans found throughout the oceans e.g. Nordic Sea, Barents Sea and as well as Antarctica. Krill is a shrimp-like macrozooplankton having 1.5 to 6 cm in length and 1-1.5 g in weight. Around 85 species have been recorded in the order Euphausiacea (Storebakken, 1988). Suontama, (2006) reported that the biomass of Northern krill (the krill of Nordic and Barents Sea) has been sketched to be between 91-161 million tons. The krill biomass in Antarctica is estimated to be >440 million tonnes (Hewitt et al., 2004). Antarctic krill, of which *Euphausia superba* is the most narrated species

has been focused vastly through its connection with Antarctic expedition in last decades (Storebakken, 1988). Krill catch has increased 40 % in 2010 compared with the years before 2009, which was estimated at 150,000-180,000 tons (Schiermeier, 2010). Four million tons of krill catching has been set as the annual limit for the Atlantic sector (Area 48), determined by CCAMLR (Convention for the Conservation of Antarctic Marine Living Resources) (Hewitt et al., 2002). Four million tons of krill can supply 400 000 tons of marine protein and 80 000 tons of marine lipids (Suontama., 2006).

Several studies have been carried out in the late 1970s and early 1980s to evaluate the potentiality of using Antarctic krill as a fish feed (Storebakken, 1988). However, commercial krill use did not flourish, due to the limitation of krill processing, economics of the krill fisheries and boosting of fish meal supplies (Ichii, 2000). Krill use as an ingredient is limited by the EU due to high levels of fluoride and copper (Hansen et al., 2010). In the course of two decades of development, technology for harvesting is improved, the price of fish meal is mounting due to scarcity, and a new EU directive has raised the tolerable fluoride level in feed for fish from 150 mg/kg (Commission dir. 2002/32/EC) to 350 mg/kg (Commission dir. 2008/76/EC), though the upper tolerable copper content in feed is still 25 mg/kg (Commission dir. 2003/100/EC) (Hansen et al., 2010). More recent research has shown that krill meal replacing fish meal at 0-25 % on a dry matter basis gave higher weight gain in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) (Anderson et al., 1997). Hansen et al. (2010) demonstrated that entirely substitution of partial deshelled krill meal with fish meal gave parallel or superior growth performance in Atlantic salmon. In contrast, feeding Atlantic salmon 100% whole krill meal instead of fish meal in the diet gave reduced growth rate (Hansen et al., 2010).

Recent findings by (Yoshitomi and Nagano, 2012) demonstrated that same growth observed in Yellowtail (*Seriola* quinqueradiata) fed with 100% deshelled krill meal as fish fed with fish meal while adverse growth with accumulation of fluoride in bones was observed in fish fed a diet with 100% whole krill meal. The majority results showed enhanced or no effect on feed intake or specific growth rate in salmon or rainbow trout fed diverse levels of krill meal (Julshamn et al., 2004; Olsen et al. 2006; Suontama et al., 2006, 2007). Krill meal has proven to be an efficient feeding attractant (Kolkovski et al., 2000). Additionally, other krill products have been reported to function as attractants in feed for juvenile Nile tilapia (*Oreochromis niloticus*) (Gaber, 2005) and yellow perch (*Perca flavescens*), walleye (*Stizostedion vitreum*), lake whitefish (*Coregonus clupeaformis*) (Kolkovski et al. 2000), when added in somewhat smaller amounts.

Gaber (2005) has shown that Nile tilapia showed improved growth performance, feed utilization and higher nutrient digestibility in soybean based diets were supplemented with krill meal at levels of 1.5, 3.0, 4.5, and 6.0 % of protein, compared with a fish meal control. Krill hydrolysates have been shown to be an effective feed attractant for fish. Hydrolysates added through surface coating have resulted in improved feed intake, as compared with adding hydrolysates to the dry feed mixture prior to pelleting (Oikawa and March, 1997, Kolkovski et al., 2000). (Tibbetts et al., 2011) has reported that freeze-dried krill contains the soluble protein fraction that should stir feeding activity to facilitate growth performance and nutrient utilization of juvenile Atlantic cod and Atlantic halibut (*Hippoglossus hippoglossus*). Krill meal was also shown to be a striking element in starter diets for largemouth bass (*Micropterus salmoides*), red sea bream (*Pagrus major*), Japanese eel (*Anguilla japonica*) and gray mullet (*Mugil cephalus*) (Allahpichay and Shimizu, 1984a).

1.6.1 Chemical compositions

Raw krill restrain around 20% dry matter. Furthermore, the dry matter contains about 60-78% crude protein, 7-26% crude fat, 12-17% ash (Storebakken, 1988; Hansen, 2011). Whole krill and krill meal contain higher ash content (on a dry matter basis) than it is normal in fish. This is due to carpace fraction, rich in ash that includes chitin, which is a nitrogenous polysaccharide (Storebakken, 1988). Complex interaction between sex, age-classes, season and area of harvest can be resulted high content of chemical variation, conversely reproductive investment of female krill created key basis of high variation in lipid content (Pond et al., 1995).

1.6.2 Crude protein, amino acids and quality of protein

The nitrogen contents in krill exoskeleton i.e. chitin will result in partiality estimates of protein. Furthermore crude protein can be estimated by the contribution of non-amino nitrogen compounds such as nucleotides, volatile bases and trimethylamine. Krill often have elevated content of non-protein compounds, especially free amino acids (7-8% of dry weight) and trimethylamine oxide because of the presence of highly reactive hydrolytic enzymes, including proteases, nucleases and phospholipases found in the krill digestive tract that start to break down the tissue (Anheller et al., 1989).These hydrolytic enzymes of krill are adapted to the Antarctic low temperature environment and even at below freezing storage temperatures, the enzymatic break down is ongoing. For this reason rapid processing of krill is necessary to inhibit these enzymes. Storebakken (1988) reviewed that the digestibility of krill meal crude protein is around 87% and amino acid protein is 92% for trout.

1.6.3 Lipids

The lipid level varies vastly in krill (Pond et al., 1995). The phospholipids in krill oil is higher than that of fish oil with level ranging between 30 and 51% of total lipids (Gigliotti et al., 2011). Phosphatidylcholine is the major group of phospholipids found in krill that is 33.3 to 35.6% of total lipids (Winther et al., 2011). Winther et al. (2010) reported that 69 different choline-containing phospholipids have been found in krill where seven probably have a *n-3* fatty acid attached to both sn-1 and sn-2 position of the glycerol molecule. This is a unique feature for krill phospholipids, and make them strongly by-polar and thereby a highly potent emulsifier during digestion of lipids.

1.6.4 Vitamins

Storebakken (1988) reviewed that Vitamin A content to be about 11-15000 IU/kg and niacin 18-28 mg/kg, riboflavin 1-2 mg/kg, pantothenic acid 6-9 mg/kg, pyridoxine about 2 mg/kg, biotin less than 0.1 mg/kg that should be sufficient to fulfil the requirements of salmon but contents of riboflavin, pyridoxine and biotin are little low for rainbow trout.

1.6.5 Carotenoids

Among krill species *E. Superba* is mostly investigated species in terms of pigmentation as well that contains carotenoids in which astaxanthin is most familiar. In krill meal the amount of carotenoids is about 15-200 mg/kg while krill oil contains remarkably rich in carotenoids: 727-1080 mg/kg (Storebakken, 1988).

1.7 Krill as an attractant

Feed attractants have been characterized and isolated from several marine organisms such as squid, marine worms, mussels, clam, krill and brine shrimp (Mackie and Mitchell, 1982). One promising reason for feeding stimulants may be to mask diverse feeding deterrents to lower the palatability of diets (Gaber, 2005). Feed attractants are also important to stimulate a high feed intake in fish (Kolkovski et al., 2000). Different compounds can be found in attractants such as free amino acids, nucleotides, nucleosides and ammonium bases (betaine) (Kolkovski et al., 2000), small peptides, amines, free amino acids (glycine, arginine, glutamic acid), and as well as taurine and aniline, proline, sarcosine and glucosamine (Shimizu et al., 1990). Alberto et al. (2006) demonstrated that attractants usually have low molecular weight, are soluble in water, they are acidic, and contain nitrogen.

The high level of water soluble protein and free amino acids (Hansen, 2011) may explain the feeding attractant property of krill as demonstrated with rainbow trout (Storebakken, 1988), Japanese eel, gray mullet (Allahpichay et al., 1984a), sea bream (Shimizu et al., 1990), largemouth bass (Kubitza et al., 1997) and Nile tilapia (Gaber, 2005). Physical and chemical stimulation should enhance food attractiveness and stimulation of ingestion, including physical and chemical stimulation (Kolkovski et al., 2000). Chemical stimulation is related to olfactory and gustatory responses of feed, while physical stimulation is related to color and movement of feed (Shimizu et al., 1990).

