Studies on the potential application of corn co-products for fishmeal-free diets of Nile tilapia Oreochromis niloticus

Doctoral Dissertation

STUDIES ON THE POTENTIAL APPLICATION OF CORN CO-PRODUCTS FOR FISHMEAL-FREE DIETS OF NILE TILAPIA *Oreochromis niloticus*

September 2016

Graduate School of Marine Science and Technology

Tokyo University of Marine Science and Technology

Doctoral Course of Applied Marine Biosciences

SANDAMALI SAKUNTHALA HERATH

Table of Contents

List of tables

List of figures

List of Abbreviations

a*	Redness
ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
b*	Yellowness
cDNA	Complementary Deoxyribonucleic acid
CGF	Corn Gluten Feed
CGM	Corn Gluten Meal
CPC	Corn Protein Concentrate
C_T	Cycle Threshold
DDGS	Distillers Dried Grains with Soluble
DHA	Decosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Decosapentaenoic acid
DWG	Daily Weigth Gain
EAA	Essential Amino Acids
Elovl	Fatty acid elongase
EPA	Eicosapentaenoic acid
FAME	Fatty Acid Methyl Esters
FCR	Feed Conversion Ratio
FM	Fish Meal
GC	Gas Chromatography
GH	Growth Hormone
GHR	Growth Hormone Receptors

HPDDGS	High Protein Distillers Dried Grains
HIS	Hepato Somatic Index
HUFA	Highly Unsaturated Fatty Acids
IGF	Insulin like Growth Factors
IPF	Intra Peritoneal Fat
K	Condition factor
L*	Lightness
LA	Linoleic Acid
LC-PUFA	Long Chain Poly Unsaturated Fatty Acids
LNA	α- Linolenic Acid
MFI	Mean Feed Intake
MWG	Mean Weight Gain
NEAA	Non Essential Amino Acids
ND	Non Detectable
NFM	Non Fish Meal
PCR	Polymerase Chain Reaction
PER	Protein Efficiency Ration
PUFA	Poly Unsaturated Fatty Acids
qRT-PCR	quantitative Real Time Polymerase Chain Reactions
RNA	Ribonucleic acid
SEM	Standard Error of the Mean
SGR	Specific Growth Rate
SR	Survival Rate
TGC	Thermal Growth Coefficient
VSI	Viscero Somatic Index

ACKNOWLEDGEMENTS

First and foremost I wish to express my deepest and sincerest gratitude to my supervisor Professor Shuichi Satoh for the opportunity gave me to complete my doctoral study under his supervision and his invaluable guidance, suggestions, encouragement and generous support throughout this study. Besides my main supervisor, sincere thanks and appreciation are extended to Dr.Yutaka Haga, for his endless support, critical comments and encouragement which shaped up this thesis. I would also like to express my gratitude to my thesis committee member, Dr. Masashi Maita, for his precious time spent to read this thesis, critical comments and constructive suggestions.

My sincere thanks also go to Dr. Masato Endo and Mrs. Yukino Tanabe in the Laboratory of Fish culture, for providing fish fingerlings in timely manner. I am much indebted to Mr. Toda, Mr.Kurimoto, Mr. Ishibashi, Miss Sasaoka and Ms.Mojena for technical support, especially during chemical analysis and all of my colleagues in the Laboratory of fish nutrition for sharing hands and thoughts during this study. Especial thanks are due to Mr. Ian Rondon in Laboratory of Genome Science for his technical assistance and generous support in gene expression study.

It is time for me to thank the Ministry of Education, Culture and Sports, Japan (MEXT) for providing scholarship to pursue study at the Tokyo University of Marine Science and Technology, Japan. Furthermore I would like to show my gratitude to the University of Ruhuna,Sri Lanka for granting study leave and all the members of the Department of Fisheries and Aquaculutre, Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna,Sri Lanka for their support during this period.

I owe my heartiest gratitude to my parents, brother and two sisters whose endless love, encouragement and sacrifices lead me to success in my every effort. Words fail me to express my appreciation to my loving husband Keerthi whose dedication, love and persistent encouragement has made this task feasible.

At last but not least, I dedicated this small piece of work to my loving son Senula with deepest love who missed lots of love and care and sacrificed part of his childhood for the success of my study especially during the last year.

[博士過程]

Doctoral Course

博士学位論文内容要旨

Abstract

専攻 Major	Applied Marine Bioscience	氏名 Name	Sandamali Sakunthala Herath
論文題目 Title	Studies on the potential application of corn co-product for fishmeal-free diets of Nile tilapia *Oreochromis niloticus*		

Several plant based ingredients have being used to replace fish meal in aqua-feed. Corn gluten meal (CGM), corn protein concentrate (CPC), distillers dried grains with soluble (DDGS) and high protein distillers dried grains (HPDDG) are corn co-products rich in digestible proteins with comparatively less amino acid deficiencies. Usage of high proportion of these co-products in fish feeds is limited by the presence of excess yellow pigments which would possibly alter the fillet color of fish. Therefore three studies were designed to, i) evaluate and compare the suitability of above four corn co-products in preparing zero fish meal diets for Nile tilapia, ii) evaluate the long-term effect of corn co-product based diets on growth, fillet color and fillet nutrient quality of Nile tilapia and iii) evaluate the combined effect of corn co-products based non-fishmeal diet and salinity on growth, feed utilization and expression level of some selected genes involved in growth, osmoregulation and fatty acid desaturation and elongation.

In the first experiment, a 12 week feeding trial was conducted to evaluate and compare the suitability of CPC, CGM, DDGS and HPDDG in preparing zero fish meal diets for Nile tilapia, *Oreochromis niloticus* juveniles by comparing the growth performance, feed utilization efficiency and fillet color of fish. Five iso-nitrogenous diets with 32% protein were prepared.

The 50% of protein in each diet was supplied by fish meal in control diet and one of the four corn co-products (CPC, CGM, HPDDG, DDGS) in other diets. Significantly highest specific growth rate (SGR) and survival rate (SR) were observed in the control and DDGS groups while feed utilization efficiencies were not affected by the treatments. Among instrumentally measured filler color, lightness (L*), redness (a*) and yellowness (b*) only L* of DDGS was significantly higher than that of control, but none of the other diets differ either from DDGS or control.

A 24-week feeding trial was conducted as a second trial(21g initial weight)to evaluate the long-term effect of corn co-product–based fishmeal-free diets on growth, fillet color, and fillet fatty acid and amino acid composition. Dietary composition in this study was almost similar with the experiment-I, except control diet. In this experiment control diet contained only 10% fishmeal. Fish fed the control, HPDDG, or DDGS diet had significantly higher ($P < 0.05$) mean weight gain, specific growth rates, mean feed intake, protein efficiency ratio, and survival than those fed the other diets. Fish in these three treatments also had the lowest food conversion ratio. The dietary treatments did not affect the lightness, redness, yellowness, or crude protein and total amino acid content of fish fillets. Fillet fatty acid composition was greatly affected by the dietary treatments. The total polyunsaturated fatty acid levels were highest in the DDGS group. Similarly, the total n-6 level was highest in the DDGS group, followed by HPDDG. The total n-3 levels and n-3: n-6 ratios in the fillets of the control group were almost double those of the corn-based dietary groups.

In third experiment, two iso-nitrogenous (32% protein) diets were formulated with and without fishmeal. Four salinity levels, i.e. 0ppt, 4ppt, 8ppt and 12ppt were used to evaluate the combined effect of fishmeal replacement with salinity (2x4 factorial designs). Duplicated group

of fish (initial weight~ 6 g) reared in four salinity levels were fed one of the two diets to near satiety twice a day for 12 weeks. At the end of the experiment, growth performance, feed utilization efficiency and relative expression of insulin like growth factor (IGF)-I, IGF-II, growth hormone (GH), growth hormone receptor (GHR), fatty acid Δ6 desaturase (Fadsd6) and fatty acid elongase (elovl5) genes of liver and gill tissues of Nile tilapia were evaluated.

Growth performance of fish was significantly affected by the salinity while no statistical differences were observed for two diets. Percentage weight gain and mean feed intake of fish reared at 4‰was significantly higher than that of others irrespective of the diet. Food conversation ratio of fish reared in 0‰ was significantly lower than the fish in 8‰ and 12‰.This study revealed the possibility of totally replacing fishmeal in Nile tilapia juveniles reared in 0‰ to 12‰ salinity without compromising growth performances. Salinity regulation of gene expression was identified only in IGF-I, IGF-II and Fadsd6 genes and dietary effects were limited only to elovl5. No interactive effects of diet and salinity were identified for gene expression.

CHAPTER 1

Chapter 1. General introduction

Aquaculture is growing more rapidly than all the other food production sectors in the world and average annual growth rate of global aquaculture is over 9% (FAO, 2014, Gatlin *et al.*, 2007). The acceleration of aquaculture production has been accompanied by combination of factors including, improved technologies of feed formulation and nutrition. The economic returns of aquaculture production are highly relying on feed cost in the intensive and semi-intensive aquaculture systems. Feed cost often accounts more than 50% of the total variable cost(Coyle *et al.*, 2004, El‐Sayed, 1998, Naylor *et al.*, 2009)and the largest portion out of that is allocated for protein supply(Azaza *et al.*, 2015, Twibell and Brown, 1998). Because, protein requirement of fish is two to three times higher than that of warm blooded vertebrates (Crab et al., 2007).

Historically, fishmeal is the principal protein source in aqua-feed formulation (Boyd *et al.*, 2007). Fishmeal usually ensures the expected growth performances and better feed utilization efficiencies due to a combination of factors including, high protein content, excellent amino acid profile with other essential nutrients such as vitamin and mineral, high palatability, high nutrient digestibility and general lack of anti-nutritional factors(El‐Sayed, 1998, Gatlin *et al.*, 2007).With the continuous expansion of global aquaculture industry, access to fishmeal become increasingly limited because of finite wild-harvest resource. Beside the sustainability of wild fisheries resources, certain other issues such as, rising demand and price, uncertainty in future supply, potential presence of organic and inorganic contaminants in fishmeal require enhanced efforts to thoroughly evaluate the possible alternatives such as various plant feedstuffs and terrestrial animal by products (Coyle *et al.*, 2004, Gatlin *et al.*, 2007, Naylor *et al.*, 2009).

Therefore, identification of environmentally sustainable and economically viable alternatives for fishmeal is a challenge for aquaculture.

When evaluating plant-feedstuff for their suitability to use in aqua-feed, certain characteristics inherent with plant material, such as presence of anti-nutritional factors, deficiency in certain amino acids and poor nutrient digestibility make them disadvantage over fishmeal (Gatlin *et al.,* 2007). To be a potential replacer of fishmeal in aqua-feed, an alternative ingredient must meet the certain criteria such as, presence of relatively high protein with favorable amino acid profile at competitive price, high nutrient digestibility and palatability, low levels of fiber, starch and anti-nutritional factors, wide availability, ease of transporting, storage and handling during feed manufacturing (Gatlin *et al.,* 2007, Naylor *et al.,* 2009). Also such ingredients need to ensure the economic growth of farmed fish. In addition, minimum pollution and ecosystem stress and ability to confer the health protective properties of fish consumption to human is also important.

Tilapia is one of the most popular warm water fish in the world and it is likely to become the most important cultured fish in the 21st century (El-Sayed *et al.,* 2005, El-Sayed *et al.,* 2003,FAO, 2014). Farming systems of tilapia is being intensified due to combinations of reasons mainly land scarcity, increasing price of land and freshwater resources and rapidly growing market for tilapia(Naylor *et al.,* 2000). Although this species is an omnivorous grazer which feed at the lower level of aquatic food chain, culture systems of tilapia has been gradually intensified over the last two decades using formulated feed containing about 5% of fishmeal (Naylor *et al.,* 2009). Even if, fishmeal inclusion rate is low in tilapia feed, total global production volumes, and thus fishmeal volumes, are large in tilapia as it is the second largest fish culture in the world (Naylor *et al.,* 2009). As aquaculture of tilapia is a booming practice, use of fish meal in diets

could also place a pressure on pelagic fisheries and ultimately harm to marine ecosystem and long term sustainability of aquaculture itself. In addition, increasing demand, coupled with significant shortage of global fishmeal production has made it more expensive. As a consequence, resulting high feed price could undermine the profitability of tilapia aquaculture as it is a low value species. Therefore, complete fishmeal replacement in tilapia diet by cheap and highly available ingredient is crucial for the expansion of its aquaculture.

Wide range of potential alternatives including rendered animal proteins and variety of plant protein sources have been evaluated as economically feasible alternative protein sources for fish meal with different degree of success(Coyle *et al.*, 2004, El‐Saidy and Gaber, 2003, Fontainhas-Fernandes *et al.*, 1999, Lim *et al.*, 2007, Richter *et al.*, 2003, Schaeffer *et al.*, 2010, Shiau *et al.*, 1987). Among a vast array of economically viable alternatives, by products/ co-products of certain industry that are basically not suitable for direct human consumption received great attention to replace fishmeal in aqua-feeds (Xu *et al.*, 2007, Naylor *et al.*, 2009).

Under this context, protein rich corn co-products that are produced in bio-fuel industry is important. The main co-products of corn milling are distillers dried grains with soluble (DDGS), high protein distiller dried grains (HPDDG), corn gluten meal (CGM), and corn protein concentrate (CPC). Bio-fuel industry could provide the push to develop the technology to maintain the consistency of their co-products if aquaculture could provide the market for such products. As both industries are growing with certain challenges, use of corn co-product of bio-fuel industry is an attractive option to ensure sustainability of tilapia aquaculture.

In addition to fishmeal replacement, there are certain other challenges in tilapia aquaculture which includes diseases and freshwater shortage. Ability of tilapia to tolerate a wide range of salinity levels has solved the problem of freshwater shortage and now tilapia culture is

expanding to the brackish water and sea water (El-Sayed *et al.*, 2005, El-Sayed *et al.*, 2003, Kamal and Mair, 2005, Yan *et al.*, 2013).Among different species of tilapia, Nile tilapia (*Oreochromis niloticus*) received a great attention due to its high growth rate and adaptability to different culture methods and conditions(Pullin and Lowe-McConnell, 1982) and it is recognized as the most important farmed tilapia in the world (El-Sayed *et al.*, 2005, El-Sayed *et al.*, 2003).Although aquaculture of Nile tilapia is expanded into the saline water, nutritional studies of fishmeal replacement in this species is basically limited to freshwater (Coyle *et al.*, 2004, El‐Sayed, 1998,Fontainhas-Fernandes *et al.*, 1999).However, the interactive effects of salinity and complete fishmeal replacement on growth performance and feed utilization parameters of Nile tilapia, which play a significant role in global aquaculture production is still not clear.

Therefore, application of single or mixture of corn co-product to completely replace fishmeal in Nile tilapia diet in freshwater and or saline water is presented in this thesis.

References

Azaza, M.S., Khiari, N., Dhraief, M.N., Aloui, N., Kraïem, M.M. & Elfeki, A. (2015) Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. Aquaculture Research, 46, 14-27.

Boyd, C.E., Tucker, C., McNevin, A., Bostick, K. & Clay, J. (2007) Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. Reviews in Fisheries science, 15, 327-360.

Coyle, S.D., Mengel, G.J., Tidwell, J.H. & Webster, C.D. (2004) Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research, 35, 365-370.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2003) Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 220, 619-632.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2005) Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 248, 187-196.

El-Sayed, A.-F.M. & Teshima, S.-i. (1992) Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. Aquaculture, 103, 55-63.

El‐Saidy, D.M. & Gaber, M. (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‐Saidy, D.M. & Gaber, M. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research, 34, 1119-1127.

El‐Sayed, A.F. (1998) Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. Aquaculture Research, 29, 275-280.

Fallahi, P., Rosentrater, K.A. & Muthukumarappan, K. (2012) Twin-Screw Extrusion Processing of Rainbow trout (*Oncorhynchus mykiss*) Feeds Using Graded Levels of High Protein Corn-Based Distillers Dried Grains (HP-DDG) and Conventional Distillers Dried Grains with Solubles (DDGS).

FAO, U. (2014) FAOstat. Retrieved Feb, 2014.

Fontainhas-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.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å. & Nelson, R. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research, 38, 551-579.

Kamal, A.H.M.M. & Mair, G.C. (2005) Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. Aquaculture, 247, 189-201.

Likongwe, J.S., Stecko, T.D., Stauffer Jr, J.R. & Carline, R.F. (1996) Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture, 146, 37-46.

Lim, C., Garcia, J.C., Yildirim-Aksoy, M., Klesius, P.H., Shoemaker, C.A. & Evans, J.J. (2007) Growth Response and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus*, Fed Diets Containing Distiller's Dried Grains with Solubles. Journal of the World Aquaculture Society, 38, 231-237.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000) Effect of aquaculture on world fish supplies. Nature, 405, 1017-1024.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J. & Hua, K. (2009) Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences, 106, 15103-15110.

Pullin, R.S. & Lowe-McConnell, R.H. (1982) The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy, WorldFish.

Richter, N., Siddhuraju, P. & Becker, K. (2003) Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, 217, 599-611.

Schaeffer, T.W., Brown, M.L., Rosentrater, K.A. & Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co‐products by Nile tilapia.Journal of animal physiology and animal nutrition, 94, e348-e354.

Shiau, S.-Y.(2002) Tilapia, Oreochromis spp. Nutrient Requirements and Feeding of Finfish for Aquaculture, 273-292.

Shiau, S.-Y., Chuang, J.-L.& Sun, C.-L. (1987) Inclusion of soybean meal in tilapia (*Oreochromis niloticus*× *O. aureus*) diets at two protein levels. Aquaculture, 65, 251-261.

Siddiqui, A.Q., Howlader, M.S. & Adam, A.A. (1988) Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. Aquaculture, 70, 63-73.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285, 146-158.

Tadesse, Z., Boberg, M., Sonesten, L. & Ahlgren, G. (2003) Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish *Oreochromis niloticus* L.–A laboratory study. Aquatic Ecology, 37, 169-182.

Twibell, R.G. & Brown, P.B. (1998) Optimal Dietary Protein Concentration for Hybrid Tilapia *Oreochromis niloticus* × *O. aureus* Fed All‐Plant Diets. Journal of the world aquaculture Society, 29, 9-16.

Wu, Y.V., Rosati, R., Sessa, D.J. & Brown, P. (1994) Utilization of protein-rich ethanol co-products from corn in tilapia feed. Journal of the American Oil Chemists' Society, 71, 1041-1043.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1996) Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. Journal of Agricultural and Food Chemistry, 44, 1491-1493.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1997) Use of corn-derived ethanol coproducts and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. Journal of Agricultural and Food Chemistry, 45, 2174-2177.

Wu, Y.V., Rosati, R.R., Sessa, D.J. & Brown, P.B. (1995) Evaluation of corn gluten meal as a protein source in tilapia diets. Journal of Agricultural and Food Chemistry, 43, 1585-1588.

Xu, Z., Lin, X., Lin, Q., Yang, Y. & Wang, Y. (2007) Nitrogen, phosphorus, and energy waste outputs of four marine cage-cultured fish fed with trash fish. Aquaculture, 263, 130-141.

Yan, B., Wang, Z.-H.& Zhao, J.-L. (2013) Mechanism of osmoregulatory adaptation in tilapia. Molecular biology reports, 40, 925-931.