1.8 Aim of this research

Nile tilapia has both commercial and societal importance, since it involved in not only fulfils the protein requirements but also eliminates poverty and generate income. The first aim of this study were to examine nutritional response to diets in tilapia where using inclusion of mixed plant protein based diets. The second aim was to assess the

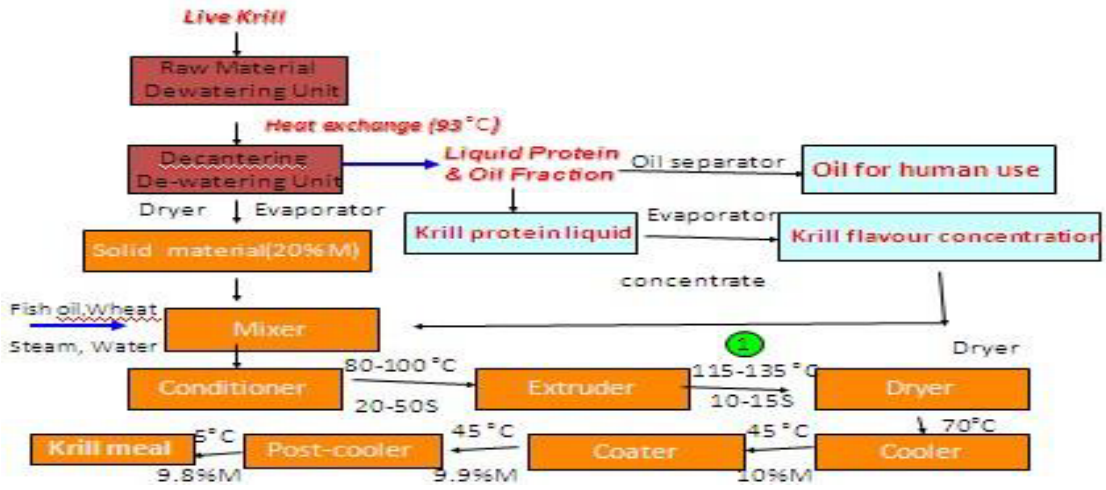
effects of using 5% of krill products krill meal (KM) or a combination of KM and krill water solubles. The parameters that observed during experiment include (i) feeding stimulation and growth performance, (ii) feed utilization competence and nutrient digestibility (iii) whole body composition and subsequent nutrient retention efficiencies.

2. Materials and methods

2.1 Feed ingredients, feed formulation and processing

2.1.1 Krill meal and krill flavor concentrate

Fresh Antarctic krill, *Euphausia superba* was used to produce experimental krill meal (KM) and krill flavor concentrate (KFC) supplied by Krillsea Group AS. The production line is schematically presented in fig. 1 and the krill product used in this experiment are characterized in Table 1.



The temperature in the last section of extruder

Fig 1. Procedure of producing krill meal and krill flavor concentrate (Krillsea Group AS)

2.1.2 Experimental diets formulation

Table 1. Formulation of the experimental diets

Diets	Diet 1	Diet 2	Diet 3
	Plant100%	Plant95%＋ KM2.5% ＋KFC2.5%	Plant95%＋ KM5%
Ingredients DM gkg-1	911	905	911
Krill meal[1],g	-	22.3	44.5
Krill flavour concentrate[2],g	-	15.9	-
Soybean meal[3],g	126	115	115
Sunflower meal[4],g	104	94.5	94.5
Rapeseed meal ,g	58.7	53.4	53.4
Pea protein Concentrate[5],g	40.6	36.9	36.9
Corn gluten meal[6],g	135	123	123
Rapeseed oil[7],g	70	70	70
Wheat[8],g	295	295	295
MCP[9],g	20	20	20
Premix[10],g	9	9	9
Methionine[11],g	11.4	10.4	10.4

Lysine[12],g	10.1	9.29	9.29
Threonine ,g	6.93	6.3	6.3
Taurine[13],g	3.47	3.15	3.15
Phenylalanine ,g	0	0	0
Tryptophan ,g	1.04	1.04	1.04
Y_2O_3[14] ,mg	0.08	0.08	0.08
Vit-C 35%[15] ,g	0.1	0.1	0.1
Sodium alginate ,g	20	20	20

1 Aker Biomarine, Oslo, Norway.

2 Aker Biomarine, Oslo, Norway

3 ADM, Netherlands.

4 Extracted Sunflower, Ukraine.

5 Agri-marine, Stavanger, Norway.

6 Felleskjøpet, Kambo, Norway.

7 Sop international Ltd Orland house.UK.

8 Felleskjøpet, Kambo, Norway.

9 Felleskjøpet, Kambo, Norway.

10 Contents per kg: Vitamin A 2500.0 IU; Vitamin D 3 2400.0 IU; Vitamin E 0.2 IU; Vitamin K3 40.0 mg; Thiamine 15.0 mg; Riboflavin 25.0 mg; d-Ca-Pantothenate 40.0 mg; Niacin 150.0 mg; Biotin 3.0 mg; Cyanocobalamine 20.0g; Folic acid 5.0 mg; Pyridoxine 15.0 mg; Vitamin C: 0.098 g (Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland); Cu: 12.0 mg; Zn: 90.0 mg; Mn: 35.0 mg; I: 2.0 mg; Se: 0.2 mg; Cd = 3.0 g; Pb = 28.0 g; total Ca: 0.915 g; total K 1.38 g; total Na 0.001 g; total Cl 1.252 g; Trouw Nutrition, LA Putten, The Netherlands.

11 Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil

12 CJ Indonesia, Jakarta, Indonesia

13 Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China.

14 Metal Rare Earth Limited, Jiaxing, China.

15 Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland

Table 1 indicates that this experiment employed three diets. Diet one contained all plant ingredients considered as a control diet in this experiment. Diets two and three contained both krill meal (KM) and krill flavour concentrate (KFC) and only krill meal (KM) respectively. Three types of mixer were produced for every diet having two common mixers for all diets that were mentioned above. However, two experimental diets had 95% of their crude protein from the plant protein mix, and approximately 5 % from the KM+KFC and KM respectively (Table 1). The plant protein ingredients: soybean meal, sunflower meal, corn gluten, rape seed meal and pea protein were mixed 2:2:2:1:1 ratio (ingredients one) to supply 382 g kg^{-1} crude protein in feed and same amount of starch content (274 g kg^{-1}) (Table 3). All plant protein diets were supplemented limiting amino acid, taurine and MCP. The efficiency of limiting amino acid formed in crystal was proven in a previous study with rainbow trout (Zhang et al., 2011a). To estimate digestibility, each diet contained 0.01% Y_2O_3 as inert marker.

2.1.3 Description of other selected ingredients

The plant protein sources used in this experiment are characterized in Table 2

Table 2 Chemical compositions of plant protein ingredients (% of crude protein)

Ingredients	Wheat[1]	Corn gluten[2]	Soybean meal[3]	Sunflower meal[4]	Rapeseed meal	Pea protein concentrate[5]
Composition, g kg-1						
Dry matter (DM),g	900	900	900	900	905	900
In DM ,g						
Crude protein ,g	172	650	480	385	385	480
Crude fat ,g	30	40	20	41	44	38
Starch ,g	680	154	0	0	28	79
Ash ,g	30	50	50	50	72	52
Amino acids, g $100CP^{-1}$						
Arginine	8.08	20.2	35.5	30.4	20.1	41.8
Histidine	4.13	13.7	13.0	9.24	8.93	13.0
Isoleucine	6.19	26.7	22.6	15.8	13.6	20.6
Leucine	11.7	106	36	24.3	22.5	34.6

Valine	7.9	30.6	23.0	19.3	16.8	22.6
Lysine	4.82	11.1	29.3	13.5	17.9	34.1
Methionine	2.75	15.0	6.24	8.47	6.43	4.32
Phenylalanine	8.26	40.3	24.0	17.3	13.2	22.6
Threonine	4.99	22.1	18.7	14.3	14.9	17.8
Tryptophan	2.06	3.90	6.72	4.62	3.85	4.80

1 Felleskjøpet, Kambo, Norway.

2 Felleskjøpet, Kambo, Norway.

3 ADM, Netherlands.

4 Extracted Sunflower, Ukraine.

5 Agri-Marine AS, Stavanger, Norway.

2.1.4 Processing of formulated diets

The all diets were produced at the feed laboratory at the Department of Animal and Aquaculture Sciences (IHA) of UMB. The two premixes containing plant ingredients were milled and mixed separately at the Centre for Feed Technology (Fortek). Before milling, macro ingredients of the formulated diet were weighed using a large weighing scale. To produce tilapia feed, all macro ingredients were milled in a Münch Hammer mill (HM 21.115, Wuppertal, Germany) and grinded to particle size of 0.5 mm using 1 mm screen.

The premixes with krill products were prepared manually by mixing vitamin and micro mineral, premix, inert marker, krill meal and calcium phosphate. Krill hydrolysate was added manually during a second mixing cycle together with 30% water, krill

hydrolysate and rapeseed oil added into the mixer. The ingredient mix was conditioned and shaped into pellets in a pasta extruder, equipped with a heater (BOE-THERM, Norway, maximum temperature 140 ^{0}C). Because of limited capacity (8 kg) of the pasta machine, the ingredient mix was processed in 3 batches. Around 6 kg of feed have been prepared for each diet. The die size used for making pellet was 0.5mm. Pasta machine (model P35A, Italy) power consumption was 1.7 KW/h, dimensions 55×102×132 cm and motor Hp 2. Following mixing extrusion occur into the machine. Each diet was extruded first two periods with 85^{o}C temperature following 100^{o}C temperature applied in last stage. The moist diets were placed onto trays and put into a hot air drying cabinet (Termaks, Norway) at 50 ^{o}C for 2-3 hours. Just after completion of drying diets were placed into the room temperature for overnight period.

At the end of the feeding period, feed ran short and new feed was produced using same parameters as mentioned above.

Table3. Theoretical chemical compositions of diets

Diets	Diet 1	Diet 2	Diet 3
	Plant100%	Plant95%＋KM2.5%＋KFC2.5%	Plant95%＋KM5%
Ingredient g kg^{-1}DM	911	905	911
In DM (g/kg)			
Protein (g)	379	383	386
Fat (g)	107	114	114
Starch (g)	276	275	273
Ash (g)	62.8	65.2	65.5
Amino acids ,g 100 CP^{-1}			
Arginine	19.1	17.9	17.8
Histidine	8.17	7.70	7.66
isoleucine	14.1	13.3	13.2
Leucine	33.6	31.4	31.3
Valine	16.2	15.3	15.2
Lysine	23.8	22.4	22.3
Methionine	18.7	17.3	17.2

Phenylalanine	17.6	16.7	16.6
Threonine	19.7	18.4	18.3
Tryptophan	4.66	4.44	4.41

Table 4. Percentage of essential AA of protein in Feed

	Requirement In Nile Tilapia	Diet 1	Diet 2	Diet 3
		Plant100%	Plant95%＋KM2.5%＋KFC2.5%	Plant95%＋KM5%
Pers of AA (%) of protein in Feed				
Arginine	4.20	5.03	4.70	4.63
Histidine	1.70	2.16	2	1.99
Isoleucine	3.10	3.72	3.50	3.43
Leucine	3.60	8.86	8.20	8.11
Valine	2.80	4.27	4	3.94
Lysine	5.10	6.28	5.80	5.78
Methionine	3.20	4.92	4.50	4.47
Phenylalanine	5.50	4.63	4.30	4.30
Threonine	3.80	5.19	4.80	4.74

Tryptophan	1	1.23	1.20	1.14

(Chowdhury, 2011)

Table 3 and table 4 give clear data lists of chemical compositions and AA in different diets which reach to the feed requirement of Nile tilapia.

It should be noted that at the ending period feed shortage arose and again need to make feed using same parameters as mentioned above.

2.1.5 Sampling

Samples of feed were collected in plastic bags after the feed was cooled and removing of fine particles. About 1 kg of each diet was collected for chemical analysis and kept in 4 ^{o}C.

2.2 Fish trial and fish performance parameters

2.2.1 Fish keeping facilities

The experiment was conceded at the fish laboratory of UMB, between 21st of February and 30th of April, 2012. The design of the recirculation system for tilapia (water treatment section) includes drum filter (removal of big particles), bio-filter (to remove ammonia), air bowler, circulating pump and electric heaters. In the recirculation system for tilapia, more than 99% water is reused. Each tank has an individual aerator to keep the oxygen at an acceptable level even if some hour central air bowler fails.

Each of 9 tanks (size: 70×50×50 cm) containing 20 Nile tilapias *Oreochromis niloticus*, from the 18th generation of genetic selection in the GIFT program were used for the trial. On the average, the initial individual fish weight was estimated at 25g and distributed randomly to all respective tanks. Before the beginning of the experiment, commercial feed was fed to the fish (ALLER AQUA, Denmark). The fish were

depleted for 24h before commencing of the trial. In order to avoid absconding of fish tanks were always sheltered with lids. Water quality parameters were monitored daily between 9.00 and 21.00h. During the experiment period, water temperature was monitored at average 27^0C and recorded daily. Fish were provided with a continuous flow of water (150 Lm^{-1}average) with continuous aeration to maintain the dissolved oxygen level above the saturation. Dissolved oxygen (DO) was measured using an YSI Model 58 oxygen meter and pH using electronic pH meter. Ammonia and nitrite were measured at a weekly interval by using DREL 2000 Spectrophotometer. To keep tank environment clean everyday floating faeces were removed daily at 22.00h.

2.2.2 Feeding and feed intake assessment

Fish in each tank were fed with one of the three experimental diets for 28 days. Each diet has given to triplicate groups. On each tank semi-automatic electronically driven band feeder and uneaten feed collector was adjusted. Feed was offered by electronically driven feeder to each tank four times a day, at 9am, 1pm, 5pm and 9pm. Each feeding lasted for a 30-min period. In order to ensure maximum voluntary feed intake feeding was 20% in excess of appetite, depend on the last 3 days records of feed intake. The boxes containing uneaten feed were weighed per day for each tank.

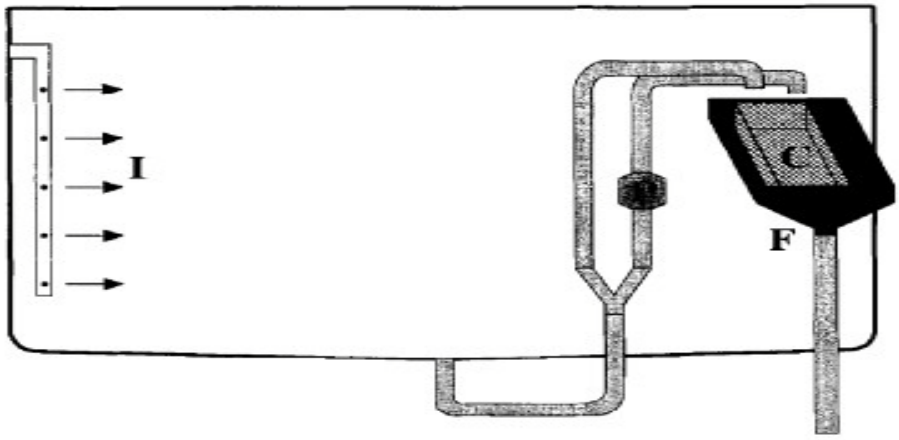


Fig 2. Waste feed collection system and tank showing water inlet pipe (I), pinch valve (P) connected to one of the two effluent water pipes, waste feed collector (C)

Every day after last feeding floating faces was taken carefully by small net from the each tank prior to keep the tank clean and to minimizing the effects of deteriorating faeces on the water quality. In addition outlet pipe was flashed to check whether any uneaten feed left or not. In addition outlet pipe was flushed daily after the last meal to check whether any uneaten feed was trapped in the system.

According to a record from an experiment booklet, problems were found with feed intake in some tanks. For instance, especially from tank 8, tank 6, tank 5, and tank 3, recycling systems did not operate smoothly. Therefore, the following solutions were implemented: brushing the pipe every time before feeding, removing faeces every day after the last meal (but not every meal, so as not to disturb fish feed intake), increasing water flow in a reasonable range, and also reducing feed increase rate (which was increased too much before). In fact, water quality was modified by implementing these solutions. However, for some severe tanks, such as tank 8 and tank 3, whole pipes connected to the outlet were changed. These tasks were done at the end of period 1.

2.2.3 Weighing and sampling

At the start and at days 14 and 29 of feeding, all fish were weighed. An anaesthetic agent (MS-222, 100mgl^{-1} in water) was used prior to weighing, sampling and for faeces collection. Fish were gently picked up by small landing net and transferred to a small bucket containing MS-222 solution. The fish weights were recorded as soon as the fish became immobilized, and the tilapias were quickly returned to the tanks. No mortalities occurred during weighing.

At the start of fish trial, three fish were taken randomly from each tank for whole body composition analyses. In addition, five fish of each tank were randomly taken out for whole body chemical analyses at the end of the experiment. The entire fish samples were stored at -20 ^{0} C until being processed for analysis.

Prior to faeces collection after fish sampling the rest of all fish was killed by an overdose of an anaesthetized agent MS-222 (300 mgl^{-1}, in water). Tank water was

reduced very slowly to diminish stress and diluted anaesthetic agent added carefully to the tank. Faeces were collected two hours after completion of the first meal of the day. The fish were not subject to unnecessary pain, as the anaesthetized fish were killed by cutting the spinal nerve in the neck before the start of dissection. The belly was opened, the gastro-intestinal tract stretched out, and the contents of the last 10 cm of the distal intestine were squeezed carefully out. Distal intestinal contents were pooled into a plastic jar for each tank of fish and kept frozen until processed for analysis.

2.2.4 Sample preparation, chemical and physical analysis

Feed was ground in a laboratory blender along with dry ice and IKA-Analytical mill (A11 basic, 230V, 50/60Hz, knife: Cutter A11.2, IKA Werke GmbH & Co. KG, Germany). Owing to high fat content, CO_2 ice was provided. The whole body samples were cut into small pieces, ground homogenously along with CO_2 ice in a food processor (Kenwood Major, UK, 220-240 V, 50/60 Hz and 1200W) and subsequently freeze-dried. Tilapia scale was not successfully ground so that tried to grind whole

body with the aid of laboratory blender (IKA A11 basic) after burning. But this approach was failed to grind the scales properly. Representative 10 g samples from each tanks has taken and put onto dryer at 550 ^{0}C about 16 hrs. In that way endeavoured to overcome the scale problem to get phosphorous content.

Diets samples, uneaten feed and freeze-dried initial and final whole body were analyzed for dry matter by drying to constant weight at 104 °C (Commission dir. 71/393/EEC). Diets, freeze-dried initial and final whole body protein content analyzed by Kjeldahl nitrogen (N) (Commission dir. 93/28/EEC) × 6.25, lipid of diets was analyzed by HCl hydrolysis followed by diethyl ether extraction (Commission dir. 98/64/EC), freeze-dried initial and final whole body for lipid without HCl hydrolysis employed. Energy in diets and freeze-dried whole body samples were measured by

bomb calorimetry (Parr 1271 Bomb calorimeter, Parr, Moline, IL, USA). Ash (Commission dir. 71/250/EEC) and starch (AOAC 996.11) were also analyzed.

From freeze-dried faeces dry matter, nitrogen and gross energy were analyzed by using the similar methods. Freeze-dried faeces were ground with a mortar and pestle. Dumas method was used to measure fecal nitrogen content. In feed and faeces concentration of Yttrium oxide and mineral (phosphorus) (ICP-AES/ICP-MS) were determined by inductively coupled plasma mass spectroscopy (ICP-MS) following the entire digestion of the homogenized and dried samples in HNO3 after cooking in a microwave oven for 1 h (Zhang et al. 2011a).