CHAPTER 2

Chapter 2. Literature review

2.1. Taxonomy and biology of Nile tilapia

Nile tilapia is taxonomically categorized as follows:

Phylum:	Chordata
Class :	Pisces
Order :	Perciformes
S.Order :	Percoideii
Family:	Cichlidae
Genus :	*Oreochromis*
Species:	*niloticus* (Linnaeus, 1758)

Tilapia is an omnivorous grazer, preferred to live in shallow fresh water which primarily depends on planktons especially blue green algae in the natural environment, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus.

2.2. Aquaculture of tilapia

Tilapia aquaculture is popular in more than 100 countries in tropical and sub tropical region. Global aquaculture production of tilapia is second only to the carp (El-Sayed *et al.*, 2005, El-Sayed *et al.*, 2003,FAO, 2014) and it is likely to become the most important cultured fish in the 21st century. Among all cultured tilapia species, Nile tilapia (*Oreochromis niloticus*) has recognized as the most important farmed tilapia in the world (El-Sayed *et al.*, 2005). General

hardiness, incredible adaptability and ability to reproduce in a wide range of physical and environmental conditions, excellent growth rates on a wide variety of natural and prepared diets, resistance to handling and disease-causing agents, and their broad consumer appeal as a food fish has made it better candidate for aquaculture (Pullin and Lowe-McConnell, 1982).

The rapid expansion of tilapia aquaculture stimulates the intensification of culture systems and consequently a great attention has been paid on feed formulations and feed manufacturing. Economic returns of the fish production highly depend upon the feed cost in intensive and semi-intensive aquaculture systems. Feed costs often accounts for more than 50% of the total variable cost of aquaculture operations (Coyle *et al.*, 2004, El‐Sayed, 1998) whereas largest portion is allocated for supplying protein (Azaza *et al.*, 2015, Twibell and Brown, 1998). Therefore successfulness and sustainability of tilapia aquaculture depends on selection of proper quality and quantity of protein. Formulation of cost effective commercial tilapia feed, therefore, is a challenge for fish nutritionist.

2.3. Nutrient requirements of tilapia

2.3.1. **Proteins and amino acids**

Sufficient dietary protein supplementation is required for better performance of aquaculture practice as protein is the main constituent of the fish body(Ahmad *et al.*, 2004). Protein requirement of tilapia has extensively studied in dose response method ((Ahmad *et al.*, 2004). Many factors like size or age, dietary protein source and quality, water quality and culture conditions are reported to be affected on protein requirement of tilapia (El Sayed, 2004). However, protein requirement for better growth performance is decreased with increasing size of the fish (Table 1). However, practical diets used in tilapia aquaculture ranges from 25-35% crude proteins where fish meal used as the main ingredient for protein.

Table 1. Protein requirement of Tilapia

Life stage	Size (g)	Requirement (% diet)	References
Fry	0.012-0.5	45	Ahmad *et al.* (2004), El-Sayed and Teshima (1992)
	0.51-0.8	40	Al Hafedh (1999), Siddiqui *et al.* (1988)
Fingerling	2.4-3.5	30-35	Wang *et al.* (1985)
Juvenile	16.5-20	30-35	Ahmad *et al.* (2004), De Silva and Radampola (1990)
Adult			
Brood stock		30-45	Al Hafedh (1999), (El-Sayed *et al.*, 2003)

Tilapia requires all ten essential amino acids as same with the other species of fish and specific requirement is described by Santiago and Lovell (1988) and are shown in the Table 2. Requirement of sulfur containing amino acid of tilapia can be met by methionine or cysteine/methionine mixture (El Sayed, 2004).

2.3.2. **Lipids and fatty acids**

Dietary lipids are the only source of essential fatty acids needed by fish for normal growth and development. Phospholipids are vital for maintaining membrane flexibility and permeability and also for cellular structures. It is also important to maintain proper balance of lipids in diets as it gives good results of protein efficiency ratio (Shiau, 2002). Studies have been shown that protein level in Oreochromis niloticus diets can be reduced from 33.2% to 25.7% by increasing dietary lipids from 5.7% to 9.4% and carbohydrate from 31.9% to 36.9% (Li et al 1991). However, 5% dietary lipid appeared to be sufficient to meet the minimal requirement of juvenile tilapia, but a level of 12% is needed for maximum growth (Shiau, 2002).Both n-3 highly unsaturated fatty acids and n-6 fatty acids require for maximum growth of tilapia (Chou and Shiau, 1996).

Table 2.Amino acid requirement of tilapia

Amino acid	Requirement (% of dietary protein)
Arginine	4.20
Histidine	1.72
Isoleucine	3.11
Leucine	3.39
Lysine	5.12
Methionine	2.68
Phenylalanine	3.75
Thereonine	3.75
Tryptophan	1.00
valine	2.80

Dietary lipids have to supply not only the energy but also the essential fatty acids required for the normal growth and development of fish. Both n-3 and n-6 fatty acids are important for fish and in Nile tilapia 18:2n-6 and 20:4n-6 are better growth promoters than that of 18:3n-3 and 20:5n-3 (Sargent *et al.*, 2002, Sargent *et al.*, 1995). Requirement of dietary highly unsaturated fatty acids (HUFA) or ability to thrive on dietary 18:2n-6 or 18:3n-3 appear to be evolutionary related to fatty acid composition of their natural diets (Agaba *et al.*, 2005). Therefore, freshwater tilapia whose vegetarian diet generally lacks HUFA has a capacity to bio-convert 18 carbon PUFA to their long chain homologs (Henderson and Tocher, 1987, Olsen *et al.*, 1990, Teoh *et al.*, 2011,Tocher *et al.*, 2002). Fatty acid composition of tilapia is closely

influenced by the dietary fatty acid inputs (Justi *et al.*, 2003, Ng *et al.*, 2013, Tadesse *et al.*, 2003,Teoh *et al.*, 2011).

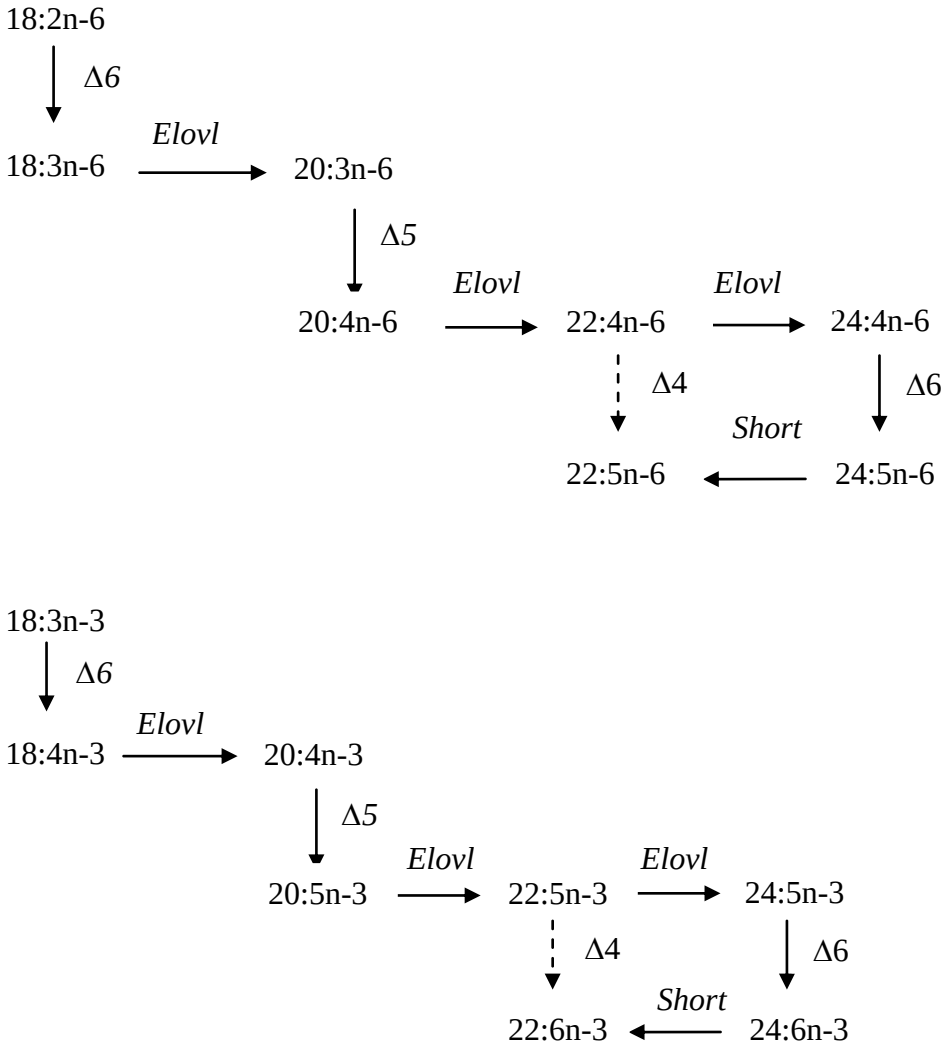


Figure 1: Pathways of highly unsaturated fatty acid (HUFA) biosynthesis from the C18 poly unsaturated fatty acids (PUFA), 18:2n-6 (linoleic acid) and 18:3n-3 (alpha linolenic acid).

Solid arrows represent steps that have been shown to occur in fish and dotted line represent steps that have not been directly demonstrated in fish. Δ6, Δ5 and Δ4 are fatty acid desaturases; Elovl is fatty acid elongases; Short is peroxisomal chain shortning.

2.3.3. **Vitamin and minerals**

Vitamin supplementation is needed for intensive tilapia system where limited or no natural foods are available. Generally natural foods provide enough vitamin and minerals in extensive and semi intensive operations. Vitamin-C is an important vitamin for Nile tilapia and it is recommended to include 420 mg kg^{-1} diet (Soliman *et al.*, 1994). Main reason for supplementation is unstable nature of ascorbic acid and therefore, use of more stable form of ascorbic acid is needed. Further, dietary vitamin E requirement of Nile tilapia is range from 50-100 mg kg^{-1} diet containing 5% lipid. Mineral requirements of Nile tilapia is not extensively reported in scientific literature. However, studies have been undertaken to quantified nutritional requirements of calcium, phosphorous, magnesium, zinc and potassium (Shiau, 2002). The lack of dietary calcium and phosphorus was found to have a significant effect on growth and feed utilization. In Nile tilapia, calcium uptake takes place in the skin, particularly by the opercula membrane (McCormick et al., 1992). In contrast to phosphorus, it seemed, therefore, that the calcium requirement could be met from the rearing water. However, supplementation of phosphorus in diets for Nile tilapia reared under semi-intensive conditions improved growth and feed utilization (Dato-Cajegas and Yakupitiyage, 1996).

2.4. Protein sources use in tilapia feed

As feed is the single nutrient source in intensive aquaculture (Cho and Bureau, 2001), formulation of aqua-feed to fulfill all the nutrient requirement of cultured fish is essential. At the beginning of aquaculture, fish meal is the widely used protein source in feed formulations because of its known nutritional properties and availability (Boyd *et al.*, 2007). Due to unstable supply, high price and many other reasons, finding alternative to replace fishmeal in aqua-feed received great attention and acquired a considerable progress over the last couple of decades. A

vast array of protein sources including terrestrial animal by-product meals, oilseed meals and by-products, aquatic plants, single-cell proteins, and legumes and cereal by-products has widely been evaluated for their suitability to partially or totally replace fishmeal from aqua-feed (Abdel-Tawwab *et al.*, 2008, Coyle *et al.*, 2004, Fontainhas-Fernandes *et al.*, 1999, Richter *et al.*, 2003, Schaeffer *et al.*, 2010, Wu *et al.*, 1996, Wu *et al.*, 1994). Among those tested ingredients, by products or co-products of certain industry which are basically not suitable for direct human consumption received great attention (Xu *et al.*, 2007). Under this context, protein rich co-products of corn milling industry play a significant role as a protein source for animal feed manufacturing including aqua-feed.

The most pressing issue is not just finding replacement ingredients for fishmeal but identifying a consistent supply of high quality ingredients with a suitable available nutrient content. Lastly, an important aspect of potential ingredients is its effect on the final product quality, including but not limited to, changes in fish texture and flavor and whether the ingredient alters or confers additional human health benefits (i.e. omega 3 fatty acids)(Barrows and Sealey).

2.5. Corn co-products and their applications in aqua-feed

Production of ethanol from corn for use as a transportation fuel is a mature technology and introduced in early 1990's in United States (Bothast and Schlicher, 2005). Corn is a versatile crop and about 70-72% of dry weight of corn kernel is comprised with starch. To produce ethanol from the starch, first the starch is converted to glucose and then glucose is fermented. Dry milling and wet milling are the two main processes used to convert corn into ethanol. Fig 2 describes the production processes involved in corn dry milling and wet milling. During this

milling process, various co-products are produced and mainly that co-products of ethanol manufacture is a valuable feed ingredient for livestock, poultry, and fish.

Palatability is important because, no matter how digestible and available the nutrients and energy from an ingredient may be, if the ingredient reduces feed intake then it will slow animal growth and have limited value as a feed ingredient. Feed intake is the key performance criteria in palatability assessments for fish(Barrows and Sealey).

The utilization of co-products from the alternative fuels industries has been of increasing interest by the animal feed industry due to potential for increased volumes of these products. Economics not only plays a role in the cost of the ingredients, but also what the consumer is willing to spend for a product fed a specific diet. Dietary fatty acid profile is of upmost importance to final product quality for its flavor and beneficial effect on human health.

2.5.1. **Use of corn co-products in tilapia diets**

Since Nile tilapia (*O. niloticus*) has an ability to utilize relatively high percentage of plant ingredients (Twibell and Brown, 1998), certain co-products of corn milling industry was tested for tilapia. Wu et al. (1995) observed that diets (36% protein) containing corn gluten meal yielded higher weight gain, higher protein efficiency ratio, and better or equal feed conversion ratio values of tilapia than a commercial feed containing fish meal. Coyle et al. (2004) reported the possibility of incorporating 30% DDGS into none fishmeal diet for Nile tilapia without affecting growth performances. Tidwell et al. (2000) compared the growth of Nile tilapia raised in cages fed diets with 97.5% of DDGS and 2.5% binder with commercial catfish pellet and observed 25% growth retardation in DDGS diet. Wu et al. (1996) observed the good weight gain and feed conversion ratio of tilapia fry fed none fishmeal diet containing 16-49% mixture of corn ethanol co-products. HPDDG is relatively new to fish nutritional studies and have not yet been

used for Nile tilapia. But it has successfully been used and showed the high nutritional value in rainbow trout diet (Prachom et al., 2013). There is no evidence of using CPC as alternative to fishmeal.

CGM is the major co-product of corn wet milling industry and it has widely been used in aqua-feed (Pereira and Oliva‐Teles, 2003). The CPC, a co-product of wet milling industry is the only corn based feed ingredient which provide higher protein content than that in fishmeal. The other two co-products, DDGS and HPDDG produce in the corn dry milling industries for fuel ethanol production. DDGS contains approximately 30% crude proteins (Fallahi et al., 2012, Lim et al., 2007). The HPDDG is a relatively new co-product of corn ethanol industry while the protein content of this product is 1.5-fold higher than that of DDGS. Further, the nutritional values of HPDDG are much more consistent than that of DDGS (Robinson et al., 2008). Compared to other plant-based protein sources such as soybean meal and cotton seed meal, corn co-products are free from anti-nutritional factors (Robinson et al., 2008, Shiau et al., 1987) and less in amino acid deficiencies (Cheng and Hardy, 2004). Moreover, lower phytate phosphorous content of corn co-product ensures the bioavailability of phosphorous in the feed and consequently minimizes nutrient loading via decreasing phosphorous excretion into environment (Fallahi et al., 2012).

Whole corn kernel is fermented in dry milling industry (Belyea et al., 2004, Weigel et al., 2005, Wu et al., 1997) while wet milling industry use steeping and fractionation steps to separate different components such as germ, fiber, gluten protein and starch (Kim et al., 2008, Weigel et al., 2005, Wu et al., 1997). Therefore wet milling industry separates protein rich gluten from starch before being fermented to produce ethanol whereas in the dry milling industry co-product recovery is done after the process of fermentation (Anderson and Lamsal, 2011, Han and Liu,

2010). Due to differences in the production process of wet milling and dry milling, corn co-products not only vary in the quantity but also the quality of proteins.

Several researches have been conducted to evaluate the potential application of certain corn co-products to replace fishmeal in aqua-feed, but comparison of wet milling and dry milling corn co-product as fishmeal alternatives is not yet reported. Although the corn co-products are used in aqua-feed formulations, many practical constraints prevails the utilization of high percentage of corn based ingredients. For example, high content of yellow xanthophylls in corn co-products has potential to alter the color of either fish flesh or skin or may be both. Commercial CGM contains xanthophylls ranging from 224 to 550 mg/kg on a dry matter basis (Wright, 1987). Even though the pigmentation provides value addition to ornamental fish and certain food fish such as trout and salmon, it could possibly reduce the consumer preference of white fish such as channel cat fish, *Ictalurus punctatus* (Li et al., 2007). However, information on the comparison of fishmeal replacement in Nile tilapia diets using different corn co-products of corn milling industry are scared to our knowledge. Further, the effect of corn co-products on fillet quality of Nile tilapia is remained unclear.

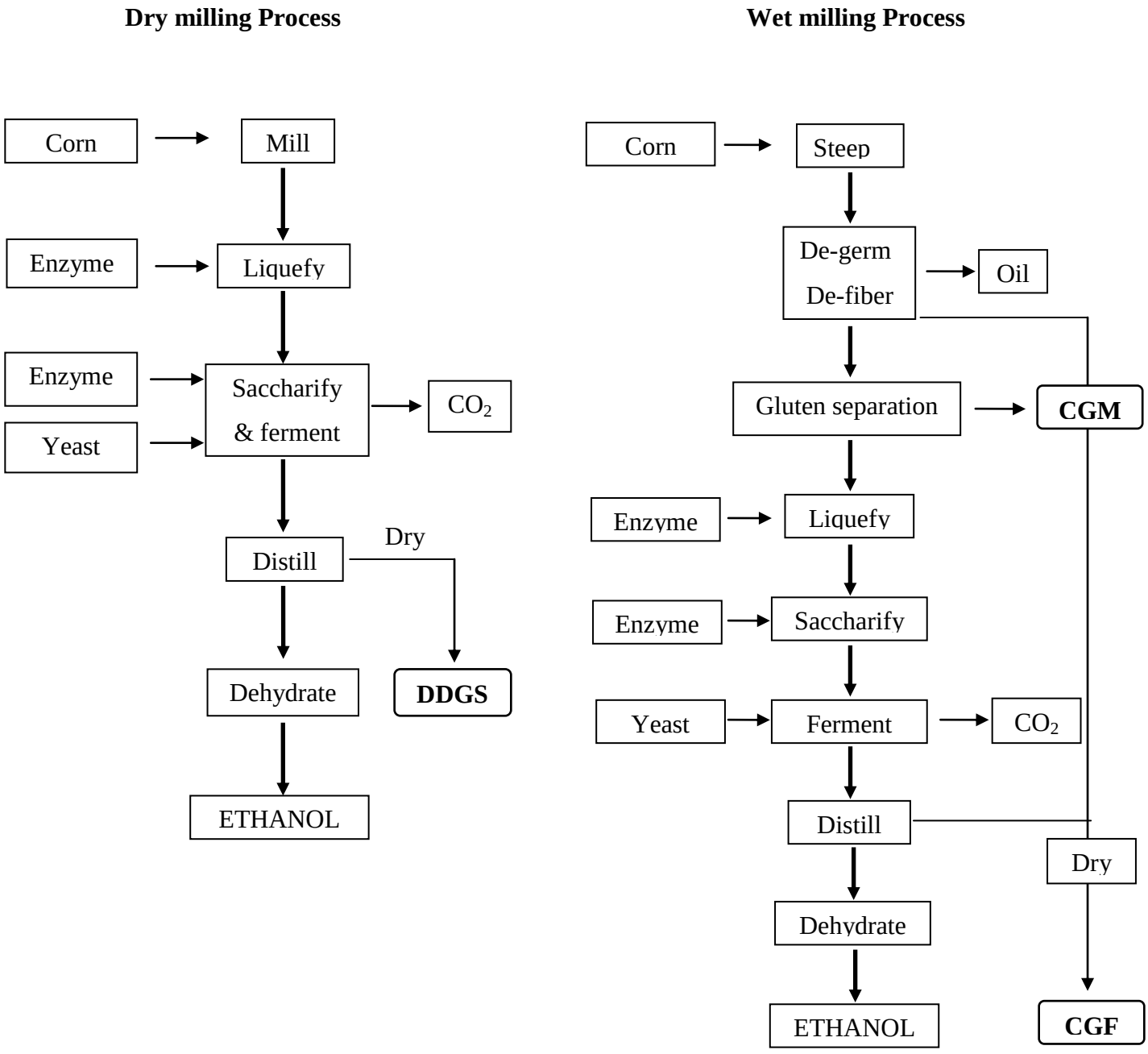


Figure 2: Corn ethanol production process.

DDGS; distillers dried grains with soluble, CGM; corn gluten meal, CGF; corn gluten feed

References

Agaba, K.M., Tocher, D.R., Zheng, X., Dickson, C.A., Dick, J.R. & Teale, A.J. (2005) Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. Comp Biochem Physiol, 142.

Ahmad, M.H., Abdel-Tawwab, M. & Khattab, Y.A. (2004) Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (Oreochromis *niloticus* L.) with different initial body weights In The sixth international symposium on tilapia in aquaculture, Manila, Philippine, pp. 249-263.

Al Hafedh, Y. (1999) Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* L. Aquaculture Research, 30, 385-393.

Azaza, M.S., Khiari, N., Dhraief, M.N., Aloui, N., Kraïem, M.M. & Elfeki, A. (2015) Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. Aquaculture Research, 46, 14-27.

Barrows, F.T. & Sealey, W.M. Feed Ingredients and the Reduction of Dependence on Marine Harvested Products for Aquaculture Feeds.

Bothast, R. & Schlicher, M. (2005) Biotechnological processes for conversion of corn into ethanol. Applied microbiology and biotechnology, 67, 19-25.

Boyd, C.E., Tucker, C., McNevin, A., Bostick, K. & Clay, J. (2007) Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. Reviews in Fisheries science, 15, 327-360.

Chou, B.-S.& Shiau, S.-Y. (1996) Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromisaureus*. Aquaculture, 143, 185-195.

Coyle, S.D., Mengel, G.J., Tidwell, J.H. & Webster, C.D. (2004) Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research, 35, 365-370.

Dato-Cajegas, C.R.S. & Yakupitiyage, A. (1996) The need for dietary mineral supplementation for Nile tilapia, *Oreochromis niloticus*, cultured in a semi-intensive system. Aquaculture, 144, 227-237.

De Silva, S. & Radampola, K. (1990) Effect of dietary protein level on the reproductive performance of *Oreochromis niloticus* In The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, pp. 559-564.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2003) Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 220, 619-632.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2005) Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 248, 187-196.

El-Sayed, A.-F.M. & Teshima, S.-i. (1992) Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. Aquaculture, 103, 55-63.

El‐Saidy, D.M. & Gaber, M. (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‐Saidy, D.M. & Gaber, M. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research, 34, 1119-1127.

El‐Sayed, A.F. (1998) Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. Aquaculture Research, 29, 275-280.

FAO, U. (2014) FAOstat. Retrieved Feb, 2014.

Fontainhas-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.

Justi, K., Hayashi, C., Visentainer, J., De Souza, N. & Matsushita, M. (2003) The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. Food Chemistry, 80, 489-493.

Ng, W.-K., Chong, C.-Y., Wang, Y. & Romano, N. (2013) Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. Aquaculture, 372, 97-110.

Olsen, R.E., Henderson, R.J. & McAndrew, B. (1990) The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by *Tilapia* (*Oreochromis*) *nilotica* in vivo. Fish Physiology and Biochemistry, 8, 261-270.

Pullin, R.S. & Lowe-McConnell, R.H. (1982) The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy, WorldFish.

Richter, N., Siddhuraju, P. & Becker, K. (2003) Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, 217, 599-611.

Santiago, C.B. & Lovell, R.T. (1988) Amino acid requirements for growth of Nile tilapia.The journal of nutrition, 118, 1540-1546.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J. & Tocher, D.R. (1995) Requirement criteria for essential fatty acids. J Appl Ichthyol, 11.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids In Fish nutrition (Halver, J.E. ed.). Academic Press, San Diego, California.

Schaeffer, T.W., Brown, M.L., Rosentrater, K.A. & Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co‐products by Nile tilapia.Journal of animal physiology and animal nutrition, 94, e348-e354.

Shiau, S.-Y.(2002) Tilapia, *Oreochromis* spp. Nutrient Requirements and Feeding of Finfish for Aquaculture, 273-292.

Shiau, S.-Y., Chuang, J.-L.& Sun, C.-L. (1987) Inclusion of soybean meal in tilapia (*Oreochromis niloticus*× *O. aureus*) diets at two protein levels. Aquaculture, 65, 251-261.

Siddiqui, A.Q., Howlader, M.S. & Adam, A.A. (1988) Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. Aquaculture, 70, 63-73.

Soliman, A., Jauncey, K. & Roberts, R. (1994) Water‐soluble vitamin requirements of tilapia: Ascorbic acid (vitamin C) requirement of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture Research, 25, 269-278.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285, 146-158.

Tadesse, Z., Boberg, M., Sonesten, L. & Ahlgren, G. (2003) Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish *Oreochromis niloticus* L.–A laboratory study. Aquatic Ecology, 37, 169-182.

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.

Tocher, D.R., Agaba, M., Hastings, N., Bell, J.G., Dick, J.R. & Teale, A.J. (2002) Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). Fish Physiol Biochem, 24.

Twibell, R.G. & Brown, P.B. (1998) Optimal Dietary Protein Concentration for Hybrid Tilapia *Oreochromis niloticus* × *O. aureus* Fed All‐Plant Diets. Journal of the world aquaculture Society, 29, 9-16.

Wang, K.W., Takeuchi, T. & Watanabe, T. (1985) Effect of dietary protein levels on growth of Tilapia nilotica.Bulletin of the Japanese Society of Scientific Fisheries (Japan).

Wu, Y.V., Rosati, R., Sessa, D.J. & Brown, P. (1994) Utilization of protein-rich ethanol co-products from corn in tilapia feed. Journal of the American Oil Chemists' Society, 71, 1041-1043.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1996) Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. Journal of Agricultural and Food Chemistry, 44, 1491-1493.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1997) Use of corn-derived ethanol coproducts and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. Journal of Agricultural and Food Chemistry, 45, 2174-2177.

Xu, Z., Lin, X., Lin, Q., Yang, Y. & Wang, Y. (2007) Nitrogen, phosphorus, and energy waste outputs of four marine cage-cultured fish fed with trash fish. Aquaculture, 263, 130-141.

Yan, B., Wang, Z.-H.& Zhao, J.-L. (2013) Mechanism of osmoregulatory adaptation in tilapia. Molecular biology reports, 40, 925-931

CHAPTER 3

Chapter 3. Potential application of corn co-products for complete fishmeal replacement in Nile tilapia diets

Abstract

A 12-week feeding trial was conducted to evaluate the effects of total fishmeal replacement with different corn co-products on growth performance, feed utilization efficiency and body composition in juvenile Nile tilapia (*Oreochromis niloticus*). Five iso-nitrogenous and iso-energetic diets were prepared. Fifty per cent of the protein they contained was obtained from fishmeal (control diet) or from one of four corn co-products, namely corn protein concentrate, corn gluten meal, high-protein distillers' dried grains (HPDDG) or distillers' dried grains with solubles (DDGS) (experimental diets). Fish with an initial mean weight of 4.5 g were fed one of the five diets twice a day to near satiety. Significantly higher ($P < 0.05$) specific growth rates and survival occurred in fish fed the control diet or DDGS, followed by HPDDG; mean feed intakes by fish in these three groups were significantly greater than those in the others. Food conversion ratio, protein efficiency ratio, and total amino acid content of fillets were not affected by changes in dietary ingredients. Fillet protein content was highest in the HPDDG group, and lipid content was highest in those fed DDGS. DDGS can be used to fully replace the fishmeal component of Nile tilapia diets.

3.1. Introduction

The search for alternatives to replace the fishmeal component of aqua-feeds has received a good deal of attention, resulting in considerable research progress, over the last couple of decades. A vast array of proteins from both plant and animal sources have been evaluated widely for their suitability for partial or total replacement of fishmeal in aqua-feeds (Lu *et al.*, 2015, Plaipetch and Yakupitiyage, 2014, Prachom *et al.*, 2013, Richter *et al.*, 2003, Schaeffer *et al.*, 2010, Wu *et al.*, 1994). Among the tested ingredients, industrial by-products or co-products that are considered unsuitable for direct human consumption have received much interest (Barrows and Sealey, Xu *et al.*, 2007). In this context, protein-rich co-products of the corn-milling industry play an important role as protein sources for the manufacture of animal feeds, including aqua-feeds.

Corn gluten meal (CGM) and corn protein concentrate (CPC) are co-products of the corn wet-milling industry, and CGM has been used widely in aqua-feeds (Pereira and Oliva‐Teles, 2003). Distillers' dried grains with solubles (DDGS) and high-protein distillers' dried grains (HPDDG) are co-products of corn dry-milling industries for fuel ethanol production. HPDDG is a relatively new product of the corn ethanol industry, and its nutritional value is much more consistent than that of DDGS (Robinson and Li, 2008). Unlike other, conventional, plant protein sources such as soybean meal and cotton seed meal, corn co-products are free from anti-nutritional factors(Li *et al.*, 2008, Robinson and Li, 2008, Shiau *et al.*, 1987)and only few amino acid deficiencies have been reported (Barrows and Sealey, Cheng and Hardy, 2004). Moreover, the low phytate phosphorus content of corn co-products ensures the bioavailability of phosphorus in the feed formula and consequently reduces water pollution by minimizing phosphorus excretion into aquatic environments (Fallahi *et al.*, 2012). However, similarities as well as

dissimilarities exist among different corn co-products owing to differences in the wet-milling and dry-milling processes.

Aquaculture of tilapia—a group of fish with herbivorous or omnivorous feeding habits—is the most widespread in the world and is second only to carp aquaculture in terms of global production (FAO, 2014). Dietary substitution of fishmeal with an alternative protein source in herbivorous or omnivorous species is considerably easier than in carnivorous species, which are nutritionally more demanding (Tacon and Metian, 2008).

Because tilapia can utilize a high percentage of dietary plant ingredients (Twibell and Brown, 1998), co-products of the corn-milling industry such as CGM and DDGS have been tested in their diets, with varying degree of success (Coyle et al., 2004, Schaeffer et al., 2010, Tidwell et al., 2000, Wu et al., 1994). However, total fishmeal replacement with a single corn co-product has not yet been evaluated in this fish, and to our knowledge no attempt has been made to compare multiple corn co-products as fishmeal alternatives in a single growth trial. Our objectives here were to 1) compare the effects of total fishmeal replacement with various single corn co-products in the diet of Nile tilapia, *Oreochromis niloticus*, juveniles, on growth performance, feed utilization efficiency, and fillet nutritional quality; and 2) determine the most suitable corn co-product for use in a zero-fishmeal diet for these fish.

3.2. Materials and Methods

3.2.1. **Experimental diets**

Five iso-nitrogenous (32% protein) experimental diets (fishmeal-based control diet and four corn co-product-based diets) were formulated to fulfill the known nutritional requirements of juvenile Nile tilapia. Proximate composition of ingredients and ingredient composition of diets are given in Table 3 and Table 4 respectively. In the control diet, 50% of the protein was

supplied by fishmeal. In the other four diets, the fishmeal component of the diet was fully replaced with one of four corn co-products, namely corn protein concentrate (CPC), corn gluten meal (CGM), high-protein distillers' dried grains (HPDDG) or distillers' dried grains with soluble (DDGS). The calculated lysine and methionine contents of the corn-based diets (calculation was based on amino acid composition of ingredient) were insufficient to meet the essential amino acid requirements of Nile tilapia(Santiago and Lovell, 1988), and therefore crystalline amino acids were used to compensate for this deficiency. The proximate composition and amino acid composition of the experimental diets is presented in Table 5. Before feed preparation, the ingredients were ground to pass through a mesh (0.5 μm) screen and then mixed by using a horizontal mixer. The mixture was then moistened by adding distilled water (~20%) and pelleted to appropriate sizes and freeze-dried. Diets were stored at 5 ˚C until use. The names of the five diets were designated according to the main protein ingredients, namely control (FM), CPC, CGM, HPDDG and DDGS.

Table 3.Proximate composition (gkg^{-1}) of protein sources used in experimental diets fed Nile tilapia *Oreochromis niloticus* (n=3)

Ingredient	Dry matter	Protein	Lipid	Ash
FM	918	682	100	113
CPC	901	798	95.8	16
CGM	933	658	130	18
HPDDG	904	461	37.7	38
DDGS	896	314	77.1	66
SBM	934	458	21	63
PBM	877	69	106	101

FM, fish meal (anchovy); CPC, corn protein concentrate, Empyreal 75®, Cargill Corn Milling; CGM, corn gluten meal; HPDDG, high protein distillers dried grains, Dakota Gold ® BFRAC™, SBM, soybean meal; PBM, poultry by product meal

Table 4.Ingredient composition (gkg^{-1}) of experimental diets fed Nile tilapia *Oreochromis niloticus*

Ingredient	**Control**	**CPC**	**CGM**	**HPDDG**	**DDGS**
Fish meal	218	0	0	0	0
Corn co-product	0	194	235	332	524
Soybean meal	162	166	168	188	211
Poultry byproduct meal	51	53	52	52	71
Wheat flour	312	329	302	225	56
Alpha starch	190	176	163	121	86
Vitamin mix	10	10	10	10	10
Mineral mix	16	16	16	16	16
Soy oil	41	44	44	46	18
Crystalline amino acids	0	12	10	10	8

CPC; corn protein concentrate based diet, CGM; corn gluten meal based diet, HPDDG; high protein distillers dried grain based diet, DDGS; distillers dried grains with soluble based diet

Vitamin mixture composition (unit kg^{-1}): Vitamin D3, 2 420 000 IU; Vitamin K3, 6050mg; thiamine, 3025 mg; riboflavin, 3630 mg; pyridoxine, 2420 mg; cyanocobalamine, 6 mg; L-ascorbic acid, 368 900mg; nicotinic acid, 24 200mg; D-pantothenic acid, 6050mg; inositol, 121 000mg; d-biotin, 363 mg; folic acid, 908 mg; para-aminobenzoic acid 3025 mg.

Mineral mixture composition (g/kg^{-1}) : Sodium chloride 50; magnesium sulphate 745; iron(lll) citrate n-hydrate 125; trace element mix 50; cellulose 30 [the trace element mixture contains(g/kg^{-1}) Zinc sulphateheptahydrate 353; manganese sulphate 162; copper (ll) sulphate pentahydrate 31; aluminium chloride hexahydrate 10; cobalt chloride 3; potassium iodate 1; cellulose 440].

Table 5.Proximate and amino acid composition of experimental diets (gkg^{-1}) fed Nile tilapia *Oreochromis niloticus* (n=3)

Components	Control	CPC	CGM	HPDDG	DDGS
Dry matter	968	963	964	961	961
Protein	319	323	321	321	315
Lipid	88.7	97.2	97.4	88.9	98.4
Ash	76	38.7	34.4	45.2	56.5
EAA					
Arginine	14.8	9.1	10.5	14.1	13.9
Histidine	7.5	3.9	4.4	6.0	5.7
Isoleucine	6.8	5.6	6.5	8.5	7.7
Leucine	17.7	19.8	23.2	23.6	22.8
Lysine	15.0	11.8	12.5	16.6	14.6
Methionine	5.3	5.9	6.1	7.7	8.1
Phenylalanine	11.0	9.9	11.7	12.5	11.6
Threonine	10.3	6.8	8.0	11.0	9.9
Tryptopan	1.0	0.7	0.8	1.0	0.6
Valine	8.4	5.7	7.5	10.4	9.5
NEAA					
Alanine	15.4	12.5	14.9	16.1	15.6
Aspartic acid	25.5	15.4	18.3	24	21.9
Cysteine	2.2	2.0	2.2	3.3	3.1
Glutamic acid	45.6	40.6	47	48.5	45
Glycine	16.3	8.4	10.1	12.8	12.6
Serine	13.0	10.4	12.2	14.4	13.2
Tyrosine	8.1	7.6	9.3	10.1	9.5

3.2.2. **Experimental fish and rearing condition**

Nile tilapia fingerlings were obtained from the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Tokyo, Japan. Before the start of the experiment, the fish were acclimated to the experimental conditions for 2 weeks. During this acclimation period, fish were hand fed to near satiety twice a day by using the control diet. After the acclimatization period food was withheld for 24 h, after which 200 fish with similar initial body weights (4.0 to 4.5 g) were selected for potential use in the experiment. Groups of 18 fish were bulk weighed and stocked into each glass tank (60 L). Five treatments, one for each diet (control, CPC, CGM, HPDDG and DDGS) in duplicate was randomly allocated to ten (2 x 5) glass tanks. Each tank was connected to a freshwater re-circulating system in which the water temperature was maintained at 28 ± 1 ˚C by using electrical heaters (immersion heaters). The water flow rate was maintained at 0.5 L min –1, and aeration was provided continuously to each tank via submerged air stones. Approximately 50% of the water in the re-circulating system was replaced with de-chlorinated tap water once every 2 weeks. Fish were hand fed to near satiety twice a day, 6 days a week for 12 weeks, and daily feed intakes were recorded.

3.2.3. **Data collection and sampling**

The 20 fish remaining from the initial stock of 200 were sacrificed by using an overdose of 2-phenoxyethanol and kept at –30 ˚C for initial carcass analysis. During the 12-week experiment, the fish in each tank were bulk weighed at 3-week intervals. At the end of the experiment, fish were starved for 24 h and anaesthetized with 0.2% 2-phenoxyethanol before being handled. The weight and length of the fish in each tank were individually measured with an electrical balance and a measuring board, respectively, to the nearest first decimal point.

Five average-size fish from each tank were euthanized and used to calculate the hepatosomatic index (HSI), viscerosomatic index (VSI) and fillet yield (FY). Four fish from each treatment and the fillet samples used for fillet yield determination were immediately frozen at –30 ˚C for final chemical analysis. Frozen samples were minced, freeze-dried and kept at –30 ˚C until analysis.