2.3 Data analysis

R Commander(R*64 2.15.1) program was used for data analyses. As it only measured effects from one class (ingredients), one-way analysis of variance (ANOVA) was suggested. Significant level P-value<0.05 was chosen and significant difference among various means were indicated by subscripts a, ab and b, doing post hoc pair-wise tests. Additionally, standard error of mean (S.E.M) was expressed for variance.

Daily feed intake was expressed by

$DFI_{DM}/g=(F*DM_F/100)-(UF*DM_{UF}/R)$

$R/g=100*((W*W_{DM})*(F*F_{DM})^{-1})$

Where:

DFI=Daily feed intake (g);

F=Feed amount per day (g), DM_F=Dry matter of feed (%), UF=Uneaten feed amount per day (g), DM_{UF}=Dry matter of uneaten feed (%);

R=Recovery rate (%);

W=Weight of waste feed collected (g), W_{DM}=DM content of waste feed (%);

F=Weight of feed (g), F_{DM}=DM content of feed (%);

Specific Growth Rate (SGR) was calculated by formula:

SGR=100× (ln(final WT)–ln(start WT))/Δt

Feed Conversion Ratio was calculated by formula:

FCR= total amount of feed/ ((final WT)- (start WT))

Where: Final WT=Final weight of fish

Start WT=Start weight of fish

Δt=Experimental days

Digestibility

Marker technique based on the relations of nutrient concentrations and marker concentrations was used for apparent digestibility coefficients (ADC) calculation.

Formula:

ADC=((a1/a2-b1/b2)/(a1/a2))*100%

Where a1,a2=Concentrations of nutrients, marker in Diets respectively.

While b1,b2=Concentrations of nutrients, marker in faeces respectively.

Utilization of the nutrients

Nutrient and energy retentions (R_N and R_E)

R_N=100%*(N_1*FBW-N_0*IBW)/(N_D*FI)

R_E=100%*(N_1*FBW-N_0*IBW)/(N_D*FI)

Where:

N_0 is represent for nutrient or energy concentration in initial fish sample

N_1 is represent for nutrient or energy concentration in final fish sample (pooled 5 fish from each tank in some diet)

Where:

FBW is expressed for fish final dry body weight

IBW is expressed for fish initial dry body weight

N_D is expressed for concentrations of nutrient or energy in diets (a_1)

FI is total feed intake during whole period

3. Results

3.1 Feed quality

Here presents the dry matter content of experimental diets, compositions content of protein, starch, fat and ash in dry matter separately, in addition to total phosphorus content and gross energy.

Table 5. Chemical analysis of experimental diets

Diets	Diet 1	Diet 2	Diet 3		
	Plant100 %	Plant95% ＋KM2.5% ＋KFC2.5%	Plant95% ＋KM5%	S.E.M pooled	P-Value
Dry matter ,g	931[a]	942[b]	937[ab]	1.53	0.0081 **
In dry matter					
Protein ,g	314	326	319		
Starch ,g	287	288	289		
Fat ,g	114	117	113		
Total P ,g	9.67[b]	7.87[a]	9.93[c]	0.33	1.45e-07 ***
Ash ,g	62.5	62.7	65.3	0.62	0.12
Energy ,MJ	20.0	20.4	20.2	0.078	0.11

Significant differences were found both in dry matter content and phosphorus content in different diets. The biggest difference in dry matter content was between Diet 1 (931g) and Diet 2 (942g), and pooled standard error of mean is 1.53g. Nevertheless, content of total P was significant lowest in Diet 2 (7.87g) which was made from plant

95%+KM2.5%+KFC2.5%. While highest P content was from Diet 3 (9.93g) made from Plant95%+KM5%. Diet 2 showed the highest protein content and starch content in dry matter, followed by Diet 3 and Diet 1 separately. When it comes to fat content, Diet 2 still provided the most, followed by Diet 1 and Diet 3. Nevertheless, Diet 3 gave the highest ash content, while Diet 2 contained the most energy.

3.2 Feed intake and growth, chemical composition of experimental fish

3.2.1 Feed intake

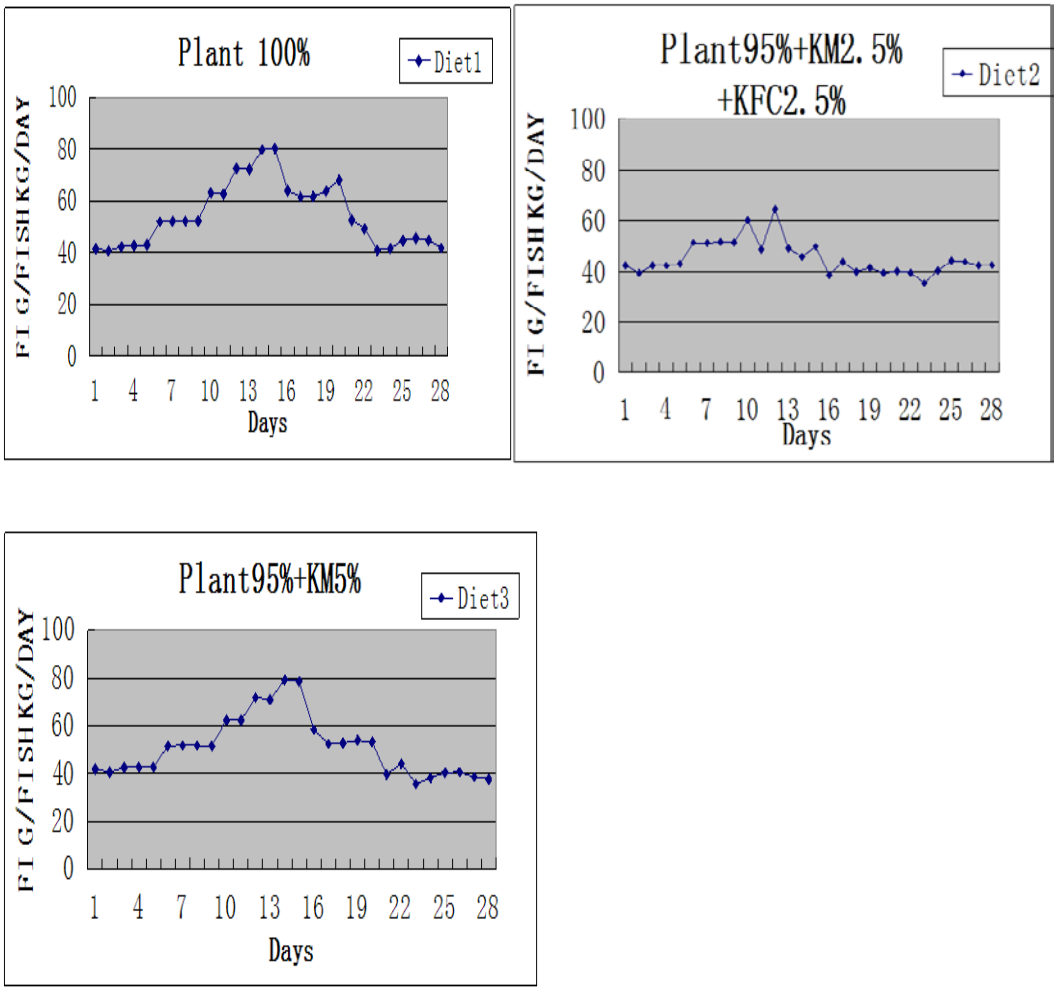


Fig 3. Feed intake (dry basis) g of per kg fish per day fed Plant100%, Plant95%＋KM2.5%＋KFC2.5%, Plant95%+KM5% diets separately in 28 days.

Daily feed intake per kg fish increased during first period (1-14D) and decreased during the second period (15-28D). From these 3 graphs, it can be readily seen that Diet 1 (Plant 100%) and Diet 3 (Plant 95%, KM 5%) give similar results, while Diet 2 (Plant 95%, KM 2.5%, KFC 2.5%) shows a little bit lower data distribution of feed intake(g/fish kg/day) during the whole period(28 days).

According to the charts from Diet 1 and Diet 3, feed intake (g/fish kg/day) started roughly from 40 grams, while there was a slight decrease of FI during 2 or 3 days. The decrease was slow, they were close to being stable. After 5 or 6 days, FI was over 50 grams with a stable increase rate. After 3 circulations, the amount of FI (g/fish kg/day) ballooned to the peak, both being up to 80 grams. While this was in the end of first period, FI showed a sharp decrease in the beginning of the second period, sinking to approximately 62 grams over 2 or 3 days. The main reason for this would likely to be from the disturbance of weighing fish (we weighed fish after the first period). Diet 1 showed a smooth rise of daily FI between the 16^{th} and 19^{th} days, dropping to the level close to beginning weight (40 grams), and after a small increase and a small decrease, reaching nearly 40 grams again at the ending point. On the other hand, Diet 3 experienced a flat trend after a sharp sinking, up to 20^{th} day; there was then a rapid decrease again, and after a small increase and a small decrease, the daily FI ending with a bottom lower than 40 grams.

On the other hand, Diet 2 had a slightly higher starting point compared with Diet 1 and Diet 3, and experienced a smooth decrease and a smooth increase, continuing for several days. Then, after two sharp increases, the daily FI reached its first peak of nearly 60 grams. After a rapid decline and a rapid rise, it reached an even higher peak of over 60 grams. Diet 2 also showed a drop after the first period, but not as much as did Diet 1 and Diet 3. Decreasing stably, the daily FI of Diet 2 ended at a point over 40 grams, which was higher than the points reached by Diet 1 and Diet 3.