Daily weight gain (DWG g day–1), percentage specific growth rate (SGR, %), food conversion ratio (FCR), survival rate (SR, %), protein efficiency ratio (PER),thermal growth coefficient (TGC), HSI, VSI, fillet yield and the coefficient of condition (K) were calculated by using the following equations to compare fish growth, nutrient utilization efficiency, and body indices among treatments.

DWG = [final weight (FW) – initial weight (IW)] / time

SGR = (ln FW – ln IW) / time x 100

FCR = feed intake (dry basis) / wet weight gain

SR = number of fish at harvest / number of fish stocked x 100

PER = body weight gain (g)/ protein intake (g)

TGC = [(FW 1/3 – IW 1/3) / (water temperature) x no. of days)] x 1000

HSI = 100 x wet liver weight / g wet body weight

VSI = 100 x wet visceral weight / wet body weight

Fillet yield = 100 x fillet weight/ wet body weight

K = 100 x mean weight (g) / (total length (cm)) 3

3.2.4. **Chemical analysis**

Feed ingredients, diets and carcass and muscle samples were analyzed in accordance with standard procedures for chemical analysis (AOAC 1990). All the samples were finely ground

and analyzed in triplicate. Dry matter content was calculated from the weight loss after drying of the sample at 105 ˚C until it reached a constant weight. Ash content was determined after the incineration of samples in a muffle furnace at 550 ˚C for 16 h. The Kjeldhal method was used for crude protein analysis (N content x N factor). Crude lipid content was determined by the gravimetric method after extraction of the lipids into a mixture of chloroform and methanol (2:1 v/v).

Amino acid content was analyzed by using an automatic amino acid analyzer (JLC-500/v; JEOL, Tokyo, Japan). For total amino acids, samples were digested at 1100 ˚C for 22 h with 4 M methanosulphonic acid (Sigma-Aldrich, St. Louis, MO, USA); the digested solution was then passed through a 0.45-µm membrane filter and injected into the analyzer. For free amino acids, samples were deproteinised with 3% sulphosalicylic acid (Wako Pure Chemical Industries, Tokyo, Japan) and the mixture was centrifuged at 12 000g for 15 min at 4 ˚C. The supernatant was decanted and passed through a 0.45-µm membrane filter; it was then injected with ninhydrin into an automatic amino acid analyzer for detection of free amino acids.

3.2.5. **Statistical analysis**

Data were initially checked for normality and equal variance by using Levene's test for equality of variances. The effects of different corn co-products on Nile tilapia growth performance, feed utilization efficiency, body indices and fillet quality were compared in a one-way analysis of variance (ANOVA) at the 5% significance level ($P < 0.05$). When ANOVA was found to be significant, Tukey's multiple range tests was used to detect differences among treatments. All statistical analyses were performed with SPSS version 16.0.

3.3. Results

3.3.1. **Growth performances and feed utilization efficiencies**

The initial wet weights of fish assigned to different treatments (4.0g- 4.5g) were not statistically different ($p > 0.05$). Final wet weight gain of fish in this experiment was significantly affected by the treatment and the pattern of weight gain over the experimental period is presented in Figure 3. According to the growth curve, differences in growth rates between fish fed the various dietary treatments were first observed after six weeks. However individual growth curve of each treatment showed the exponential growth curve.

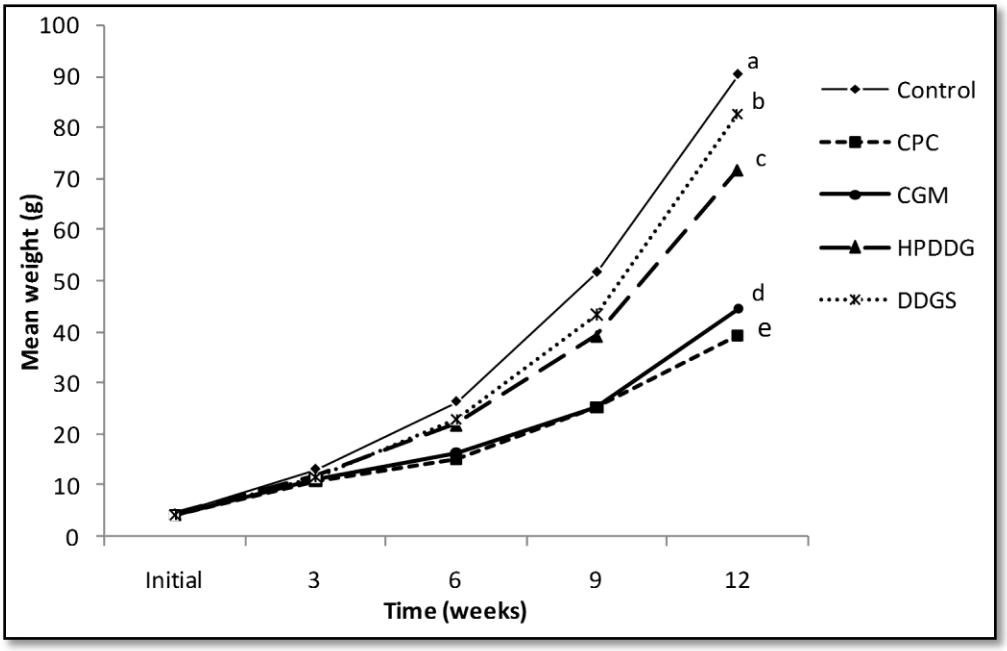


Figure 3.Mean weight of fish over experimental period (n=2, different letters indicate significant differences, $p < 0.05$)

Final wet weight gain of fish in five treatments was significantly different from each other. The highest weight gain was observed in control followed by DDGS and the lowest wet weight gain was observed in CPC treatment. Among the corn based test diets, DDGS showed

significantly greater value for weight gain followed by HPDDG. Weight gain of DDGS was almost double compared to CPC. Weight of fish in CPC and CGM treatment was almost overlapped until 9th week and at the end of the experiment CGM reported significantly higher weight gain compared to CPC. Growth performance and feed utilization efficiencies of fish are shown in Table 6.

Table 6.Growth performances and feed utilization efficiencies of Nile tilapia fed diets containing corn co-products over 12 weeks (mean ± SD,n=2)

	Control	CPC	CGM	HPDDG	DDGS	p
% SGR	3.56± 0.01^{a}	2.63± 0.01^{d}	2.75± 0.20 ^{c}	3.30± 0.03^{b}	3.53± 0.06^{a}	*
TGC	1.21 ± 0.01 ^{a}	0.76 ± 0.01^{e}	0.81 ± 0.01^{d}	1.06 ± 0.01^{c}	1.16 ± 0.00^{b}	*
FI (g)	84.05± 0.78^{a}	38.80± 0.28^{b}	40.2± 0.14^{b}	71.05± 9.85^{a}	81.20± 1.20^{a}	*
FCR	1.00 ±0.00	1.10 ±0.00	1.00 ±0.00	1.05 ±0.15	1.05 ±0.07	ns
PER	3.20± 0.01	2.84± 0.11	3.10± 0.08	2.99± 0.50	3.06± 0.06	ns
Protein retention (%)	49.62 ± 0.13^{a}	38.42 ± 0.78^{c}	42.02±1.63^{bc}	46.17±2.91^{ab}	46.70±0.81^{ab}	*
% Survival	100.0± 0.0^{a}	75.0± 4.0^{c}	66.6± 7.8^{c}	80.6± 3. 9^{bc}	97.2± 3.9^{ab}	*

Mean values in same row withdifferent superscripts are statistically different ($p < 0.05$)

% SGR: percentage specific growth rate;TGC : thermal growth coefficient; FI, feed intake; FCR: food conversion ratio; PER: protein efficiency ratio; ns : not significant, *: $p < 0.05$)

Specific growth rate (% SGR) of control and DDGS is significantly higher than that of others and lowest was observed in CPC followed by CGM. Mean feed intake was significantly highest in control, DDGS and HPDDG. Feed utilization efficiencies in terms of food conversion ratio (FCR) and protein efficiency ratio (PER) was independent from the dietary treatments. Survival rate of fish was significantly affected by the treatment. The CPC, CGM and HPDDG showed statistically similar and lower survival rate than that of control. However survival rate of DDGS was not different either from control or HPDDG.

3.3.2. **Whole body and fillet proximate composition**

Proximate composition of whole body and fillet is presented in Table 7. Moisture content of whole body was not influenced by the different diets having different corn co-products or fish meal as the main protein source. Significantly higher crude protein content was observed in control, HPDDG and DDGS which showed higher feed intake and growth rate than that of other two treatments. Whole lipid content of all the corn based significantly higher than that of control. Ash content was also greatly affected by the dietary treatments and it was significantly highest in control followed by DDGS.

Table 7.Proximate composition of whole body and fillet (% wet basis) of Nile tilapia fed experimental diets over 12 weeks (Mean ± SD, n=3)

Treatment	Moisture	Protein	Lipid	Ash
Whole body				
Control	69.4 ± 1.6	15.5 ± 1.1^{b}	8.5 ±0. 6^{b}	6.9 ±0. 5^{a}
CPC	71.6 ±0.8	13.9 ±0.3^{d}	9.6 ±1.3^{a}	5.0 ±0. 1^{d}
CGM	70.9 ± 1.0	14. 6±1.0^{c}	9.8.5±2.9^{a}	4.0 ±0. 4^{e}
HPDDG	68.9 ± 0.7	16. 7± 1.9^{a}	9.9 ±3.6^{a}	5.4 ±1.2^{c}
DDGS	69.7 ± 1.2	15.4 ± 0.3^{b}	10.0 ±0. 4^{a}	5.7 ±0. 4^{b}
Fillet				
Control	78.2 ±1.2	18.8 ±0. 6^{b}	1.6 ±0. 2^{c}	1.4 ±0.5
CPC	78.5 ± 1.6	18.7 ±2.8^{b}	1.9 ±0. 1^{bc}	1.4 ±0. 4
CGM	77.9 ±0.8	19.2 ±2.4^{b}	2.2 ± 0.8^{b}	1.3 ±0.1
HPDDG	76.2 ±1.3	19.8 ±1.2^{a}	2.4 ±0.5^{b}	1.2±0.7
DDGS	77.2 ±0.6	18.3 ±0. 9^{b}	3.08±0.8^{a}	1.3±0.1

Mean values in same row with different superscripts are statistically different ($p < 0.05$)

The data for proximate analysis of fillet samples reflects that there are no treatment effects on percentage moisture and ash. In both whole body carcass and fillet, crude protein content in HPDDG is significantly higher than others while the crude lipid in fillet was highest in DDGS. However, the amount of protein in whole body or fillet of control was not different from DDGS which showed almost similar growth performances. In general, all the corn based treatment had significantly higher lipid content compared to fishmeal based control and whole body ash component in control was highest among treatments. Total amino acid composition of fish fillet at the end of 12 week period is presented in Table 8.

According to the results; total amino acid composition of fillet was independent from the treatment. Morphometry indices and haematocrit value of different treatments is presented in Table 9. Differences in all the evaluated morphometry indices, i.e; viscerosomatic index, hepatosomatic index, fillet yield, heamatocrit value and coefficient of condition (K) of fish in different dietary group were not statistically significant. The results of this study suggested that feed ingredients does not affect on the viscerosomatic index, hepatosomatic index, fillet yield, coefficient of condition and haematocrit value of Nile tilapia juveniles.

Table 8.Whole body amino acid composition (gkg^{-1}wet basis) of Nile tilapia fed experimental diets over 12 weeks (Mean ± SD, n=3).

Amino acid	Control	CPC	CGM	HPDDG	DDGS	p value
EAA						
Arginine	10.3 ± 1.2	9.1 ± 0.5	10.2 ± 2.2	10.8 ± 0.1	10.2 ± 1.2	0.747
Histidine	3.4 ± 0.4	2.9 ± 0.1	3.4 ± 0.8	3.4 ± 0.1	3.4 ± 0.5	0.758
Isoleucine	5.1± 0.8	4.4 ± 0.1	5.8 ± 1.3	4.5 ± 0.3	5.4 ± 0.6	0.443
Leucine	11.5 ± 1.4	10.6 ± 0.4	12.4 ± 2.8	11.2 ± 0.5	11.8 ± 1.8	0.695
Lysine	10.2 ± 1.1	9.4 ± 1.3	10.8 ± 2.3	11.0 ±0.1	10.5 ± 1.5	0.774
Methionine	3.5 ± 0.4	3.1 ± 0.2	4.0 ± 0.9	3.2 ± 0.1	3.7 ± 0.4	0.467
Phenylalanine	6.2 ± 0.8	5.2 ± 0.4	6.6 ± 1.4	5.6 ± 0.4	6.4 ± 1.1	0.586
Threonine	7.7 ± 0.9	6.6 ± 0.8	8.1 ± 1.8	7.3 ± 0.5	7.8 ± 1.2	0.690
Valine	6.2 ± 0.9	5.4 ± 0.7	6.8 ± 1.6	5.7 ± 1.4	6.4 ± 0.6	0.537
NEAA						
Alanine	12.2 ± 1.3	10.5 ± 0.4	11.8 ± 2.5	12.8 ± 0.3	11.9 ± 1.6	0.642
Aspartic acid	16.2 ± 1.8	14.2 ± 0.1	13.4 ± 2.6	16.4 ± 0.5	16.3 ± 2.4	0.405
Cysteine	0.8 ± 0.1	0.6 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.195
Glutamic acid	24.0 ± 2.6	21.0 ± 0.1	24.6 ± 5.3	24.7 ± 0.4	23.8 ± 3.3	0.748
Glycine	15.4 ± 1.9	12.2 ± 1.1	13.4 ± 2.9	15.8 ± 0.6	14.0 ± 1.3	0.334
Serine	7.7 ± 0.9	6.6 ± 0.1	7.9 ± 1.6	7.6 ± 0.2	7.8 ± 1.0	0.688
Taurine	2.1 ± 0.1	2.8 ± 0.8	3.0 ± 0.7	3.8 ± 0.2	2.7 ± 0.1	0.151
Tyrosine	5.0 ± 0.6	4.4 ± 0.4	5.4 ± 1.3	4.4 ± 0.4	5.3 ± 0.8	0.591

Table 9.Body indices of Nile tilapia fed diets containing corn co-products over 12 weeks ((Mean ± SD, n=10)

Morphometry index	Control	CPC	CGM	HPDDG	DDGS
VSI	10.8± 0.5	12.8± 1.4	12.1± 0.5	11.6± 0.9	12.9± 1.6
HSI	3 .0 ± 0.2	2 .0 ± 0.8	2 .2 ± 1.4	2.1 ± 0.1	2.7 ± 0.7
FY	30.4 ±2.6	28.3 ±1.1	31.9 ±2.6	30.8 ±1.1	32.4 ±1.9
K	2.0 ± 0.1	1.9± 0.1	1.8± 0.1	2.0± 0.2	2.0± 0.1

VSI, viscerosomatic index = 100 x visceral weight (g)/ body weight(g); HSI, hepatosomatic index = 100 x liver weight (g)/body weight (g); FY, fillet yield = 100 x fillet weight (g) /body weight (g) ; K, coefficient of condition = 100 x body weight (g)/ total length $(cm)^3$

3.4. Discussion

Growth in terms of the weight of juvenile fish usually follows a sigmoid curve, as we observed here in Nile tilapia. Although fish in all treatments had similar growth patterns, growth rates differed among all treatments. Growth of fish is a function of feed intake and capacity of fish to utilize ingested food(Carter et al., 2001). Since the feed intake in this study showed significant differences among treatments, the observed variations in growth rate were likely explained by the factors affected on feed intake.

Dietary availability of essential nutrients such as amino acids is a factor which determine the feed intake of fish(De la Higuera, 2001).Therefore differences in feed intake in this study can be explained by the dietary essential amino acid composition. Even if diets were formulated to fulfill the essential amino acid requirement of Nile tilapia (Santiago and Lovell, 1988)(estimated using values in(Prachom *et al.*, 2013)), analytical data showed that some amino acids such as threonine and valine are deficient in CPC and CGM diets. Essential amino acid composition of control, HPDDG and DDGS was almost similar except histidine and those were the treatment group which showed higher growth performances and feed intakes compared to CPC and CGM.

Feed intake of fish is known to reduce when dietary essential nutrients are deficient(De la Higuera, 2001, Gómez-Requeni *et al.*, 2004). Therefore differences in amino acid compositions in diets are the most plausible responsible factor for observed variation in feed intakes and thereby for differences in growth rate of fish as previously observed for Nile tilapia (Wu *et al.*, 1997).

The % SGRs of fish in the control, DDGS and HPDDG groups were similar to those reported for Nile tilapia of similar size by (He *et al.*, 2014, Lim *et al.*, 2007)but slightly higher than those observed by (Likongwe *et al.*, 1996). Even though the % SGRs were lower in the CPC and CGM treatments than in the other treatments, our values were consistent with the reported values in the literature for Nile tilapia of similar size reared at 28 ˚C in fresh water(Likongwe *et al.*, 1996, Fontainhas-Fernandes *et al.*, 1999).

Feed utilization efficiencies in terms of FCR and PER were independent of the treatment type despite the large differences in growth parameters. These findings agreed with those of a previous study(Wu *et al.*, 1997, He *et al.*, 2014).However, dietary ingredients seemed to affect on protein retention of this study and values are almost similar to what was reported in literature(Furuya *et al.*, 2004). Fish can utilize organic macronutrients such as protein, lipid and carbohydrate as a direct metabolic fuel or store for utilization in later date or deposit in the structural materials which represent as growth (Jobling, 2001). Protein retention in this study showed close relationship with weight gain of fish and indicated the differences in nutrient retention are partly responsible for differences of growth. Although it was difficult to distinguish statistical differences of protein retention among corn based treatments, fishmeal based control which had highest final body weight showed the higher protein retention compared to that of CPC which had lowest final weight. However difficulties in finding differences in feed

utilization parameters among corn based treatment groups which were significant for growth parameters may be due to low statistical power in this study which arises with limited number of replicates used.

The whole body and fillet proximate compositions of our experimental fish were affected by the dietary ingredients. However whole body and fillet composition of corn based diets was equal to or higher than that in the control. This result suggests that complete replacement of fishmeal in the tilapia diet with corn co-products does not negatively affect the final nutritional quality of fish products. Because the total amino acid content of the whole body did not differ significantly among treatments, complete replacement of fishmeal with corn co-products had no negative impact even from the perspective of essential amino acids. Our fillet yield varied in a narrow range (28% to 32%) and was not affected by treatment. This indicates that Nile tilapia are capable of converting corn co-products and other ingredients into edible muscle mass at rates similar to those on a fishmeal-based diet.

3.5. Conclusion

Total fishmeal replacement with different corn co-products had different effects on the growth performance and proximate composition of the fish's whole body and fillets. Among the corn co-products tested, DDGS was the best choice for a zero fishmeal diet for Nile tilapia juveniles. Because we focused here only on the juvenile stage of Nile tilapia with duplicate, a long-term growth trial with more replicates and balanced amino acid profiles in diets should be done to confirm the effects of corn co-products on the growth performance and fillet quality of marketable-size fish.

References

Abdel-Tawwab, M., Abdel-Rahman, A.M. & Ismael, N.E.M. (2008) Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, Oreochromis niloticus (L.) challenged in situ with Aeromonas hydrophila. *Aquaculture,***280,** 185-189.

Agaba, K.M., Tocher, D.R., Zheng, X., Dickson, C.A., Dick, J.R. & Teale, A.J. (2005) Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. *Comp Biochem Physiol,***142**.

Ahmad, M.H., Abdel-Tawwab, M. & Khattab, Y.A. (2004) Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (Oreochromis niloticus L.) with different initial body weights In *The sixth international symposium on tilapia in aquaculture, Manila, Philippine*, pp. 249-263.

Al Hafedh, Y. (1999) Effects of dietary protein on growth and body composition of Nile tilapia, Oreochromis niloticus L. *Aquaculture Research,***30,** 385-393.

Azaza, M.S., Khiari, N., Dhraief, M.N., Aloui, N., Kraïem, M.M. & Elfeki, A. (2015) Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, Oreochromis niloticus L., in response to dietary starch to protein ratios. *Aquaculture Research,***46,** 14-27.

Barrows, F.T. & Sealey, W.M. Feed Ingredients and the Reduction of Dependence on Marine Harvested Products for Aquaculture Feeds.

Bothast, R. & Schlicher, M. (2005) Biotechnological processes for conversion of corn into ethanol. *Applied microbiology and biotechnology*,**67,** 19-25.

Boyd, C.E., Tucker, C., McNevin, A., Bostick, K. & Clay, J. (2007) Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. *Reviews in Fisheries science,***15,** 327-360.

Cheng, Z.J. & Hardy, R.W. (2004) Nutritional Value of Diets Containing Distiller's Dried Grain with Solubles for Rainbow Trout, Oncorhynchus mykiss. *Journal of Applied Aquaculture,***15,** 101-113.

Cho, C. & Bureau, D. (2001) A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture research,***32,** 349-360.

Coyle, S.D., Mengel, G.J., Tidwell, J.H. & Webster, C.D. (2004) Evaluation of growth, feed utilization, and economics of hybrid tilapia, Oreochromis niloticus× Oreochromis aureus, fed diets containing different protein sources in combination with distillers dried grains with solubles. *Aquaculture Research,***35,** 365-370.

Dato-Cajegas, C.R.S. & Yakupitiyage, A. (1996) The need for dietary mineral supplementation for Nile tilapia, Oreochromis niloticus, cultured in a semi-intensive system. *Aquaculture,***144,** 227-237.

De la Higuera, M. (2001) Effects of nutritional factors and feed characteristics on feed intake. *Food Intake in Fish,***827,** 250-268.

De Silva, S. & Radampola, K. (1990) Effect of dietary protein level on the reproductive performance of Oreochromis niloticus In *The Second Asian Fisheries Forum. Asian Fisheries Society, Manila*, pp. 559-564.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2003) Effects of dietary protein level on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock reared at different water salinities. *Aquaculture,***220,** 619-632.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2005) Effects of dietary lipid source on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock reared at different water salinities. *Aquaculture,***248,** 187-196.

El-Sayed, A.-F.M. & Teshima, S.-i. (1992) Protein and energy requirements of Nile tilapia, Oreochromis niloticus, fry. *Aquaculture,***103,** 55-63.

El‐Saidy, D.M. & Gaber, M. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, Oreochromis niloticus (L.) diets. *Aquaculture Research,***34,** 1119-1127.

El‐Sayed, A.F. (1998) Total replacement of fish meal with animal protein sources in Nile tilapia, Oreochromis niloticus (L.), feeds. *Aquaculture Research,***29,** 275-280.

Fallahi, P., Rosentrater, K.A. & Muthukumarappan, K. (2012) Twin-Screw Extrusion Processing of Rainbow trout (Oncorhynchus mykiss) Feeds Using Graded Levels of High Protein Corn-Based Distillers Dried Grains (HP-DDG) and Conventional Distillers Dried Grains with Solubles (DDGS).

FAO, U. (2014) FAOstat. *Retrieved Feb,***2014**.

Fontainhas-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.

Furuya, W.M., Pezzato, L.E., Barros, M.M., Pezzato, A.C., Furuya, V.R. & Miranda, E.C. (2004) Use of ideal protein concept for precision formulation of amino acid levels in fish‐meal‐free diets for juvenile Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research,***35,** 1110-1116.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å. & Nelson, R. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research,***38,** 551-579.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J., Médale, F., Martin, S., Houlihan, D., Kaushik, S. & Pérez-Sánchez, J. (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (Sparus aurata). *Aquaculture,***232,** 493-510.

He, J.-y., Han, B., Tian, L.-x., Yang, H.-j., Zeng, S.-l. & Liu, Y.-j. (2014) The sparing effect of cystine on methionine at a constant TSAA level in practical diets of juvenile Nile tilapia Oreochromis niloticus. *Aquaculture Research,* n/a-n/a.

Henderson, R.J. & Tocher, D.R. (1987) The lipid composition and biochemistry of freshwater fish. *Progress in lipid research,***26,** 281-347.

Jobling, M. (2001) Feed composition and analysis. *Food Intake in Fish,***827,** 1-24.

Justi, K., Hayashi, C., Visentainer, J., De Souza, N. & Matsushita, M. (2003) The influence of feed supply time on the fatty acid profile of Nile tilapia (Oreochromis niloticus) fed on a diet enriched with n-3 fatty acids. *Food Chemistry,***80,** 489-493.

Kamal, A.H.M.M. & Mair, G.C. (2005) Salinity tolerance in superior genotypes of tilapia, Oreochromis niloticus, Oreochromis mossambicus and their hybrids. *Aquaculture,***247,** 189-201.

Li, L., Hao, S., Diao, S., Cen, J., Yang, X., Shi, H. & Chen, S. (2008) PROPOSED NEW COLOR RETENTION METHOD FOR TILAPIA FILLETS (O. NILOTICUS ♀ × O. AUREUS ♂) BY EUTHANATIZING WITH REDUCED CARBON MONOXIDE. *Journal of Food Processing and Preservation,***32,** 729-739.

Likongwe, J.S., Stecko, T.D., Stauffer Jr, J.R. & Carline, R.F. (1996) Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia Oreochromis niloticus (Linneaus). *Aquaculture,***146,** 37-46.

Lim, C., Garcia, J.C., Yildirim-Aksoy, M., Klesius, P.H., Shoemaker, C.A. & Evans, J.J. (2007) Growth Response and Resistance to Streptococcus iniae of Nile Tilapia, Oreochromis niloticus, Fed Diets Containing Distiller's Dried Grains with Solubles. *Journal of the World Aquaculture Society,***38,** 231-237.

Lu, F., Haga, Y. & Satoh, S. (2015) Effects of replacing fish meal with rendered animal protein and plant protein sources on growth response, biological indices, and amino acid availability for rainbow trout Oncorhynchus mykiss. *Fisheries Science,***81,** 95-105.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000) Effect of aquaculture on world fish supplies. *Nature,***405,** 1017-1024.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J. & Hua, K. (2009) Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences,***106,** 15103-15110.

Ng, W.-K., Chong, C.-Y., Wang, Y. & Romano, N. (2013) Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. *Aquaculture,***372,** 97-110.

Olsen, R.E., Henderson, R.J. & McAndrew, B. (1990) The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by Tilapia (Oreochromis) nilotica in vivo. *Fish Physiology and Biochemistry,***8,** 261-270.

Pereira, T. & Oliva‐Teles, A. (2003) Evaluation of corn gluten meal as a protein source in diets for gilthead sea bream (Sparus aurata L.) juveniles. *Aquaculture Research,***34,** 1111-1117.

Plaipetch, P. & Yakupitiyage, A. (2014) Effect of replacing soybean meal with yeast‐fermented canola meal on growth and nutrient retention of Nile tilapia, Oreochromis niloticus (Linnaeus 1758). *Aquaculture Research,***45,** 1744-1753.

Prachom, N., Haga, Y. & Satoh, S. (2013) Impact of dietary high protein distillers dried grains on amino acid utilization, growth response, nutritional health status and waste output in juvenile rainbow trout (Oncorhynchus mykiss). *Aquaculture Nutrition,***19,** 62-71.

Pullin, R.S. & Lowe-McConnell, R.H. (1982) *The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy,* WorldFish.

Richter, N., Siddhuraju, P. & Becker, K. (2003) Evaluation of nutritional quality of moringa (Moringa oleifera Lam.) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus L.). *Aquaculture,***217,** 599-611.

Robinson, E.H. & Li, M.H. (2008) Replacement of soybean meal in channel catfish, Ictalurus punctatus, diets with cottonseed meal and distiller's dried grains with solubles. *Journal of the World Aquaculture Society,***39,** 521-527.

Santiago, C.B. & Lovell, R.T. (1988) Amino acid requirements for growth of Nile tilapia. *The journal of nutrition,***118,** 1540-1546.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J. & Tocher, D.R. (1995) Requirement criteria for essential fatty acids. *J Appl Ichthyol,***11**.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids In *Fish nutrition* (Halver, J.E. ed.). Academic Press, San Diego, California.

Schaeffer, T.W., Brown, M.L., Rosentrater, K.A. & Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co‐products by Nile tilapia. *Journal of animal physiology and animal nutrition,***94,** e348-e354.

Shiau, S.-Y. (2002) Tilapia, Oreochromis spp. *Nutrient Requirements and Feeding of Finfish for Aquaculture,* 273-292.

Shiau, S.-Y., Chuang, J.-L. & Sun, C.-L. (1987) Inclusion of soybean meal in tilapia (Oreochromis niloticus× O. aureus) diets at two protein levels. *Aquaculture,***65,** 251-261.

Siddiqui, A.Q., Howlader, M.S. & Adam, A.A. (1988) Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, Oreochromis niloticus. *Aquaculture,***70,** 63-73.

Soliman, A., Jauncey, K. & Roberts, R. (1994) Water‐soluble vitamin requirements of tilapia: Ascorbic acid (vitamin C) requirement of Nile tilapia, Oreochromis niloticus (L.). *Aquaculture Research,***25,** 269-278.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture,***285,** 146-158.

Tadesse, Z., Boberg, M., Sonesten, L. & Ahlgren, G. (2003) Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish Oreochromis niloticus L.–A laboratory study. *Aquatic Ecology,***37,** 169-182.

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.

Tocher, D.R., Agaba, M., Hastings, N., Bell, J.G., Dick, J.R. & Teale, A.J. (2002) Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (Danio rerio) and tilapia (Oreochromis niloticus). *Fish Physiol Biochem,***24**.

Twibell, R.G. & Brown, P.B. (1998) Optimal Dietary Protein Concentration for Hybrid Tilapia Oreochromis niloticus× O. aureus Fed All‐Plant Diets. *Journal of the world aquaculture Society,***29,** 9-16.

Wang, K.W., Takeuchi, T. & Watanabe, T. (1985) Effect of dietary protein levels on growth of Tilapia nilotica. *Bulletin of the Japanese Society of Scientific Fisheries (Japan).*

Wu, Y.V., Rosati, R., Sessa, D.J. & Brown, P. (1994) Utilization of protein-rich ethanol co-products from corn in tilapia feed. *Journal of the American Oil Chemists' Society,***71,** 1041-1043.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1996) Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. *Journal of Agricultural and Food Chemistry,***44,** 1491-1493.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1997) Use of corn-derived ethanol coproducts and synthetic lysine and tryptophan for growth of tilapia (Oreochromis niloticus) fry. *Journal of Agricultural and Food Chemistry,***45,** 2174-2177.

Xu, Z., Lin, X., Lin, Q., Yang, Y. & Wang, Y. (2007) Nitrogen, phosphorus, and energy waste outputs of four marine cage-cultured fish fed with trash fish. *Aquaculture,***263,** 130-141.

Yan, B., Wang, Z.-H. & Zhao, J.-L. (2013) Mechanism of osmoregulatory adaptation in tilapia. *Molecular biology reports*,**40,** 925-931.

CHAPTER 4

Chapter 4. Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*

Abstract

A 24-week feeding trial was conducted with Nile tilapia *Oreochromis niloticus* to evaluate the effects of long-term feeding of corn co-product–based diets on growth, fillet color, and fillet fatty acid and amino acid composition. Five iso-nitrogenous diets were prepared (34% protein). The control diet included 10% fishmeal. Fishmeal was eliminated from the other four diets, and 50% of the dietary protein was supplied by one of four corn co-products, namely high-protein distillers' dried grains (HPDDG), distillers' dried grains with solubles (DDGS), corn gluten meal (CGM), and corn protein concentrate (CPC). Fish with an initial mean weight of 21 g were fed one of the five diets twice a day to near satiety. Fish fed the control, HPDDG, or DDGS diet had significantly higher ($P < 0.05$) mean weight gain, specific growth rates, mean feed intake, protein efficiency ratio, and survival than those fed the other diets. Fish in these three treatments also had the lowest food conversion ratio. The dietary treatments did not affect the lightness, redness, yellowness, or crude protein and total amino acid content of fish fillets. Fillet lipid and ash content were highest in the CGM group. Fillet fatty acid composition was greatly affected by the dietary treatments. The CGM and CPC groups had significantly highest palmitic acid (16:0) and total saturated fatty acid levels, whereas linoleic acid (18:2n-6) and total polyunsaturated fatty acid levels were highest in the DDGS group. Similarly, the total n-6 level was highest in the DDGS group, followed by HPDDG. The total n-3 levels and n-3: n-6 ratios in the fillets of the control group were almost double those of the corn-based dietary groups. Our results suggest that dietary inclusion of HPDDG or DDGS in non-fishmeal diets at up to 50% of

dietary protein does not negatively affect growth performance, feed utilization efficiency, or fillet color and amino acid composition, but further improvement of n-3 fatty acid composition is necessary to ensure human health benefits.

4.1. Introduction

In response to the rising demand and cost of fishmeal, the use of dietary alternatives is becoming a common practice in aqua-feed formulations. Among the categories of alternatives, plant-based ingredients have long received great attention (Gatlin et al., 2007, Hansen et al., 2006, Olsen et al., 2007, Vilhelmsson et al., 2004). Corn co-products such as corn gluten meal (CGM), distillers' dried grains with solubles (DDGS), and high-protein distillers' dried grains (HPDDG) are among the plant-based ingredients rich in digestible proteins. Unlike conventional plant protein sources such as soybean meal, these co-products are free from anti-nutritional factors (Robinson and Li, 2008, Shiau et al., 1987) and deficient in only a few amino acids (Cheng and Hardy, 2004). Yellow pigments inherent in the corn, however, limit the proportion of corn co-products that is acceptable for use in feeding food fishes. For instance, xanthophylls in corn-based ingredients could alter the fish fillet color (Gatlin et al., 2007).

Fillet color is an important sensory attribute of food fish that directly determines the acceptability of the product (Dhanapal et al., 2013, Gatlin et al., 2007, Ross, 2000). It is also among the attributes of fish quality that could easily be altered by changes in the pigments in dietary ingredients (Gatlin et al., 2007, Skonberg et al., 1998). The effects of dietary pigments on fillet color have been reported in rainbow trout and some other food fish species, including salmon (Akhtar et al., 1999, Buttle et al., 2001, Li et al., 2007, Skonberg et al., 1998). Changes in flesh color caused by feed ingredients can have a negative effect on sale if the resulting product does not meet consumers' expectations (Park et al., 1997, Skonberg et al., 1998). Pigmentation or coloration is considered a desirable trait in certain food fishes such as trout and salmon (Li et al., 2007), whereas the same attribute is highly undesirable in some white-fleshed fish such as channel catfish (Li et al., 2011, Li et al., 2007) and tilapia (Girao et al., 2012).

Even though fillet color is among the attributes that could be strongly influenced by the pigments present in the protein source (Gatlin et al., 2007), it has been among the criteria that have received the least attention during the evaluation of alternatives for fishmeal. A variety of corn-based ingredients have been tested in tilapia (Coyle et al., 2004, Schaeffer et al., 2010, Suprayudi et al., 2015, Tidwell et al., 2000, Wu et al., 1997, Wu et al., 1996, Wu et al., 1994); almost all of the studies have focused primarily on evaluating the fishes' growth performance and feed utilization efficiencies. To our knowledge, not a single nutritional study has evaluated the effects of the pigments in corn-based ingredients on the fillet color of Nile tilapia.

Another important area that needs to be addressed in evaluating alternative ingredients in fish feeds is the nutritional profile of the resulting fish flesh. Fish is a good source of long-chain n-3, highly unsaturated fatty acids (HUFA), essential amino acids, and micronutrients such as vitamins and minerals, and this unique nutritional composition has led to a rising demand for fish and fish products worldwide (Dergal et al., 2013, Vagner and Santigosa, 2011). Moreover, fish is a source of affordable, quality protein for people in developing countries, including the world's most populous countries (such as China, India, Indonesia, Pakistan, and Bangladesh), which are home to the bulk of the world's undernourished people (FAO, 2014). Compared to fishmeal, the plant-based ingredients used as protein sources for aqua-feeds contain relatively low levels of n-3 polyunsaturated fatty acids (PUFA) (Gatlin et al., 2007, Ng et al., 2013) and are poor in certain essential amino acids (Gatlin et al., 2007, Olsen et al., 2007).

Our objectives here, therefore, were to evaluate the effects of long-term feeding of zero-fishmeal diets containing different corn co-products on the growth performance, fillet color, and fillet fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*.

4.2. Materials and Methods

4.2.1.1. Experimental diets

Five iso-nitrogenous (34% protein) experimental diets (fishmeal-based control diet and four corn co-product–based diets) were formulated to fulfill the known nutritional requirements of juvenile Nile tilapia. The proximate compositions of the main protein sources and the ingredient compositions of the diets are given inTable 10. Proximate compositions together with amino acid and fatty acid compositions of the diets are given in Table 11andTable 12, respectively. The control diet included 10% fishmeal. Fishmeal was eliminated from the other four diets, and 50% of the dietary protein was supplied by one of four corn co-products, namely high-protein distillers' dried grains (HPDDG), distillers' dried grains with solubles (DDGS),corn gluten meal (CGM), or corn protein concentrate (CPC).Crystalline amino acids(L-lysine,DL-methionine and L-tryptophan)were used to compensate for the essential amino acid deficiencies in the corn-based diets (Table 11). Before they were pelletized, the ingredients were ground to pass through a screen (0.5-µm mesh) and then mixed by using a horizontal mixer. The mixture was then moistened by adding distilled water (~20%), pelletized and freeze-dried in a vacuum freeze-drier (RLE-206; Kyowa Vacuum Tec, Saitama, Japan). Diets were stored at 5 °C until use. The five diets were named according to the main protein ingredients, namely control (FM), HPDDG, DDGS, CGM, and CPC.

4.2.1.2. Experimental fish and rearing conditions

Nile tilapia fingerlings were obtained from the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Tokyo, Japan. Fish were acclimated to the experimental conditions for 2 weeks before the start of the experiment. During this acclimation

period, fish were hand-fed the control diet to near satiety twice a day. After the acclimatization period, food was withheld for 24 h, after which 200 fish with similar initial body weights (21.0 to 22.0 g) were selected for the experiment. Groups of 18 fish were bulk weighed and stocked into 10 glass tanks (60 L). Five treatments in duplicate were randomly allocated to the 10 tanks. Each tank was connected to a freshwater re-circulating system in which the water temperature was maintained at 25 ± 1 °C by using electric heaters. The water flow rate was maintained at 0.5 L min^{-1} and aeration was provided continuously to each tank via submerged airstones. Approximately 50% of the water in the re-circulating system was replaced with de-chlorinated tap water once every 2 weeks. Fish were hand-fed to near satiety twice a dayfor 24 weeksand daily feed intakes were recorded.