Table 6. Feed intake and FCR of experimental Nile Tilapia during 28days

Diets	Diet 1 Plant100%	Diet 2 Plant95%＋KM2.5%＋KFC2.5%	Diet 3 Plant95%＋KM5%	S.E.M[1] pooled	P-Value
FI(1-14days) of SW[2]	1.1[b]	0.96[a]	1.11[b]	0.025	0.00065***
FI(15-28days) of MW[3]	0.98[b]	0.76[a]	0.87[ab]	0.038	0.014 *
FI(1-28days) of SW	3.32[b]	2.53[a]	3.06[b]	0.13	0.0024 **
FCR					
1-14days	0.88	0.91	0.91	0.01	0.48
15-28days	1.15[b]	0.98[a]	1.05[ab]	0.03	0.02 *
1-28days	1.04	0.95	0.99	0.019	0.12

1. S.E.M: Mean of standard error. The different superscript letter[a,b] denotes significant differences within a row ($P<0.05$)

2. SW: Start Weight

3. MW: Mean Weight

Results from FI of the starting weight during the first period, FI of the middle weight during the second period, and FI of the starting weight during the course of the whole period, separately illustrated that there were significant differences among the various periods. It is believed that the different results depended on different diets at the level of 95%.

It is shown that the strongest significant difference was from the FI of starting weight during the course of the first 14 days (period 1), while the slightest significant

difference was from the FI of middle weight during the course of the later 14 days (period 2). Nevertheless, among all diets, ingredients in Diet 2, consisting of plant 95%, KM 2.5% and KFC 2.5%, accounted in the main for its different results as compared with those of Diet 1 and Diet 3. For instance, taking the first period as an example, it can readily be seen from the table that feed intake of Diet 2 based on biomass was around 0.98g, while FI were 1.1g and 1.11g from Diet 1 and Diet 3, respectively.

No significant differences of FCR were observed in either the first period or the whole period, but there was a slight significant difference during the course of period 2. Feed conversion rates (FCRs) of the first period were 0.88, 0.91, and 0.91 of Diet 1, Diet 2 and Diet 3, respectively, with the mean in close proximity to 0.90. The average of FCR from the whole period was at the level of 0.99 roughly. From the second period, however, the table demonstrates that the main difference in FCR was between fish fed by Diet 1 and Diet 2, separately. Furthermore, fish fed by Diet 2 were the most efficient users of feed.

3.2.2 Fish growth

No fish died during the course of the experiment. The result of fish weight shows that there was no significant difference between the starting weight of fish fed by the three different feeds. The fish in the experiment had an average weight of approximately 24.8 g. However, some changes occurred after feeding, both after 14 days and after 28 days. It can be seen from the table that there was no significant difference in connection with Diet 1 (100%Plant) and Diet 3 (95%Plant+5%KM), but results from Diet 2 which consisted of 95%Plant+2.5%KM+2.5%KFC, showed a much lower fish weight compared with Diet 1 and Diet 3.

Table 7. Weight Increase and SGR of experimental Nile Tilapia

Diets	Diet 1	Diet 2	Diet 3	S.E.M[1]	
	Plant100%	Plant95%＋	Plant95%＋	pooled	P-Value

		KM2.5%＋ KFC2.5%	KM5%		
Fish Weight g/Fish					
Start, g	24.8	24.8	24.8	0.017	0.42
14 Days, g	55.8[b]	51.0[a]	55.2[b]	0.80	0.0017 **
28 Days, g	103[b]	90.6[a]	101[b]	2.12	0.0017 **
Body Weight Increase g/fish					
1-14 Days,g	31.0[b]	26.3[a]	30.4[b]	0.8	0.0015 **
15-28 Days,g	47.6[b]	39.6[a]	46.0[b]	1.35	0.0054 **
1-28 Days,g	78.6[b]	65.8[a]	76.4[b]	2.1	0.0016 **
SGR(Specific Growth Rate)					
1-14 Days	5.8[b]	5.16[a]	5.72[b]	0.11	0.0011 **
15-28 Days	4.41	4.1	4.32	0.06	0.061
1-28 Days	5.11[b]	4.63[a]	5.02[b]	0.08	0.0011**

1 S.E.M: Mean of standard error. Different superscript letter[a,b] denotes significant differences within a row ($P<0.05$)

When it comes to BWI (Body Weight Increase), the fish body weight increasing most rapidly was from fish fed by 100% Plant, that is, from Diet 1. However, while there was

no significant difference compared with fish fed by Diet 3, there was a significant difference compared with fish fed by Diet 2. The table shows the results from the first period, second period and whole period separately, and they were all significant; while at the same time, weight diversity from the first period was more obvious than that from the second period.

SGR (Specific Growth Rate) was observed from the table as well; significant differences occurred both in the first period and the whole period. However, the results did not show any significant differences in SGR. In short, period 1 was the most significant time when SGR was affected by diets. From the whole period, fish fed by Diet 2 carried the lowest SGR (4.63%), while fish fed by Diet 3 had higher SGR (5.02%), and fish fed by Diet 1 showed the highest SGR (5.11%). Therefore, the results of Diet 2 were significantly different from those of Diet 3 and Diet 1.

In general, compared with initial whole body fish fatty acids content(% of total fatty acid), the final whole body fish fatty acid content (% of total fatty acid) was lower in fatty acids, with carbon atom numbers less than 18, but it increased in fatty acids with carbon atom numbers equal or more than 18. Among the results of increased poly-unsaturated fatty acid (PUFA), C18:1(n-9)c showed an extreme increase in percent of total fatty acids in final fish compared with initial fish. Significant differences among fish fed by 3 different diets were only found from C18:3(n-6), C18:3(n-3) and C20:4 (n-6) separately. Both of C18:3(n-6) and C20:4 (n-6), percentages are highest in fish fed by Diet 1 while lowest in fish fed by Diet 2. However, when it comes to C18:3(n-3), its percentage is highest in fish fed by Diet 2, while lowest in fish fed by Diet 3.

3.2.3 Fatty acids composition

Table 8. Fatty acids composition (% of total fatty acids) of the initial and final whole body fish

Diets		Diet 1	Diet 2	Diet 3		
Fatty acids	Initial fish	Plant100 %	Plant95 %＋ KM2.5% ＋ KFC2.5 %	Plant95 %＋ KM5%	S.E.M [1]	P-value
C14:0	5.13	1.66	1.82	1.84	0.05	0.21
C16:0	23.4	17.2	16.9	17.3	0.11	0.31
C16:1(n-7)	5.76	3.15	3.13	3.17	0.025	0.87
C17:1	0.35	0.13	0.11	0.15	0.009	0.32
C18:0	4.44	3.89	3.72	3.9	0.042	0.14
C18:1(n-9)t	0.31	0.10	0.12	0.11	0.008	0.59
C18:1(n-9)c	29.0	44.3	44.0	43.8	0.15	0.49
C18:1(n-7)c	4.44	3.86	4.14	4.12	0.074	0.25
C18:2(n-6)c	6.88	12.3	12.1	12.1	0.074	0.62
C18:3(n-6)	0.24	0.75^{b}	0.62^{a}	0.66^{ab}	0.023	0.02 *
C18:3(n-3)	5.16	4.68^{ab}	4.83^{b}	4.63^{a}	0.035	0.03*
C20:1	0.56	0.43	0.43	0.4	0.007	0.27
C20:2	0.31	0.36	0.37	0.34	0.006	0.22
C20:3(n-6)	0.21	0.48	0.5	0.47	0.007	0.13
C22:1(n-11)	2.94	0.34	0.37	0.34	0.015	0.65
C22:1(n-9)/C20:3(n-3)?	0.46	0.35	0.37	0.36	0.005	0.30
C20:4 (n-6)	0.34	0.64^{b}	0.56^{a}	0.58^{ab}	0.014	0.04*
C20:5(n-3)	0.5	0.26	0.28	0.27	0.009	0.56
C22:5(n-3)	1	0.53	0.59	0.57	0.024	0.62
C22:6(n-3)	1.86	1.33	1.6	1.59	0.069	0.19

1. S.E.M: Mean of standard error. Different superscript letter[a,b] denotes significant differences within a row (P<0.05)
?. Question mark. Machine could not identify which fatty acid clearly.

3.2.4 Chemical compositions of fish body and fish nutrient digestibility

Table 9. Chemical compositions of fish body and fish nutrient digestibility

Fish body compositions					
Diets	Diet 1 Plant100%	Diet 2 Plant95%＋KM2.5%＋KFC2.5%	Diet 3 Plant95%＋KM5%	S.E.M[1] pooled	P-Value
Dry matter (Freezer dried) ,g/kg	321	321	327	1.6	0.16
Protein ,g	140	143	145	1.09	0.22
Lipids ,g	133	135	139	1.22	0.24
Ash .g	30.6[b]	27.4[a]	31.9[b]	0.74	0.0055**
P ,g	13.7	11.8	13.7	0.14	0.081
Apparent Digestibility(Y_2O_3),%					
Nitrogen	82.4	85.9	82.4	1.05	0.32
Phosphorus	30.2	33.0	37.3	1.86	0.34

1. S.E.M: Mean of standard error. Different superscript letter[a,b] denotes significant differences within a row ($P<0.05$)

Results from fish body compositions did not show significant differences of dry matter, protein, lipids, and phosphorus contents, while they did show significant differences from ash content in dry matter. The lowest ash content was from Diet 2 (27.4g), while the highest ash content was from Diet 3 (31.9g). Moreover, nitrogen apparent digestibility was around 82.4%, 85.9% (highest) and in 82.4% of fish fed by Diet 1,

Diet 2, and Diet 3, respectively; and from the result of phosphorus digestibility, fish fed by Diet 3 showed the highest digestibility (37.3%).