4.2.2. **Data collection and sampling**

During the 24-week experiment, the fish in each tank were bulk-weighed at 4-week intervals. At the end of the experiment, fish were starved for 24 h and anaesthetized with 0.2% 2-phenoxyethanol before being handled. The weight and length of the fish in each tank were individually measured with an electric balance and a measuring board, respectively, to the nearest first decimal place.

Five fish from each tank were euthanized and used to calculate the hepatosomatic index (HSI), viscerosomatic index (VSI), intra-peritoneal fat (IPF) ratio, and fillet yield (FY). The fillet samples used for fillet yield determination were immediately frozen at –30 °C for final chemical analysis. Frozen samples were minced in a centrifugal mill (Model ZM 1; Retsch, Haan, Germany), freeze-dried in a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tec), and kept at –30 °C until analysis.

Table 10.Proximate composition of main ingredients[1]and formulation of experimental diets[1]for Nile tilapia, *Oreochromis niloticus* (gkg^{-1}dry matter, *n*=3)

Component	**Fishmeal**	**HPDDG**	**DDGS**	**CGM**	**CPC**
Dry matter	918	904	896	933	901
Crude protein	682	461	314	658	798
Crude lipid	100	37.7	77.1	103	95.8
Crude Ash	113	38	66	41	16
Ingredient	**Control**	**HPDDG**	**DDGS**	**CGM**	**CPC**
Fishmeal	100	0	0	0	0
Corn co-product	0	332	524	235	194
Soybean meal	278	188	211	168	166
Poultry by-product meal	104	52	71	52	53
Wheat flour	308	225	56	302	329
Alpha starch	152	121	86	175	176
Vitamin mix[2]	10	10	10	10	10
Mineral mix[3]	16	16	16	16	16
Soy oil	41	46	18	32	44
L-lysine	0	5	4	6	7
DL-methionine	0	4	5	3	4
L-tryptophan	0	1	1	1	1

[1]HPDD,high-protein distillers' dried grain/HPDDG-based diet; DDGS, distillers' dried grains with solubles/DDGS-based diet; CGM, corn gluten meal/CGM-based diet;CPC, corn protein concentrate/CPC-based diet

[2]Vitamin mixture composition (unit kg^{-1}): Vitamin D3, 2,420,000 IU; Vitamin K3, 6050mg; thiamine, 3025 mg; riboflavin, 3630 mg; pyridoxine, 2420 mg; cyanocobalamin, 6 mg; L-ascorbic acid, 368,900mg; nicotinic acid, 24,200mg; D-pantothenic acid, 6050mg; inositol, 121,000mg; d-biotin, 363 mg; folic acid, 908 mg; para-aminobenzoic acid, 3025 mg

[3]Mineral mixture composition (gkg^{-1}): Sodium chloride, 50; magnesium sulfate, 745; iron(III) citrate n-hydrate, 125; cellulose, 30; trace-element mixture, 50.The trace-element mixture contained (gkg^{-1}) zinc sulfateheptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminum chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose, 440.

Table 11.Proximate and amino acid composition of experimental diets for Nile tilapia, *Oreochromis niloticus* (g kg^{-1}dry matter, *n*=3)

Component	**Control**	**HPDDG**	**DDGS**	**CGM**	**CPC**
Dry matter	947.3	976.5	949.0	967.4	965.3
Crude protein	342.9	348.7	350.9	336.4	343.2
Crude lipid	78.3	66.8	69.6	93.8	87.8
Ash	68.6	34.4	38.7	55.6	45.2
EAA[1]					
Arginine	11.7	13.7	12.1	11.0	10.2
Histidine	4.1	4.2	3.9	3.6	3.4
Isoleucine	5.7	5.8	6.2	8.3	5.7
Leucine	15.8	22.6	21.8	29.4	25.7
Lysine	12.0	16.0	13.1	13.1	14.0
Methionine	4.6	5.2	5.2	5.1	5.3
Phenylalanine	10.0	12.9	12.4	15.2	13.9
Threonine	7.2	11.2	9.5	10.5	8.8
Tryptophan	0.9	1.0	1.1	1.0	1.0
Valine	7.5	8.4	7.7	10.6	8.0
NEAA[2]					
Alanine	9.0	17.9	16.4	19.2	18.0
Aspartic acid	24.9	26.4	22.5	21.2	20.6
Cysteine	1.6	3.0	2.8	2.6	2.4
Glutamic acid	52.6	60.8	54.2	65.6	63
Glycine	14.6	15.2	13.0	11.7	11.5
Serine	6.6	14.7	12.5	13.1	13.0
Tyrosine	6.8	10.9	9.8	11.8	10.6

[1]EAA, essential amino acids; [1]NEAA, non-essential amino acids

Table 12.Fatty-acid composition of experimental diets (area %, *n*=3)

Fatty acid[1]	Control	HPDDG	DDGS	CGM	CPC
14:0	1.13	0.15	0.15	0.16	0.18
16:0	16.58	15.08	15.41	16.79	14.67
18:0	3.85	3.34	2.74	2.99	3.33
18:1n-9	23.05	23.91	24.97	22.71	23.77
18:2n-6	42.06	49.72	50.60	49.80	49.16
18:3n-3	3.74	4.08	2.61	3.45	3.90
20:1n-9	0.02	0.12	0.02	0.22	0.03
20:4n-6	0.29	0.05	0.11	0.09	0.10
20:5n-3	1.19	ND[32]	ND	0.11	0.03
22:6n-3	0.98	0.07	0.08	ND	0.04
Σ saturates	21.56	18.57	18.30	19.94	18.18
Σ monoenes	27.11	26.31	27.03	25.20	26.34
Σ PUFA[43]	48.66	53.91	53.41	53.49	53.28
n-3:n-6	0.15	0.08	0.05	0.07	0.08

[1]Some fatty acids are not shown (16:1n-7,18:1n-7, 18:4n-3, 20:1n-11, 20:4n-3, 22:1n-11, 22:1n-13, 22:4n-9, 22:5n-3); [2]ND, not detectable; [3]PUFA, polyunsaturated fatty acids

Mean weight gain (MWG, g), percentage specific growth rate (SGR, %), mean feed intake (MFI,g), food conversion ratio (FCR), percent survival (SR, %), protein efficiency ratio (PER), HSI, VSI, IPF ratio, FY, and the coefficient of condition (K) were calculated by using the following equations to compare fish growth, nutrient utilization efficiency, and body indices among treatments.

MWG (g) = [final weight (FW, g) – initial weight (IW, g)]

SGR (%) = (ln FW – ln IW)/(time, days)× 100

MFI(g) = total feed intake of fish (g)/number of fish

FCR = feed intake (g)/weight gain (g)

SR (%)= number of fish at harvest/number of fish stocked × 100

PER = bodyweight gain (g)/protein intake (g)

HSI = 100 × liver weight (g)/bodyweight (g)

VSI = 100 × visceral weight (g)/bodyweight (g)

IPF = 100 × weight of peritoneal fat (g)/bodyweight (g)

Fillet yield = 100 × fillet weight (g)/bodyweight (g)

K = 100 ×body weight (g)/(total length [cm])^3

4.2.3. **Colorimetric analysis of fillets**

Fillet color was assessed instrumentally by using a tristimulus colorimeter (Chroma meter, model CR 13; Minolta, Osaka, Japan), which compares the reflectance of light from an object (fish fillet) with that of a standard calibration plate (number 12456). Lightness (L*, negative for blackness and positive for whiteness), red–green chromaticity (a*, negative for greenness and positive for redness),and yellow–blue chromaticity (b*, negative for blueness and positive for yellowness) were measured for each fillet. Five fish per tank (right-side fillet of each fish) were used for the instrumental colorimetric analysis. The colorimetric values of the white muscle in five places (three dorsal, two ventral) in a single fillet were analyzed and the average value was used for calculations. Values were obtained from the same area of the fillet for all fish analyzed. Values for a* and b*, representing redness and yellowness respectively, were used to calculate the hue and chroma. Hue is the relationship between redness and yellowness, which is expressed as $H_{ab}° = \tan^{-1}(b^*/a^*)$(Hunt, 1977).Hue is an angular measurement of color where 0°and 90° denote red and yellow hues, respectively. Chroma is expressed as $C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$ and gives information about the clarity and intensity of the color. The total color difference between the control and corn-based treatments is given by ΔE, which is calculated as $\Delta E = (\Delta L^{*2} + \Delta a^{*2} +$

$\Delta b^{*2})^{1/2}$ where ΔL*, Δa*and Δb* refer to the differences between the control and corn-based treatments.

4.2.4. **Chemical analysis**

Feed ingredients, diets, and muscle samples were analyzed in accordance with standard procedures for chemical analysis (AOAC, 2005).All samples were finely ground and analyzed in triplicate. Dry-matter content of each sample was calculated from the weight loss after drying at 105 °C to a constant weight. Ash content was determined after the incineration of samples in a muffle furnace at 550 °C for 16 h. The Kjeldhal method was used for crude protein analysis (N content ×6.25).Total lipids in the samples were extracted after homogenization in a chloroform:methanol mixture (2:1, v:v) according to the method of Folch et al. (1957).

For fatty acid extraction, lipids were saponified with 1 mL 50% KOH in 15 mL100% ethanol at 80 °C for 60 min. The saponifiable matter was then esterified with 7% boron trifluoride (BF_3) in methanol (Morita Chemical Industries Co., Ltd., Osaka, Japan) and heated for 20 min at 80 °C. Fatty acid methyl ester (FAME) preparations were made up in hexane (20 mg/mL) and analyzed by using a gas chromatograph (GC) (GC-2025; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector and a silica capillary column (L × I.D., 30 m × 0.32 mm, df 0.25 μm; Supelcowax 10 Fused Silica Capillary GC column; Supelco, Bellefonte, PA, USA).Helium was used as the carrier gas and the thermal program was set to increase the column temperature from 170 to 260 °C at 2 °Cmin^{-1}. The temperature in both the injection port and the detector was adjusted to 260 °C. FAME peaks were identified by comparing the retention time of each with that of the appropriate FAME standard. Amino acid content was analyzed by using an automatic amino acid analyzer (JLC-500/v; JEOL, Tokyo, Japan).For total amino acids, samples were digested at 110 °C for 22 h with 4 M methanosulphonic acid (Sigma-

Aldrich, St. Louis, MO, USA); the digested solution was then passed through a 0.45-µm membrane filter and injected into the automatic amino acid analyzer.

4.2.5. **Statistical analysis**

Data were initially checked for normality and equal variance by using Levene's test. The effects of different corn co-products on Nile tilapia growth performance, feed utilization efficiency, body indices, and fillet quality were compared in a one-way analysis of variance (ANOVA) at the 5% significance level ($P< 0.05$). When ANOVA was found to be significant, Tukey's multiple range test was used to detect differences among treatments. Statistical analysis of fillet fatty acid composition (area %) was performed on arcsine-transformed data. All statistical analyses were performed with SPSS version 16.0.

4.3. Results

4.3.1. **Growth performance and feed utilization efficiencies**

The experimental diets affected growth performance and feed utilization efficiencies of fish (Table 13). Compared with the control, CGM and CPC showed significantly lower MWG, % SGR, and MFI. These responses in the HPDDG and DDGS treatments were not different from those in the control. The significantly lowest percent survival was observed in CPC; there were no significant differences in survival between the other treatments. Feed utilization efficiencies in terms of FCR and PER were also affected by the diet treatments. Fish fed HPDDG and DDGS performed equally well in terms of FCR and PER compared with fish fed the control diet based on fishmeal. The highest FCRs and lowest PERs were observed in the CGM and CPC groups.

Table 13.Growth performance and feed utilization efficiencies of Nile tilapia fed experimental diets for 24 weeks (*n*=2)

Parameter[1]	Control	HPDDG	DDGS	CGM	CPC	*P*-value
MWG (g)	162.2 ±16.8[a]	160.7 ±6.4[a]	161.4 ±10.0[a]	88.3 ±20.9[b]	74.9± 5.2[b]	0.005
%SGR	1.27 ±0.04[a]	1.26± 1.11 [a]	1.27 ±0.03[a]	0.96± 0.12[b]	0.90 ±0.03[b]	0.009
MFI ($gfish^{-1}$)	216.2±21.1[a]	222.2 ±3.5[a]	225.5± 1.2[a]	148.8±26.5[b]	124.1± 9.4[b]	0.008
FCR	1.33 ±0.01[b]	1.38± 0.07[b]	1.4 ±0.08[b]	1.72± 0.15[a]	1.66 ±0.03[a]	0.017
PER	2.31± 0.01[a]	2.12± 0.11[a]	2.30± 0.12[a]	1.69± 0.15[b]	1.68± 0.01[b]	0.004
% Survival	97.2 ±3.9[a]	97.2± 3.9[a]	97.2 ±3.9[a]	91.7 ±11.8[a]	52.7± 11.8[b]	0.010

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P*> 0.05) by the Tukey's test

[1]MWG, mean weight gain; % SGR, specific growth rate (%); MFI, mean feed intake; FCR, food conversion ratio; PER, protein efficiency ratio

Long-term feeding with corn co-product–based diets did not affect certain body indices such as the IPF ratio, VSI, and FY (Table 14). There were significant differences in the HSI among treatments. The highest and lowest HSIs were observed in the CGM and DDGS treatments, respectively (Table 14). However, the HSI in the control group was not statistically different from that in either HPDDG or CPC. Significantly highest condition factor (K) was observed in the control group (Table 14).

4.3.2. **Fillet color**

The chromatic parameters of fillets from Nile tilapia were not affected by the dietary treatments (Table 15). The lightness (L*) value ranged from 41.5 to 48.0. Even though redness (a*) and yellowness (b*) were numerically highest in the CPC and control groups, respectively, there were no observed statistical differences among treatments.

Table 14**.** Body indices of Nile tilapia fed experimental diets for 24 weeks (*n*=10)

Parameter[1]	Control	HPDDG	DDGS	CGM	CPC	*P*-value
IPF ratio	1.88±0.09	2.22 ±0.64	1.50 ±0.44	2.02 ±1.05	1.34 ±0.35	0.612
HSI	2.70 ±0.32^{b}	2.70±0.15^{b}	1.93 ±0.30^{c}	3.45 ±0.21^{a}	2.30±0.26^{bc}	0.014
VSI	9.33 ±0.92	10.92±1.27	9.44 ±0.35	11.62 ±1.06	11.50 ±0.30	0.116
Fillet yield	28.16 ±0.87	27.52±0.05	27.34 ±0.68	27.14 ±0.18	26.37 ±0.82	0.080
K	2.01 ±0.01^{a}	1.83±0.00^{c}	1.89 ±0.02^{bc}	1.94 ±0.05^{b}	1.87±0.01^{bc}	0.006

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P*> 0.05) by the Tukey's test. [1]IPF, intraperitoneal fat; HIS, hepatosomatic index; VSI, viscerosomatic index; K, condition factor

Table 15.Tristimulus color parameters of fillets from Nile tilapia fed experimental diets for 24 weeks (*n*=10)

Parameter[1]	Control	HPDDG	DDGS	CGM	CPC	*P*-value
L*	47.8 ± 3.7	48.0 ± 0.7	47.8 ± 0.6	41.5 ± 2.4	41.8 ± 0.9	0.061
a*	1.3 ± 0.3	0.7 ±0.3	1.2 ±0.2	1.8 ± 0.5	2.3 ±0.5	0.450
b*	3.2 ± 0.6	2.3 ± 0.2	2.3 ± 1.2	1.3 ± 0.7	2.2 ±0.5	0.516
Chroma	3.5 ± 0.3	2.4 ± 0.2	2.7 ± 0.9	1.9 ± 1.2	3.3 ± 0.7	0.574
Hue (°)	67.5 ± 7.6	74.0 ± 5.6	54.5 ± 18.7	53.6 ± 13.1	43.3 ± 12.1	0.501
ΔE	0	1.11	0.97	6.61	6.22	

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P*> 0.05) by the Tukey's test. [1]L*,lightness; a*, redness; b*,yellowness; chroma, intensity of color; hue angle = 0° for redness and90°foryellowness; ΔE, total color difference compared with control

4.3.3. **Nutritional quality of fillets**

The nutritional quality of fillets was determined on the basis of proximate composition, total fatty acid composition, and amino acid composition. Proximate fillet compositions are given in Table 16.

Table 16.Proximate compositions of fillets from Nile tilapia fed experimental diets for 24 weeks (% wet basis, *n*=3)

Component	Control	HPDDG	DDGS	CGM	CPC	*P*-value
Moisture	77.85±0.21	77.60± 0.14	77.35 ± 0.21	77.30 ± 0.14	77.70 ± 0.14	0.096
Crude protein	19.60 ± 0.28	19.60± 0.00	19.65 ± 0.35	19.40 ± 0.14	19.35 ± 0.21	0.637
Crude lipid	1.80 ± 0.00 ^{b}	2.05 ± 0.07^{ab}	2.20 ± 0.00 ^{ab}	2.35 ± 0.07 ^{a}	2.05 ± 0.21^{ab}	0.024
Ash	1.30 ± 0.05^{b}	1.30 ± 0.00 ^{b}	1.26 ± 0.06 ^{b}	1.60 ± 0.08^{a}	1.50 ± 0.14 ^{ab}	0.013

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different ($P > 0.05$) by the Tukey's test

There were no statistical differences in dry matter or crude protein contents of fillets from fish in the different dietary groups. CGM treatment gave the highest lipid content ($P < 0.05$) compared with the control. The other three treatments were not significantly different from either CGM or the control in terms of crude lipid content of fillets. The highest ash content was also obtained with CGM; the other treatments gave statistically similar ash contents (Table 16).

Nineteen different fatty acids were identified in fish fillets (Table 17). Linoleic acid (LA,18:2n-6), oleic acid (18:1n-9), and palmitic acid (16:0) occurred in highest proportions in all treatments; these three fatty acids collectively accounted for two-thirds of the total fatty acid pool. With the exception of two fatty acids (oleic acid [18:1n-9], and α-linolenic acid [LNA, 18:3n-3]), the fatty acid profile of the Nile tilapia fillets was significantly affected by the treatments.

Among five dietary groups, linoleic acid was significantly highest in the DDGS group, followed by the HPDDG. The highest and the lowest fillet percentages of stearic acid (18:0) were observed in CPC and DDGS, respectively. Among C-20 and C-22 PUFA, the level of arachidonic acid (ARA, 20:4n-6) was significantly higher with all of the corn-based treatments than with the control. Fillet levels of adrenic acid (22:4n-6) and decosapentaenoic acid (22:5n-6) were highest in DDGS, whereas the fillet level of adrenic acid was lowest in CGM. Fillets of both the control and CGM groups had the lowest levels of decosapentaenoic acid (22:5n-6). Interestingly, the levels of eicosapentaenoic acid (EPA, 20:5n-3), decosapentaenoic acid (DPA, 22:5n-3), and decosahexaenoic acid (DHA, 22:6n-3) were significantly highest in the fillets of the fishmeal-based control. The proportion of individual saturated fatty acids (SFA) was significantly highest in the fillets of the CGM and CPC groups, whereas the proportion of PUFA was highest in the DDGS-group fillets. The ratio of n-3 PUFA to n-6 PUFA in the fillets of the control group given fishmeal was more than double that of the fillets from the corn-based treatments. The calculated PUFA to SFA ratio was above 0.83 in all treatments.

There were no significant differences in total amino acid composition of Nile tilapia fillets among different dietary groups. Among the total amino acids, glutamic acid, aspartic acid, and lysine were found at relatively high percentages in tilapia fillets, whereas tryptophan was the least available amino acid (Figure 4).

Table 17.Fatty-acid[1]compositions of fillets of Nile tilapia fed experimental diets[2]for 24 weeks (area %, *n*=3)

Fatty acid	**Control**	**HPDDG**	**DDGS**	**CGM**	**CPC**	***P*-value**
14:0	2.02 ± 0.09[a]	1.94 ± 0.05 [a]	1.40 ± 0.02 [b]	2.29 ± 0.19 [a]	2.30 ± 0.08 [a]	0.002
16:0	21.95 ± 0.40[bc]	22.61 ± 0.29 [b]	20.19 ± 0.08 [c]	26.65 ± 1.01 [a]	25.74 ± 0.13 [a]	0.000
18:0	6.82 ± 0.02 [b]	6.35 ± 0.05 [b]	5.61 ± 0.02 [c]	6.66 ± 0.32 [b]	7.51 ± 0.00 [a]	0.000
18:1n-9	22.13 ± 0.04	22.01 ± 0.03	21.29 ± 0.84	23.04 ± 1.04	21.38 ± 0.19	0.067
18:2n-6	23.33 ± 0.07 [c]	25.41 ± 0.12 [b]	29.93 ± 0.00 [a]	21.17 ± 1.00 [d]	20.93 ± 0.05 [d]	0.000
18:3n-3	1.35 ± 0.17	1.34 ± 0.11	1.09 ± 0.09	0.91 ± 0.04	0.96 ± 0.08	0.084
20:4n-6	2.37 ± 0.09 [b]	2.92 ± 0.05 [a]	3.31 ± 0.07 [a]	3.12 ± 0.20 [a]	3.09 ± 0.03 [a]	0.000
20:5n-3	0.27 ± 0.01 [a]	0.06 ± 0.00 [d]	0.08 ± 0.00 [cd]	0.16 ± 0.00 [b]	0.11 ± 0.01 [bc]	0.000
22:4n-6	0.73 ± 0.01 [b]	0.77 ± 0.07 [ab]	0.92 ± 0.05 [a]	0.48 ± 0.05 [c]	0.71 ± 0.02 [b]	0.000
22:5n-6	1.18 ± 0.03 [d]	2.36 ± 0.13 [b]	2.70 ± 0.11 [a]	1.25 ± 0.12 [d]	2.01 ± 0.08 [c]	0.001
22:5n-3	0.96 ± 0.03 [a]	0.29 ± 0.03 [b]	0.30 ± 0.02 [b]	0.19 ± 0.00 [c]	0.34 ± 0.01 [b]	0.000
22:6n-3	3.61 ± 0.13 [a]	1.33 ± 0.09 [c]	1.30 ± 0.04 [cd]	1.02 ± 0.02 [d]	1.65 ± 0.06 [b]	0.000
Σ saturates	30.80 ± 0.47 [b]	30.90 ± 0.29 [b]	27.20 ± 0.08 [c]	35.60 ± 0.88 [a]	35.55 ± 0.04 [a]	0.000
Σ monoenes	28.99 ± 0.15	27.74 ± 0.07	26.14 ± 0.02	30.06 ± 0.94	28.05 ± 0.60	0.057
Σ PUFA	33.86 ± 0.23 [b]	34.04 ± 0.35 [b]	37.73 ± 0.06 [a]	29.70 ± 1.06 [c]	30.70 ± 0.32 [c]	0.008
n-3	6.25 ± 0.16[a]	2.98 ± 0.08[b]	2.76 ± 0.05[b]	2.28 ± 0.06[c]	3.07 ± 0.08[b]	0.000
n-6	27.61 ± 0.07[c]	31.06 ± 0.18 [b]	34.96 ± 0.11 [a]	27.42 ± 1.00 [c]	27.64 ± 0.24 [c]	0.000
n-3:n-6	0.23	0.10	0.08	0.08	0.11	
PUFA:SFA	1.10	1.10	1.39	0.83	0.86	

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P*> 0.05) by the Tukey's test; Standarderrors below 0.01 are not given. ND, not detectable; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids;[1]Some fatty acids are not shown (16:1n-7, 18:1n-7, 18:4n-3, 20:1n-9, 20:1n-11, 20:4n-3, 22:1n-11)

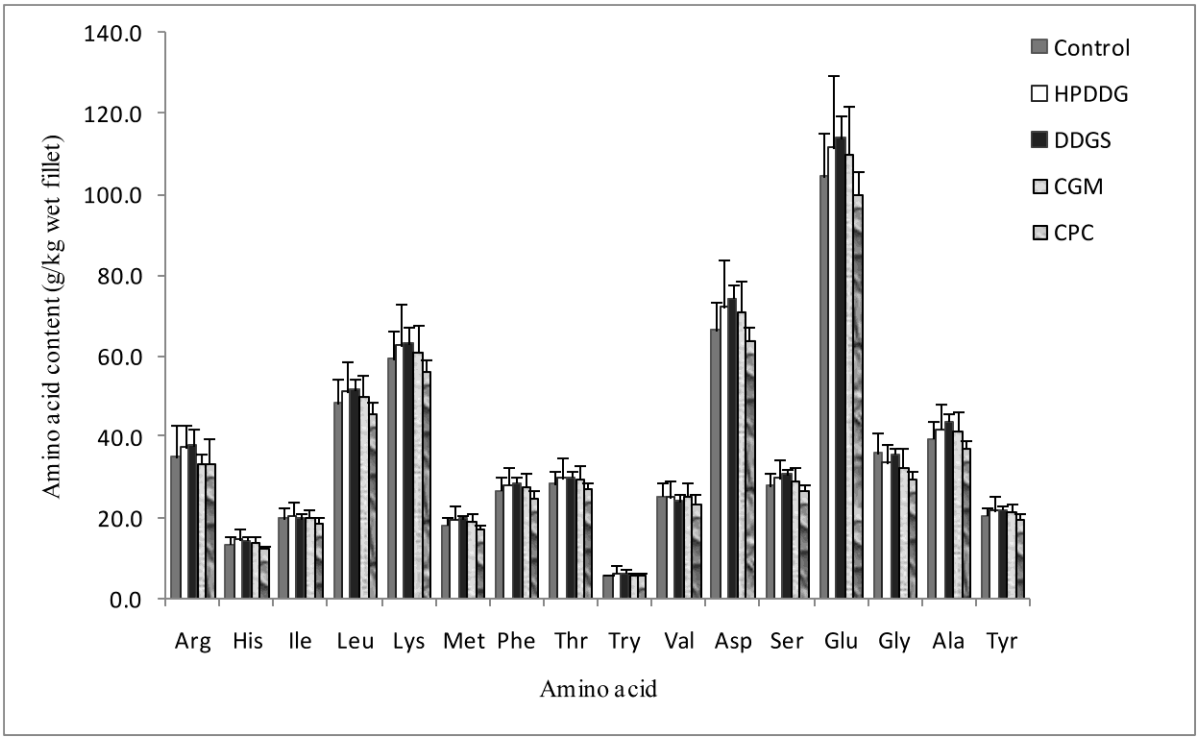


Figure 4.Total amino acid compositions of Nile tilapia fillets (n = 3).

Bars represents the standard error of the mean and values were not significantly different among dietary groups (P>0.05), Tukey's test

4.4. Discussion

The growth rates of fish were affected by the dietary treatments. Growth is a consequence of feed intake and the capacity of the fish to utilize ingested food (Carter et al., 2001). We clearly observed this relationship: the control, HPDDG, and DDGS groups showed feed intake levels higher than those of the CGM and CPC groups, and the effects of lower feed intake in the latter treatments were clearly noticed in the lower specific growth rates. The observed variations in growth rates are therefore likely explained by the differences in treatments.

Dietary availability of essential nutrients such as amino acids is an important determinant of feed intake in fish (De la Higuera, 2001). Dietary amino acid profiles in this study were not equal among treatments, and the differences in feed intake observed here can be explained by the differences in dietary amino acids. Even if the diets were formulated to fulfill the essential amino acid requirements (Santiago and Lovell, 1988) of Nile tilapia, the analytical data showed that CGM and CPC were deficient in dietary arginine and histidine. Feed intake is known to decrease when dietary essential nutrients are deficient (De la Higuera, 2001, Gómez-Requeni et al., 2004). Therefore, differences in amino acid composition among diets are, plausibly, responsible for the observed variation in feed intake and thereby for differences in growth performance.

In addition, HPDDG and DDGS, which yielded growth performances similar to the control, are co-products of a corn dry-milling process, whereas CGM and CPC are co-products of corn wet-milling. Because all four corn co-products were made from the same ingredient, the observed differences in feed intake and growth are likely attributable to difference in the corn processing method. Unlike wet-milling, dry-milling processes the whole corn kernel, which is subjected to subsequent saccharification and fermentation before the co-products are processed (Weigel et al., 2005, Weigel et al., 1997, Wu et al., 1997). Dry-milling co-products such as

HPDDG and DDGS usually contain fermentation residues, including yeast cells (*Saccharomyces cerevisiae*) (Belyea et al., 2004, Han and Liu, 2010, Weigel et al., 1997) and other unidentified nutrients formed during the fermentation–distillation process (Weigel et al., 1997). Corn wet-milling uses steeping and fractionation technology to separate the corn kernel into protein, starch, oil, and fiber components, and the protein portion is not subjected to fermentation (Weigel et al., 1997, Wu et al., 1997). Therefore, the greater feed intake and growth performance observed in the HPDDG and DDGS groups than in the CGM and CPC groups could be associated with the availability of yeast and other unidentified fermentation residues remaining in the dry-milled co-products. This hypothesis is supported by the work of Abdel-Tawwab et al. (2008), who reported improved growth performance of Nile tilapia juveniles fed diets supplemented with commercial live yeast (*S. cerevisiae*); they noted that the yeast enhanced appetite and consequently improved feed intake. Lim et al. (2007) also reported improved feed consumption and growth of Nile tilapia fed diets containing DDGS.

Although we found here that the % SGRs were lower in the CPC and CGM treatments than in the other treatments, our observations are consistent with the reported literature for Nile tilapia of similar size (Fontainhas-Fernandes et al., 1999, Likongwe et al., 1996). Our highest PERs were in the control, HPDDG, and DDGS groups, which showed higher growth rates compared to other two treatments, indicating that these groups had the most efficient feed utilization for growth. There were no significant differences in fillet yields of tilapia in the different dietary treatments; this shows that Nile tilapia are capable of converting corn co-products and other ingredients into edible muscle mass at rates similar to those when the fish are fed a fishmeal-based diet.

The lightness of Nile tilapia fillets, as measured instrumentally, has been reported as 55.2 and 52.7 by Girao et al. (2012) and Lima et al. (2015), respectively, whereas we obtained values here of 41.5 to 48.0. In contrast, the redness measured in these two earlier studies was comparable to our values. Yellowness was reported as 5.5 by Lima et al. (2015) and 9.7 by Girao et al. (2012), whereas our values ranged from 1.3 to 3.2. This indicates that dietary inclusion of corn-based ingredients does not enhance the generally observed yellowness in tilapia fillets. Xanthophyll retention in fish is a affected by a wide array of factors such as uptake, absorption, transport, metabolism and excretion (Torrissen et al., 1989). Many fish species have the ability to convert one pigment into another (Kaisuyama and Matsuno, 1988, Matsuno, 1991, Yamashita et al., 1996) and this ability varies considerably between species. The most important metabolic products of carotenoids in some animals are the retinoids; Matsuno (1991) reported the direct bioconversion of xanthophylls astaxanthin, zeaxanthin, lutein, and tunaxanthin into 3-dehydroretinol in Nile tilapia. The instrumentally measured fillet color values in our study clearly indicate that the yellow pigments in corn-based ingredients are not deposited in the fillet to the extent that the fillets exhibit clear visual differences in color. Further studies are required to confirm this observation.

After 24 weeks of growth trials, the fatty acid composition of Nile tilapia fillets mirrored that of their respective diets. This agrees similar previous observations in Nile tilapia and red hybrid tilapia (Justi et al., 2003, Ng et al., 2013, Tadesse et al., 2003, Teoh et al., 2011). In general, LC-PUFA such as 20:4n-6, 22:5n-3 and 22:6n-3 show selective deposition and retention in fish fillets, as their concentrations in fillets are always higher than those in the diet. We saw a opposite selective utilization of 18:2n-6 and 18:3n-3 in which the dietary concentration was always higher than that of fillet. This result also suggests that 18:2n-6 and 18:3n-3 is readily

utilized by fish when present at high concentrations. The dietary long-chain polyunsaturated fatty acids (LC-PUFA) made up less than 0.25% of the four corn-based diets; the LC-PUFA concentration in the fishmeal-based control diet was 10 times greater. However, at the end of 24 weeks, the LC-PUFA level in fillets from the corn-based treatments was almost two-thirds that of the control. This indicates the effectiveness of fatty acid desaturase and elongase enzymes in the herbivorous Nile tilapia.

The individual fatty acid compositions of fillets from fish in the corn-based treatments in this study, however, further indicated that the activity of fatty acid desaturase and elongase in the bio-conversion of 18:3n-3 to its long-chain homologs was insufficient to maintain the proportions of EPA and DHA in fish fed non-fishmeal diets at the same levels as in fish fed the fishmeal-based control diet. We did not use fish oil here: fishmeal was the only marine-derived feedstuff used and it was present only in the control. This result might therefore expose the possible effects of using fishmeal- and fish oil-free diets in commercial tilapia culture on the fatty acid composition of the fish produced. However, the PUFA:SFA ratio in all treatments ranged between 0.83 and 1.39 which is well above the minimum value of 0.45 recommended by the Department of Health of the UK (HMSO, 1994) and confirms that fish fed corn co-product are suitable for human consumption.

4.5. Conclusions

A 24-week feeding trial suggested that, among the four corn co-products tested as dietary supplements, HPDDG and DDGS were the best choices for a zero-fishmeal diet for Nile tilapia juveniles. Furthermore, the dietary inclusion of a high percentage of corn co-products did not negatively affect fillet color or amino acid profile. Although HPDDG and DDGS are suitable for zero fishmeal diets, the relatively poor n-3 highly unsaturated fatty acid profiles of the resulting

fish fillets are a potential drawback for these diets from the perspective of human nutrition. To mitigate this problem and to optimize the balance between cost-effectiveness of feed and the human-health protective properties of fish, a fish oil–based finishing diet could be applied. Further studies are required to demonstrate the effectiveness of this approach.

References

Abdel-Tawwab, M., Abdel-Rahman, A.M. & Ismael, N.E.M. (2008) Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture, 280, 185-189.

Akhtar, P., Gray, J.I., Cooper, T.H., Garling, D.L. & Booren, A.M. (1999) Dietary Pigmentation and Deposition of α - Tocopherol and Carotenoids in Rainbow Trout Muscle and Liver Tissue. Journal of food science, 64, 234-239.

AOAC (2005) Official methods of analysis of AOAC International, AOAC International.

Belyea, R.L., Rausch, K.D. & Tumbleson, M.E. (2004) Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresource Technology, 94, 293-298.

Boonyoung, S., Haga, Y. & Satoh, S. (2013) Preliminary study on effects of methionine hydroxy analog and taurine supplementation in a soy protein concentrate - based diet on the biological performance and amino acid composition of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. Aquaculture Research, 44, 1339-1347.

Buttle, L., Crampton, V. & Williams, P. (2001) The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon, *Salmo salar* L. Aquaculture Research, 32, 103-111.

Carter, C., Houlihan, D., Kiessling, A., Médale, F. & Jobling, M. (2001) Physiological effects of feeding.Food intake in fish, 297-331.

Cheng, Z.J. & Hardy, R.W. (2004) Nutritional Value of Diets Containing Distiller's Dried Grain with Solubles for Rainbow Trout, *Oncorhynchus mykiss*. Journal of Applied Aquaculture, 15, 101-113.

Coyle, S.D., Mengel, G.J., Tidwell, J.H. & Webster, C.D. (2004) Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research, 35, 365-370.

De la Higuera, M. (2001) Effects of nutritional factors and feed characteristics on feed intake. Food Intake in Fish, 827, 250-268.

Dhanapal, K., Reddy, G., Nayak, B., Venkateshwarlu, G., Balasubramanian, A., REDDY, A.D. & Basu, S. (2013) Changes in instrumental and sensory characteristics of tilapia fish steaks during cold blanching and Cooking treatments. CHANGES, 8, 887-892.

FAO, I. (2014) WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition.Food and Agriculture Organization (FAO), Rome.

Folch, J., Lees, M. & Sloane-Stanley, G. (1957) A simple method for the isolation and purification of total lipids from animal tissues.J biol chem, 226, 497-509.

Fontainhas-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.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å. & Nelson, R. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research, 38, 551-579.

Girao, P.M., Pereira da Silva, E.M. & de Melo, M.P. (2012) Dietary lycopene supplementation on Nile Tilapia (*Oreochromis niloticus*) juveniles submitted to confinement: effects on cortisol level and antioxidant response. Aquaculture Research, 43, 789-798.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J., Médale, F., Martin, S., Houlihan, D., Kaushik, S. & Pérez-Sánchez, J. (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture, 232, 493-510.

Hansen, A.C., Rosenlund, G., Karlsen, Ø., Olsvik, P.A. & Hemre, G.I. (2006) The inclusion of plant protein in cod diets, its effects on macronutrient digestibility, gut and liver histology and heat shock protein transcription. Aquaculture Research, 37, 773-784.

HMSO, U. (1994) Nutritional aspects of cardiovascular disease (report on health and social subjects No. 46). London: HMSO.

Hunt, R. (1977) The specification of colour appearance. I. Concepts and terms. Color Research & Application, 2, 55-68.

Justi, K., Hayashi, C., Visentainer, J., De Souza, N. & Matsushita, M. (2003) The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. Food Chemistry, 80, 489-493.

Kabeya, N., Takeuchi, Y., Yamamoto, Y., Yazawa, R., Haga, Y., Satoh, S. & Yoshizaki, G. (2014) Modification of the n-3 HUFA biosynthetic pathway by transgenesis in a marine teleost, nibe croaker. Journal of Biotechnology, 172, 46-54.

Kaisuyama, M. & Matsuno, T. (1988) Carotenoid and vitamin A, and metabolism of carotenoids, β-carotene, canthaxanthin, astaxanthin, zeaxanthin, lutein and tunaxanthin in tilapia *Tilapia nilotica*. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 90, 131-139.

Li, M.H., Robinson, E.H., Oberle, D.F., Lucas, P.M., Peterson, B.C. & Bates, T.D. (2011) Clearance of yellow pigments lutein and zeathanxin in channel catfish, Ictalurus *punctatus*, reared at different water temperatures. Journal of the World Aquaculture Society, 42, 105-110.

Li, M.H., Robinson, E.H., Oberle, D.F. & Zimba, P.V. (2007) Effects of various dietary carotenoid pigments on fillet appearance and pigment absorption in channel catfish, *Ictalurus punctatus*. Journal of the World Aquaculture Society, 38, 557-563.

Likongwe, J.S., Stecko, T.D., Stauffer Jr, J.R. & Carline, R.F. (1996) Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture, 146, 37-46.