3.2.5 Utilization of the nutrients

Table 10. Nitrogen, energy and phosphorus retention of juvenile Nile tilapia fed with plant100%, plant95%+KM2.5%+KFC2.5%, and Plant95%+KM5% respectively.

Diets	Diet 1 Plant100%	Diet 2 Plant95%＋KM2.5%＋KFC2.5%	Diet 3 Plant95%＋KM5%	S.E.M[1] pooled	P-Value
Nitrogen Retention(%)	41.7	43.7	42.9	0.78	0.64
Energy Retention(%)	44.0	47.0	45.2	1.04	0.57
Phosphorus Retention(%)	45.4	49.3	45.7	2.0	0.72

1 S.E.M: Mean of standard error. Different superscript letter[a,b] denotes significant differences within a row ($P<0.05$)

No significant difference was found from the retention of nutrients. However, fish fed by Diet 2 (Plant95%+KM2.5%+KFC2.5%) showed the highest results of all in nitrogen, energy, and phosphorus retention, followed by fish fed by Diet 3 and Diet 1, separately.

4. Discussion

4.1 Water quality

Rapid and efficient solid waste removal is a key factor to maintain water quality in recirculation aquaculture systems (RAS). This was achieved by cleaning pipes and removing feces after feeding every day, and the water remained clear throughout the experiment. The temperature of 26 ± 1 ^{0}C during the experiment was almost same as like experiment carried out by the Suresh and Lin, (1992) studying the effects of stocking density on water quality and production of red tilapia in recirculated water system. It has been reported that a temperature is 26-28^{o}C optimum for 10-1000 g Nile tilapia growth (Bergheim, 2007). PH (7.2) was also within acceptable limits (6.5 to 7) for recirculation systems (Bergheim, 2007) during whole experiment period.

4.2 Feed intake and growth

This experiment demonstrated a capacity for tremendous voluntary feed intake following rapid growth by using 100% plant protein without resulting observable macroscopic irregularities. The growth from 25 g to a final body weight of103g in 28 days of the fish fed by control diet and final weight fish fed by Diet 3 have been recorded which were significantly higher than fish fed by Diet 2 containing KFC. Higher feed intake resulted in higher weight gain, in keeping with Gaber(2005). The high feed intake may be explained by palatable diets, balanced IAA profiles, and high energy and nutrient utilization of dietary protein, achieved by balancing the protein according to the ideal amino acid concept (Hansen et al., 2007). The Nile tilapia has previously showed improved feed intake and growth when fed by soyabean meal or full fat soyabean diets supplemented with $_{DL}$-methionine and $_{L}$-lysine (Goda et al., 2007). Soyabean meal plus methionine could be used without impairing growth of

Nile tilapia (El-Saidy and Gaber, 2002).

Recent processing technologies have prevailed over many of the obstacles and ANFs in most plant protein sources (Oliva-Teles et al., 1994) and this was one reason behind the rapid growth during experiment. Supplementation of methionine and lysine enhance the growth of tilapia that observed in present experiment which was sometimes reversal (Sintayehu et al., 1996). Higher growth rate of hybrid tilapia by using total plant protein also experienced by (Viola et al., 1988). Different results were stated by (Fontaínhas-Fernandes et al., 1999) who observed lower weight gain and growth of tilapia fed by 100% plant protein. And similar result observed in Nile tilapia by El-Saidy and Gaber, (2003) by using 100% plant protein mixture though 25% plant protein mixture showed better weight gain and growth.

Inclusion of krill hydrolysate in the diet presented significant lower feed intake than the plant protein diet and one supplemented KM in this experiment. Therefore KFC was the reason that brought significant differences of feed intake observed among the three diets in whole period of experiment. Gaber, (2005) has used 1.5% to 6% KM with plant protein in juvenile Nile tilapia diet and demonstrated higher feed intake and growth performance compare to whole plant protein. That is not in line with this experiment that appears almost same feed intake between fish fed by Diets 1 and 3. By using of 5% of krill product (3.5% KM+1.5% KFC) in fish meal free Diets for rainbow trout Zhang et al. (2011c) illustrated enhanced feed intake and growth performance compared to FM control. Kolkovski et al. (2000) used krill hydrolysate as a feed attractant for larvae and juvenile of three species of freshwater fish and reported that amplified ingestion rate compared to commercial trout start control diet. It is interesting to point out that the experimental Diet 2 yielded lower feed intake and lower growth while Diet 3 gave close result as plant diet brought. Similar result observed by the Kolkovski et al. (2000) who has formulated experimental diets for walleye included 2% spray-dried krill hydrolysate.

Higher content of attractant may not necessarily elevate the feeding response that indicated the probable reason by Kolkovski et al. (2000).

FCR in the present study was 1.0±0.5 in all experimental diets fed to tilapia. The range of FCR was lower than earlier studies in Nile tilapia (El-Saidy and Gaber, 2003; Gaber, 2005). During the first period FCR was not significantly different in all three diets while from middle and whole period FCR is significantly different ($P<0.05$) among Diet 1 and Diet 3 compared to Diet 2. It means that Diet 2 was the most efficiently utilized by fish. The quality of the source of protein affected the FCR. The higher FCR value in plant diet tended to be elevated owing to use of small tanks and the relatively slow feeding habits of Nile tilapia (El-Saidy and Gaber, 2002). The value of FCR decreases with increasing dietary protein levels from 8-56% for *O. mossambicus* fry (Jauncey, 1982). FCR has been improved by adding different levels of krill product to Nile tilapia in comparison to plant control observed by Gaber, 2005. Thus the same phenomenon observed in this experiment. Proline, glycine and glucosamine from Antarctic krill meal can improve FCR in sea bream (Shimizu et al., 1990).

The diets were passed through the pasta machine several times, probably stimulating gelatinization of starch, and offering hydro-thermal treatment and mild denaturation of protein. Gelatinization of starch can be described as a swelling driven process, defined as the irreversible destruction of crystalline order in a starch granule, so that the surface of every molecule is made accessible to solvents or reactants, including digestive enzymes in the gastrointestinal tract (Sørensen, 2003) Proteins are easily denatured and can have strong bonding properties in the denatured stage and significant contribute to pellet strength (Zimonja and Svihus, 2009). However, gelatinization of starch and mild denaturation of protein make improves access by amylase and proteases, and inactivate protease inhibitors in plant proteins (Storebakken, pers. comm.).

4.3 Fatty acids composition

The fatty acid compositions in final fish body changed. Fatty acids (% of total fatty acids) with less 18 carbons decreased in final fish body compared with initial fish body. While higher fatty acids with equal or more than 18 carbons percent were found in final fish body compared with initial fish body. This result is in line with the result fromAl-Souti et al. (2012). Moreover, plant oils such as rapeseed oil, soybean oil have been reported as good lipid sources for tilapia because they are rich in C18:3*(n-3)*, C18:2 *(n-6)* and C18:1*(n-9)* (Lim et al., 2011, Bell et al., 2001). This can explain the result why final whole body fish present especially higher C18:3*(n-6)*, C18:1*(n-9)* and C18:2 *(n-6)* concentration compared with initial fish body as addition with 7% rapeseed oil in all diets. Additionally, tilapia possess the ability to convert C18:2*(n-6)* into C20:4*(n-6)* (Lim et al., 2011), that also explained why the content of C20:4*(n-6)* got higher in final fish body.

While significantly highest C18:3*(n-3)* content was found in fish fed by Diet 2, this is converse from the previous result that plant oil s are rich in C18:3*(n-3)* (Lim et al., 2011). On the other hand, both of C18:3*(n-6)* and C20:4 *(n-6)* percent are significant highest in fish fed by Diet 1 while lowest in fish fed by Diet 2 (Table 8). This can probability be explained by the effect from KFC, as experimental fish showed lowest feed intake from Diet 2 which is with 2.5%KFC.

4.4 Fish body compositions

The results of body composition did not show significant differences with the exception of the ash content. The lowest body ash content was displayed by fish fed

with Diet 2 and this is aligned with the ash content of this diet which was the lowest. The same trends can be seen with fish fed Diet 3 which displayed higher ash content in accordance with the highest ash content of this diet. Result from fish fed by Diet 3 agree the statement that krill contains much ash and is bulky due to the chitin-containing carapace (Storebakken, 1988). While it does not really give any indication to why fish fed by Diet 2 contained less ash, or which mineral elements caused this.

However while comparing Diet 1 and Diet 2 which present equal amount of ash content, the two groups of fish fed respectively by those two diets displayed different final body ash content. This could have been interpreted as the diet containing 100% plant protein can enhance the absorption of minerals but there is no evidence in this investigation supporting this statement. Additionally, fish can also absorb minerals from water.

Nevertheless a close look on the phosphorous content clearly showed that there is a slight correlation between the ash content of the body fish and the phosphorous content of the diets in the experimental conditions that were used during this trial.

4.5 Digestibility

The digestibility of crude protein did not significantly differ among diets. The nitrogen apparent digestibility varied from 82.4 for Diet 3 to 85.9 for Diet 2. A slight increasing tendency was found in fish fed by from Diet 3 to Diet 2 which contained 2.5% KFC. The result showed almost same digestibility both in fish fed by Diet 1 and Diet 3, which is not in line with the previous study that digestibility of nutrient of diets increased with increasing levels of krill meal(Gaber, 2005).

The apparent digestibility of phosphorous varied non-significantly from 30.2% for Diet 1 to 37.3% for Diet 3. The P-digestibility was lower than those obtained from

MCP-supplemented plant protein concentrate diets fed to rainbow trout by Zhang et al., (2012). This indicates inefficient uptake of P from diets by tilapia. Phosphorus is an essential minerals that have to be supplied via the diets. Phosphorus (together with calcium) is a structural component for bones, teeth and scales(Dieterich et al., 2012).

In addition, it plays a role in several metabolic processes. Reduced growth rate, reduced feed efficiency and bone deformations are the most common signs of (digestible) phosphorus deficiency (Bueno et al., 2012). The rapid growth observed in this experiment indicates that chronical P-deficiency was not manifest in the tilapias.

4.6 Nutrient retentions

Nitrogen, energy and phosphorous retention are critical parameter to evaluate fish performance because these retentions to some extent summarized net protein utilization for growth (Ribeiro et al., 2012, Jabir et al., 2012). Pollution from fish farming is also indicated by the inverse of retention values. No significant differences were observed, and high retention of nitrogen, phosphorous and energy indicates that the plant diet was very efficient, and that no achievements were made by supplementing with a marine source of protein.

5. Conclusions

The present study has showed that, juvenile Nile tilapia were able to eat much, grow rapidly, and utilize a 100% plant protein diet efficiently. Inclusion of 2.5% KM+ 2.5% KFC in plant protein diets exhibited lower feed intake, resulting retarded fish growth. Poor SGR and inferior digestibility also observed in 2.5%KM+2.5%KFC diet. Inclusion of 5% KM also showed reduced feed intake as well as lower growth compared to the plant protein diet but no big differences. Better result has been observed diet having 5% KM in comparison to 2.5%KM+2.5%KFC diet. Fish fed by

all diets had similar FCR in whole period. There were no significant differences found from chemical compositions of fish body (except ash content). Both of C18:3*(n-6)* and C20:4 *(n-6)* percent are significant highest in fish fed by plant protein. No significant differences in digestibility of nitrogen and phosphorus, and utilization of the nutrients, such as nitrogen retention, energy retention and phosphorus retention. In a nutshell, these observable outcomes were not satisfactorily meeting the paramount expectation as marine ingredients have high cost.

6. References

ABDELGHANY, A. E. & AHMAD, M. H. 2002. Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. *Aquaculture Research,* 33**,** 415-423.

ACOSTA, B. & GUPTA, M. 2010. The Genetic Improvement of Farmed Tilapias Project: Impact and Lessons Learned. *In:* SILVA, S. & DAVY, F. B. (eds.) *Success Stories in Asian Aquaculture.* Springer Netherlands.

AHMED, M. & LORICA, M. H. 2002. Improving developing country food security through aquaculture development—lessons from Asia. *Food Policy,* 27**,** 125-141.

AL-SOUTI, A., AL-SABAHI, J., SOUSSI, B. & GODDARD, S. 2012. The effects of fish oil-enriched diets on growth, feed conversion and fatty acid content of red hybrid tilapia, Oreochromis sp. *Food Chemistry,* 133**,** 723-727.

ALLAHPICHAY, I. & SHIMIZU, C. 1984a. Supplemental effect of the whole body krill meal and the non-muscle krill meal of *Euphausia superba* in fish diet. 50**,** 815-820.

ANHELLER, J. E., HELLGREN, L., KARLSTAM, B. & VINCENT, J. 1989. Biochemical and biological profile of a new enzyme preparation from antarctic krill (E. superba) suitable for debridement of ulcerative lesions. *Archives of Dermatological Research,* 281**,** 105-110.

BELL, J. G., MCEVOY, J., TOCHER, D. R., MCGHEE, F., CAMPBELL, P. J. & SARGENT, J. R. 2001. Replacement of Fish Oil with Rapeseed Oil in Diets of Atlantic Salmon (Salmo salar) Affects Tissue Lipid Compositions and Hepatocyte Fatty Acid Metabolism. *The Journal of Nutrition,* 131**,** 1535-1543.

BERGHEIM, A. 2007. Water quality criteria in recirculation systems for Tilapia.

. *Aquacultural Engineering and Environment***,** 21-47.

BUENO, G. W., FEIDEN, A., NEU, D. H., LUI, T. A., WACHTER, N. & BOSCOLO, W. R. 2012. Digestibility of phosphorus in feed as a nutritional strategy for reduce of effluents from tilapia culture. *Arquivo Brasileiro De Medicina Veterinaria E Zootecnia,* 64**,** 183-191.

CHAMBERLAIN, A. 2011. *Fish meal and fish oil the facts, figures, trends and IFFO's responsible supply standard* [Online]. International Fish meal and Fish oil Organigation

website www.iffo.net.

CHOWDHURY, D. K. 2011. *Optimal feeding rate for Nile tilapia (Oreochromis niloticus),* [Ås], [D.K. Chowdhury].

CHOWDHURY, K. M. A., P., D., BUREAUA, MANIK L. BOSEB & MADAN DEYB 2007. RELEVANCE OF A RAPID APPRAISAL APPROACH TO IDENTIFY LOCALLY AVAILABLE FEED INGREDIENTS TO SMALL-SCALE NILE TILAPIA (*Oreochromis niloticus L.*) AQUACULTURE. 11**,** 151-169.

DENG, J., MAI, K., AI, Q., ZHANG, W., WANG, X., XU, W. & LIUFU, Z. 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, Paralichthys olivaceus. *Aquaculture,* 258**,** 503-513.

DIETERICH, F., BOSCOLO, W. R., LOSH, J. A., FEIDEN, A., FURUYA, W. M. & SIGNOR, A. A. 2012. Phosphorus sources in organic diets for fingerlings and juveniles of Nile tilapia. *Pesquisa Agropecuaria Brasileira,* 47**,** 417-424.

EKNATH, A. E. & ACOSTA, B. O. 1998. Genetic improvement of farmed Tilapias (GIFT). *Project Final report, March 1988 to december 1997.* Instituation center for Livivng Aquatic Resources Management, Makati city, philipines.

EL-SAIDY, D. M. S. D. & GABER, M. M. A. 2002. Complete Replacement of Fish Meal by Soybean Meal with Dietary L-Lysine Supplementation for Nile Tilapia Oreochromis niloticus (L.) Fingerlings. *Journal of the World Aquaculture Society,* 33**,** 297-306.

EL-SAYED, A.-F. M. & KAWANNA, M. 2004. Effects of photoperiod on the performance of farmed Nile tilapia Oreochromis niloticus: I. Growth, feed utilization efficiency and survival of fry and fingerlings. *Aquaculture,* 231**,** 393-402.

EL-SAYED, A.-F. M. & TACON, A. G. J. 1997. Fish meal replacer for Tilapia. v.22**,** 205-224.

ERS 2010. U.S tilapia imports, volume by selected sources

FAO 2002. Fishery information, Data and Statistics unit. Fishstat plus Universal software for fishery stastics Unit.

FAO 2004. The state of world fisheries and aquaculture Fishstat plus Universal software for fishery stastics Unit.

FAO 2009. Fishery information, Data and Statistics unit. Fishstat plus Universal software for fishery stastics Unit.

FAO 2010. The state of world fisheries and aquaculture Rome, Italy.

FAO 2011. Fishery information, Data and Statistics unit. Fishstat plus Universal software for fishery stastics Unit.

FITZSIMMONS, K. 2010. Potential to increase tilapia production Presentation paper of global outlook fot aquaculture leadership,Kuala lumpur.

FONTAÍNHAS-FERNANDES, A., GOMES, E., REIS-HENRIQUES, M. A. & COIMBRA, J. 1999. Replacement of Fish Meal by Plant Proteins in the Diet of Nile Tilapia: Digestibility and Growth Performance. *Aquaculture International,* 7**,** 57-67.

GABER, M. M. A. 2005. The effect of different levels of krill meal supplementation of soybean-based diets on feed intake, digestibility, and chemical composition of juvenile Nile tilapia Oreochromis niloticus, L. *Journal of the World Aquaculture Society,* 36**,** 346-353.

GIGLIOTTI, J. C., DAVENPORT, M. P., BEAMER, S. K., TOU, J. C. & JACZYNSKI, J. 2011. Extraction and characterisation of lipids from Antarctic krill (Euphausia superba). *Food Chemistry,* 125**,** 1028-1036.

GODA, A. M. A. S., WAFA, M. E., EL-HAROUN, E. R. & KABIR CHOWDHURY, M. A. 2007. Growth performance and feed utilization of Nile tilapia Oreochromis niloticus (Linnaeus, 1758) and tilapia galilae Sarotherodon galilaeus (Linnaeus, 1758) fingerlings fed plant protein-based diets. *Aquaculture Research,* 38**,** 827-837.

HAN, C. Y., WEN, X. B., ZHENG, Q. M. & LI, H. B. 2011. Effects of dietary lipid levels on lipid deposition and activities of lipid metabolic enzymes in hybrid tilapia (Oreochromis niloticus × O. aureus). *Journal of Animal Physiology and Animal Nutrition,* 95**,** 609-615.

HANSEN, A.-C., ROSENLUND, G., KARLSEN, Ø., KOPPE, W. & HEMRE, G.-I. 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (Gadus morhua L.) I — Effects on growth and protein retention. *Aquaculture,* 272**,** 599-611.

HANSEN, J. Ø. 2011. *Antarctic krill (Euphausia superba) as a feed ingredient for ssalmonids with focus on the shell fraction and fluride.* Phd thesis, Norwegian University of Life Sciences.

HANSEN, J. Ø., PENN, M., ØVERLAND, M., SHEARER, K. D., KROGDAHL, Å., MYDLAND, L. T. & STOREBAKKEN, T. 2010. High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (Salmo salar). *Aquaculture,* 310**,** 164-172.

HEWITT, R. P., WATKINS, J., NAGANOBU, M., SUSHIN, V., BRIERLEY, A. S., DEMER, D., KASATKINA, S., TAKAO, Y., GOSS, C., MALYSHKO, A., BRANDON, M., KAWAGUCHI, S., SIEGEL, V., TRATHAN, P., EMERY, J., EVERSON, I. & MILLER, D. 2004. Biomass of Antarctic krill in the Scotia Sea in January/February 2000 and its use in revising an estimate of precautionary yield. *Deep Sea Research Part II: Topical Studies in Oceanography,* 51**,** 1215-1236.

HEWITT, R. P., WATKINS, J. L., NAGANOBU, M., TSHERNYSHKOV, P., BRIERLEY, A. S., DEMER, D. A., KASATKINA, S., TAKAO, Y., GOSS, C., MALYSHKO, A., BRANDON, M. A., KAWAGUCHI, S., SIEGEL, V., TRATHAN, P. N., EMERY, J. H., EVERSON, I. & MILLER, D. G. M. 2002. Setting a precautionary catch limit for Antarctic krill. 15(3)**,** 26-33.

HISHAMUNDA, N. & RIDLER, N. B. 2006. Farming fish for profits: A small step towards food security in sub-Saharan Africa. *Food Policy,* 31**,** 401-414.

HUGHES, S. G. & HANDWERKER, T. S. 1993. Formulating for tilapia: all vegetable protein feeds. 55-60.

ICHII, T. 2000. Krill Harvesting *Wiley publishers* 228-258 pp.

JABIR, M. D. A., RAZAK, S. A. & VIKINESWARY, S. 2012. Chemical Composition and Nutrient Digestibility of Super Worm Meal in Red Tilapia Juvenile. *Pakistan Veterinary Journal,* 32**,** 489-493.

JAUNCEY, K. 1982. The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (Sarotherodon mossambicus). *Aquaculture,* 27**,** 43-54.

KOLKOVSKI, S., CZESNY, S. & DABROWSKI, K. 2000. Use of Krill Hydrolysate as a Feed Attractant for Fish Larvae and Juveniles. *Journal of the World Aquaculture Society,* 31**,** 81-88.

KUBITZA, F., LOVSHIN, L. L. & LOVELL, R. T. 1997. Identification of feed enhancers for juvenile largemouth bass Micropterus salmoides. *Aquaculture,* 148**,** 191-200.

LIM, C., YILDIRIM-AKSOY, M. & KLESIUS, P. 2011. Lipid and Fatty Acid Requirements of Tilapias. *North American Journal of Aquaculture,* 73**,** 188-193.

MACKIE, A. M. & MITCHELL, A. I. 1982. Further studies on the chemical control of feeding behaviour in the Dover Sole, Solea solea. *Comparative Biochemistry and Physiology Part A:*

Physiology, 73**,** 89-93.

MAMUN, S. M., FOCKEN, U. & BECKER, K. 2007. Comparative digestion efficiencies in conventional, genetically improved and genetically male Nile tilapia, Oreochromis niloticus (L.). *Aquaculture Research,* 38**,** 381-387.

MUNDHEIM, H., AKSNES, A. & HOPE, B. 2004. Growth, feed efficiency and digestibility in salmon (Salmo salar L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities. *Aquaculture,* 237**,** 315-331.

MUZINIC, L. A., THOMPSON, K. R., METTS, L. S., DASGUPTA, S. & WEBSTER, C. D. 2006. Use of turkey meal as partial and total replacement of fish meal in practical diets for sunshine bass (Morone chrysops × Morone saxatilis) grown in tanks. *Aquaculture Nutrition,* 12**,** 71-81.

NGUYEN, T. N., DAVIS, D. A. & SAOUD, I. P. 2009. Evaluation of Alternative Protein Sources to Replace Fish Meal in Practical Diets for Juvenile Tilapia, Oreochromis spp. *Journal of the World Aquaculture Society,* 40**,** 113-121.

OLIVA-TELES, A., GOUVEIA, A. J., GOMES, E. & REMA, P. 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, Oncorhynchus mykiss. *Aquaculture,* 124**,** 343-349.

OLSEN, R. E., SUONTAMA, J., LANGMYHR, E., MUNDHEIM, H., RINGØ, E., MELLE, W., MALDE, M. K. & HEMRE, G. I. 2006. The replacement of fish meal with Antarctic krill, Euphausia superba in diets for Atlantic salmon, Salmo salar. *Aquaculture Nutrition,* 12**,** 280-290.

POND, D. W., PRIDDLE, J., SARGENT, J. R. & WATKINS, J. L. 1995. Laboratory studies of assimilation and egestion of algal lipid by Antarctic krill — methods and initial results. *Journal of Experimental Marine Biology and Ecology,* 187**,** 253-268.

PONZONI, R. W., KHAW, H. L., NGUYEN, N. H. & HAMZAH, A. 2010. Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (Oreochromis niloticus). *Aquaculture,* 302**,** 42-48.

RIBEIRO, F. B., LANNA, E. A. T., BOMFIM, M. A. D., DONZELE, J. L., QUADROS, M., CUNHA, P. D. L., TAKISHITA, S. S. & VIANNA, R. A. 2012. Apparent and true digestibility of protein and amino acid in feedstuffs used in Nile Tilapia feed as determined by the technique of dissection. *Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science,* 41**,** 1075-1081.

SCAHAWS 2003. The use of fish by-products in aquaculture *Report of the Scientific Committee on Animal Health and Animal Welfare***.** European Commission.

SCHIERMEIER, Q. 2010. Ecologists fear Antarctic krill crisis. *Nature,* 467**,** 15.

SHIMIZU, C., IBRAHIM, A., TOKORO, T. & SHIRAKAWA, Y. 1990. Feeding stimulation in sea bream, Pagrus major, fed diets supplemented with Antarctic krill meals. *Aquaculture,* 89**,** 43-53.

SINTAYEHU, A., MATHIES, E., MEYER-BURGDORFF, K. H., ROSENOW, H. & GÜNTHER, K. D. 1996. Apparent digestibilities and growth experiments with tilapia (Oreochromis niloticus) fed soybean meal, cottonseed meal and sunflower seed meal. *Journal of Applied Ichthyology,* 12**,** 125-130.

SØRENSEN, M. 2003. *Nutritional and physical quality of fish feeds extruded at various temperatures* Phd Doctor Scientiarium Thesis, Agriculture University of Norway.

STOREBAKKEN, T. 1988. Krill as a potential feed source for salmonids. *Aquaculture,* 70**,** 193-205.

SUONTAMA, J. 2006. *Macrozooplankton as feed source for farmed fish growth, product quality and safety* Phd Phd thesis, University of Bergen, Bergen, Norway.

TEOH, C.-Y., TURCHINI, G. M. & NG, W.-K. 2011. Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. *Aquaculture,* 312**,** 126-136.

THODESEN, J., RYE, M., WANG, Y.-X., YANG, K.-S., BENTSEN, H. B. & GJEDREM, T. 2011. Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth of Nile tilapia (Oreochromis niloticus) after six generations of multi-trait selection for growth and fillet yield. *Aquaculture,* 322–323**,** 51-64.

TIBBETTS, S. M., OLSEN, R. E. & LALL, S. P. 2011. Effects of partial or total replacement of fish meal with freeze-dried krill (Euphausia superba) on growth and nutrient utilization of juvenile Atlantic cod (Gadus morhua) and Atlantic halibut (Hippoglossus hippoglossus) fed the same practical diets. *Aquaculture Nutrition,* 17**,** 287-303.

TUNG, P.-H. & SHIAU, S.-Y. 1991. Effects of meal frequency on growth performance of hybrid tilapia, Oreochromis niloticus × O. aureus, fed different carbohydrate diets. *Aquaculture,* 92**,** 343-350.

VIOLA, S., ARIELI, Y. & ZOHAR, G. 1988. Animal-protein-free feeds for hybrid tilapia (Oreochromis niloticus × O. aureus) in intensive culture. *Aquaculture,* 75**,** 115-125.

WANG, Y., LIU, Y.-J., TIAN, L.-X., DU, Z.-Y., WANG, J.-T., WANG, S. & XIAO, W. P. 2005. Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, Oreochromis niloticus×O. aureus. *Aquaculture Research,* 36**,** 1408-1413.

WINTHER, B., HOEM, N., BERGE, K. & REUBSAET, L. 2011. Elucidation of Phosphatidylcholine Composition in Krill Oil Extracted from Euphausia superba. *Lipids,* 46**,** 25-36.

YOSHITOMI, B. & NAGANO, I. 2012. Effect of dietary fluoride derived from Antarctic krill (Euphausia superba) meal on growth of yellowtail (Seriola quinqueradiata). *Chemosphere,* 86**,** 891-897.

YUE, Y.-R. & ZHOU, Q.-C. 2008. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, Oreochromis niloticus × O. aureus. *Aquaculture,* 284**,** 185-189.

ZHANG, Y. X., OVERLAND, M., SHEARER, K. D., SORENSEN, M., MYDLAND, L. T. & STOREBAKKEN, T. 2012. Optimizing plant protein combinations in fish meal-free diets for rainbow trout (Oncorhynchus mykiss) by a mixture model. *Aquaculture,* 360**,** 25-36.

ZIMONJA, O. & SVIHUS, B. 2009. Effects of processing of wheat or oats starch on physical pellet quality and nutritional value for broilers. *Animal Feed Science and Technology,* 149**,** 287-297.