Lim, C., Garcia, J.C., Yildirim-Aksoy, M., Klesius, P.H., Shoemaker, C.A. & Evans, J.J. (2007) Growth Response and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus*, Fed Diets Containing Distiller's Dried Grains with Solubles. Journal of the World Aquaculture Society, 38, 231-237.

Lima, D.P., Fuzinatto, M.M., Andretto, A.P., Braccini, G.L., Mori, R.H., Canan, C., de Oliveira, C.A., Ribeiro, R.P. & Vargas, L. (2015) Physical, chemical and microbiological quality of fillets and mechanically separated meat, and sensory evaluation of fillets of Nile Tilapia treated with homeopathic product. African Journal of Pharmacy and Pharmacology, 9, 738-744.

Matsuno, T. (1991) Xanthophylls as precursors of retinoids. Pure and Applied Chemistry, 63, 81-88.

Morrison, W.R. & Smith, L.M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol.Journal of lipid research, 5, 600-608.

Ng, W.-K., Chong, C.-Y., Wang, Y. & Romano, N. (2013) Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. Aquaculture, 372, 97-110.

Olsen, R.E., Hansen, A.-C., Rosenlund, G., Hemre, G.-I., Mayhew, T.M., Knudsen, D.L., Eroldoğan, O.T., Myklebust, R. & Karlsen, Ø. (2007) Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) II—Health aspects. Aquaculture, 272, 612-624.

Park, H., Flores, R.A. & Johnson, L.A. (1997) Preparation of fish feed ingredients: reduction of carotenoids in corn gluten meal. Journal of Agricultural and Food Chemistry, 45, 2088-2092.

Robinson, E.H. & Li, M.H. (2008) Replacement of soybean meal in channel catfish, *Ictalurus punctatus*, diets with cottonseed meal and distiller's dried grains with solubles. Journal of the World Aquaculture Society, 39, 521-527.

Ross, M.P. (2000) The influence of exposure to carbon monoxide on the quality attributes for yellowfin tuna muscle. University of Florida.

Santiago, C.B. & Lovell, R.T. (1988) Amino acid requirements for growth of Nile tilapia.The journal of nutrition, 118, 1540-1546.

Schaeffer, T.W., Brown, M.L., Rosentrater, K.A. & Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co‐products by Nile tilapia.Journal of animal physiology and animal nutrition, 94, e348-e354.

Shiau, S.-Y., Chuang, J.-L.& Sun, C.-L. (1987) Inclusion of soybean meal in tilapia (*Oreochromis niloticus*× *O. aureus*) diets at two protein levels. Aquaculture, 65, 251-261.

Skonberg, D.I., Hardy, R.W., Barrows, F.T. & Dong, F.M. (1998) Color and flavor analyses of fillets from farm-raised rainbow trout (*Oncorhynchus mykiss*) fed low-phosphorus feeds containing corn or wheat gluten. Aquaculture, 166, 269-277.

Suprayudi, M.A., Yaniharto, D., Priyoutomo, N., Kurnianto, A., Ekasari, J., Jusadi, D. & Haga, Y. (2015) Evaluation of Practical Diets Containing High Levels of Corn Distillers Dried Grains with Soluble on Red Tilapia Floating Net Cage Production Performance. Pakistan Journal of Nutrition, 14, 708-711.

Tadesse, Z., Boberg, M., Sonesten, L. & Ahlgren, G. (2003) Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish *Oreochromis niloticus* L.–A laboratory study. Aquatic Ecology, 37, 169-182.

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.

Tidwell, J.H., Coyle, S.D., VanArnum, A., Weibel, C. & Harkins, S. (2000) Growth, Survival, and Body Composition of Cage‐Cultured Nile Tilapia *Oreochromis niloticus* Fed Pelleted and Unpelleted Distillers Grains with Solubles in Polyculture with Freshwater Prawn *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society, 31, 627-631.

Torrissen, O., Hardy, R. & Shearer, K. (1989) Pigmentation of salmonids-carotenoid deposition and metabolism.CRC Crit. Rev. Aquat. Sci, 1, 209-225.

Vagner, M. & Santigosa, E. (2011) Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review. Aquaculture, 315, 131-143.

Vilhelmsson, O.T., Martin, S.A., Médale, F., Kaushik, S.J. & Houlihan, D.F. (2004) Dietary plant-protein substitution affects hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition, 92, 71-80.

Weigel, J., Loy, D. & Kilmer, L. (2005) Feed co-products of the dry corn milling process.Iowa State University and Iowa Corn Promotion Board.

Weigel, J.C., Loy, D., Kilmer, L.H., Association, I.C.G., Association, R.F. & Association, N.C.G. (1997) Feed co-products of the dry corn milling process, Iowa Corn Growers Association.

Wu, Y.V., Rosati, R., Sessa, D.J. & Brown, P. (1994) Utilization of protein-rich ethanol co-products from corn in tilapia feed. Journal of the American Oil Chemists' Society, 71, 1041-1043.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1996) Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. Journal of Agricultural and Food Chemistry, 44, 1491-1493.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1997) Use of corn-derived ethanol coproducts and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. Journal of Agricultural and Food Chemistry, 45, 2174-2177.

Yamashita, E., Arai, S. & Matsuno, T. (1996) Metabolism of xanthophylls to vitamin A and new apocarotenoids in liver and skin of black bass, *Micropterus salmoides*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 113, 485-489.

Zar, J.H. (1999) Biostatistical analysis, Pearson Education India.

CHAPTER 5

Chapter 5. Influence of salinity on complete fishmeal replacement in Nile tilapia, *Oreochromis niloticus*

Abstract

A 12 week feeding trial was conducted to evaluate the combined effect of fishmeal replacement and salinity on growth, feed utilization efficiencies and expression level of genes related to growth, osmoregulation and fatty acid desaturation and elongation of Nile tilapia. Two iso-nitrogenous and iso-energetic diets were prepared to contain 15% fishmeal in control (FM) diet and fishmeal content in control was completely replaced by the mixture of poultry by-product meal, high protein distillers dried grains and distillers dried grains with soluble in non fishmeal diet (NFM). The NFM diet was supplemented with DL-methionine and L-lysine. Duplicated group of fish(initial weight :6 g)reared in four salinity levels (0ppt, 4ppt, 8ppt and 12ppt) were fed one of the two diets to satiety twice a day for 12 weeks. The %SGR, MFI and FCR was not affected by the diets while effects were significant for salinity. The highest % SGR and MFI was observed in 4pptwhile the lowest FCR was recorded in the 0ppt. The survival rate and crude protein and lipid contents of muscles were independent from treatments. Significantly highest linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) contents were observed in NFM group and salinity slightly modified the effects. TheFM group showed almost three times higher levels of eicosapentaenoic acid (20:5n-3) and decosahexaenoic acid (22:6n-3) than that of NFM and changes were not significant for the salinity. Relative expression of hepatic IGF-I and IGF-II gene of Nile tilapia showed that its expression is regulated by the salinity there were no dietary or dietary salinity interactive effects on IGF expression in liver. Gene expression of growth hormone receptor was not affected either by diet or salinity. Hepatic fatty acid Δ6 desaturase expression in 4‰ was up-regulated in compared to freshwater and gene expression of fatty acid

elongase (elovl5) of NFM group showed up-regulation compared to its FM counterparts at 8 and 12 ‰. Present finding revealed the possibility of total fishmeal replacement in saline waters (0ppt to 12ppt) without negative effects on growth, feed utilization and body composition of fish.

5.1. Introduction

Tilapia has become one of the fastest growing and economically important group of fish in the global aquaculture (El-Sayed et al., 2005, FAO, 2014). Ability of tilapia to tolerate a wide range of salinity levels has attracted the attention of fish farmers and now tilapia culture is expanding to the brackish water and sea water (El-Sayed et al., 2005, El-Sayed et al., 2003, Kamal and Mair, 2005, Yan et al., 2013). Among different species of tilapia, Nile tilapia (*Oreochromis niloticus*) received a great attention due to its high growth rate and adaptability to different culture methods and conditions (Pullin and Lowe-McConnell, 1982) and it is recognized as the most important farmed tilapia in the world (El-Sayed et al., 2005, El-Sayed et al., 2003).

Similar to other vertebrates, growth and development of fish is controlled by internal factors such as endocrinological and neuro-endocrinological as well as external factors such as temperature and salinity (Bœuf and Payan, 2001, Rubio et al., 2005). The energetic costs of respiration and osmoregulation determine the metabolic energy available for growth of fish (Prunet and Bornancin, 1989) and the influence of water salinity on growth of fish has been reported for many species (Likongwe et al., 1996, Tandler et al., 1995, Tsuzuki et al., 2007, Watanabe et al., 1989). Salinity is not only affect on the growth of fish, but also it appear to modify several other growth related aspects such as feed intake (Imsland et al., 2001, Kilambi, 1980, Rubio et al., 2005, Wang et al., 1997), macronutrient selection (Rubio et al., 2005), feed conversion efficiency (Imsland et al., 2001, Lambert et al., 1994, Likongwe et al., 1996), digestive enzyme activity (Bœuf and Payan, 2001, Moutou et al., 2004) and hormones involved in the growth of fish (Bœuf and Payan, 2001, Link et al., 2010, Magdeldin et al., 2007, Mancera and McCormick, 1998, Reinecke et al., 2005).

Growth of fish is a function of feed intake and capacity of fish to utilize ingested food (Carter et al., 2001). Impacts of salinity on feed intake of freshwater fish species are different from that of marine fish species. Wang et al. (1997) reported the diminishing feed intake with increasing salinity from freshwater to 6.5‰ in common carp (*Cyprinus carpio*) while enhanced feed intake and feed utilization efficiency was observed at higher salinity in Florida red hybrid tilapia (*Oreochromis urolepis honarum* x *O. mossambicus*) (Watanabe et al., 1988). Feed intake of grass carp (*Ctenopharyngodon idella*) was highest at 5‰ compared to higher or lower salinities (Kilambi, 1980).

Nile tilapia is a freshwater fish and it is amongst the least saline tolerant tilapia (Baysoy et al., 2013, Kamal and Mair, 2005). Although aquaculture of Nile tilapia is expanded into the saline water, nutritional studies of fishmeal replacement in this species is basically limited to freshwater (Coyle et al., 2004, El‐Saidy and Gaber, 2003, El‐Sayed, 1998, Fontainhas-Fernandes et al., 1999, Schaeffer et al., 2010, Stadtlander et al., 2013). However the effects of non-fishmeal diets on feed intake of this species are controversial. El‐Saidy and Gaber (2003) reported a reduced feed intake of juvenile Nile tilapia when fishmeal is completely replaced by a mixture of plant proteins while enhanced feed intake was reported by other authors (El‐Saidy and Gaber, 2002, Fontainhas-Fernandes et al., 1999). Differential effects of dietary protein sources and salinity on intestinal transport of nutrient were observed for Atlantic salmon and rainbow trout (Nordrum et al., 2000). However, the interactive effects of salinity and complete fishmeal replacement on growth performance and feed utilization parameters of this Nile tilapia, which play a significant role in global aquaculture production is still not clear.

Variety of hormones such as growth hormones (GH), insulin like growth factor-I (IGF-I) are also involved in the regulation of fish growth through endocrine, paracrine and/ or autocrine

manner (Cruz et al., 2006, Kajimura et al., 2001, Ueda, 2005). Highly conserved IGF-I gene is found to be the most promising indicator of the growth of fish (Cruz et al., 2006, Ueda, 2005, Kajimura et al., 2001).Understanding the genetic basis of growth related genes is important in selecting favorable alleles for aquaculture. Therefore, this study aimed to examine the effects of complete fishmeal replacement on growth performance, feed utilization efficiency, body composition and relative gene expression of IGF-1, IGF-2 and growth hormone receptors under different salinity levels to assess the nutritional and environmental regulation of those genes in Nile tilapia, *Oreochromis niloticus* juveniles.

5.2. Materials and Methods

5.2.1. **Experimental diets**

Two isonitrogenous (32% protein) experimental diets (15% fishmeal diet/FM diet and 0% fishmeal diet/NFM diet) were prepared to meet the known nutritional requirement of juvenile Nile tilapia. NFM diet was supplemented with DL-methionine, L-lysine and L-tryptophan to satisfy the essential amino acid requirement of fish (Santiago and Lovell, 1988). The ingredient composition, proximate composition and amino acid compositions of experimental diets are given in Table 18. Fatty acid composition of two diets is shown in Table 18.

5.2.2. **Experimental fish, experimental conditions and feeding**

Nile tilapia juveniles were obtained from the Laboratory of Fish culture, Tokyo University of Marine Science and Technology, Tokyo, Japan. Four closed water re-circulating systems connected to 16 glass tanks (4tanks in one system) were used in this experiment.

Table 18.Ingredient, proximate and amino acid composition of experimental diets (g kg^{-1})

Component	FM diet	NFM diet
Fish meal	150	0
DDGS	100	200
HPDDG	50	100
Soybean meal	300	300
Poultry by-product meal	0	100
Wheat flour	240	160
Alpha starch	120	90
Crystalline amino acids	0	10
Soy oil	20	20
Vitamins & mineral mix*	20	20
Dry matter	956.1	958.4
Crude protein	318	323
Crude lipid	76.6	81.3
Ash	64.3	39.4
EAA		
Arginine	14.8	13.7
Histidine	4.9	4.6
Isoleucine	8.8	8.6
Leucine	20.1	20.7
Lysine	14.2	14.5
Methionine	7.6	8.0
Phenylalanin	13.7	12.6
Threonine	10.6	10.0
Tryptophan	1.0	1.0
Valine	8.9	8.0

*Composition of vitamin and mineral mixture was same as experiment II

Table 19**.**Percentage of individual fatty acids ± SD (% of all fatty acids in total lipids) together with total saturates, total monoenes, polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA and n-3 to n-6 ratio in two diets (n=3)

Fatty acid	FM diet	NFM diet
14:0	1.78 ± 0.13	0.20 ± 0.02
16:0	16.78 ± 0.35	16.22 ± 0.47
16:1*n*-7	1.70 ± 0.20	0.89 ± 0.04
18:0	2.81 ± 0.01	3.15 ± 0.00
18:1*n*-9	18.59 ± 0.71	25.40 ± 0.10
18:1*n*-7	1.58 ± 0.02	1.39 ± 0.06
18:2*n*-6	42.94 ± 1.10	46.86 ± 0.46
18:3*n*-3	3.34 ± 0.01	2.87 ± 0.04
18:4*n*-3	0.40 ± 0.02	0.02 ± 0.00
20:1*n*-9	ND	0.02 ± 0.00
20:1*n*-11	0.33 ± 0.01	0.21 ± 0.00
20:4*n*-6	0.24 ± 0.01	0.16 ± 0.01
20:4*n*-3	0.04 ± 0.00	ND
20:5*n*-3	2.42 ± 0.15	0.06 ± 0.01
22:1*n*-11	0.22 ± 0.01	0.09 ± 0.01
22:5*n*-3	0.28 ± 0.00	ND
22:6*n*-3	2.05 ± 0.06	0.05 ± 0.01
Total saturates	21.37 ± 0.47	19.56 ± 0.49
Total monoenes	22.55 ± 0.54	28.10 ± 0.24
Total PUFA	51.71 ± 0.86	50.02 ± 0.53
n-3	8.52 ± 0.23	3.00 ± 0.06
n-6	43.18 ± 1.10	47.02 ± 0.47
n-3/*n*-6	0.20	0.06

ND, non-detectable

Fish were initially stocked in those glass tanks (60L) filled with dechlorinated tap water. A 2 x 4 factorial experiment was conducted with two dietary treatments (FM diet and NFM diet) and four salinity levels (0‰, 4‰, 8‰ and 12‰). Before the start of the experiment, the fish were acclimated to the experimental conditions for two weeks and fed control diet.

During the acclimation period, water salinity was increased by 2‰ day $^{-1}$until reached the desired salinity using artificial sea water (Sea Life®, Tokyo, Japan). To begin an experiment, fish in similar salinity levels were pooled, anesthetized and individually measured to nearest 0.1g. A group of 20 fish were stocked in each tank and dietary treatments were randomly allocated to duplicate tanks in each salinity level. Initial mean weight of fish was 6.5 ± 0.1 g. Each re-circulating system was equipped with electric heaters (100W) and water temperature and flow rate of experimental systems was maintained at 26.0 ± 1.0 ˚C and 0.5 L min^{-1}respectively. Approximately 50% of the water in the re-circulating system was replaced weekly with the dechlorinated water of correct salinity (Sea Life®, Tokyo, Japan). Fish were hand fed to near satiety twice a day for 12 weeks.

5.2.3. **Sample collection**

Twenty fish from initial stock were kept at -30 ˚C for initial body composition analysis. During the experimental period, fish in each tank were bulk weighted every three weeks. Upon termination of the experiment, fish were starved for 24 h, anaesthetized with 0.2% 2-phenoxyethanol (Wako Pure Chemical Industries, Osaka, Japan) and weight of individual fish was measured to nearest first decimal point. Five fish per tank was used for filleting and liver of same five fish was collected for fatty acid analysis. Fillet samples, liver tissue and five fish per tank were immediately frozen at -30 ˚C for chemical analysis. Frozen fillet and whole body were

minced by a centrifugal mill (Retsch ZM 1, Haan, Germany) fitted with a 0.25 mm screen and kept at -30 ˚C until analysis.

Final mean weight (FMW g), percentage specific growth rate (SGR, %), mean feed intake (MFI), food conversion ratio (FCR), survival rate (SR, %)and protein efficiency ratio (PER) were calculated by using following equations to compare fish growth and nutrient utilization efficiency among treatments.

$$FMW = Final\ weight\ (Fw) - Initial\ Weight\ (IW)$$

$$\%\ SGR = (\ln FW - lnIw)/time\ x\ 100$$

$$MFI = \frac{Total\ feed\ intake\ of\ fish\ (g)}{Number\ of\ fish}$$

$$FCR = \frac{Fed\ Intake(g)}{Weight\ gain\ (g)}$$

$$SR = \frac{Number\ of\ fish\ at\ harvest\ X\ 100}{Number\ of\ fish\ stock}$$

$$PER = \frac{Body\ weight\ gain\ (g)}{Protein\ gain\ (g)}$$

5.2.4. **Chemical analysis**

Feed ingredients, diets, whole body and muscle samples were analyzed in accordance with standard procedures for chemical analysis(AOAC, 2005). All the samples were finely ground and analyzed in triplicate. Dry matter content was calculated from the weight loss after drying sample at 105 ˚C until it reached a constant weight. Ash content was determined after the incineration of samples in a muffle furnace at 550 ˚C for 16 h. The Kjeldhal method was used for

crude protein analysis (N content x6.25).Total lipids in the samples were extracted after homogenization in chloroform: methanol (2:1) mixture according to Folch et al. (1957).

For the fatty acid extraction, lipids were saponified using 1 ml of 50% KOH in 15 ml ethanol at 80 ˚C for 60 min. The saponifiable matter was then esterified using 7% boron trifluoride (BF3) in methanol (Morita Chemical Industries Co., Ltd., Osaka, Japan) and heated for 20 min at 80 ˚C (Morrison and Smith, 1964). Fatty acid methyl esters (FAME) preparations were made up in hexane (20 mg/ml) and analyzed by gas chromatography (GC) (GC-2025; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector and a silica capillary column (L × I.D. 30 m × 0.32 mm, df 0.25 µm, Supelcowax® 10 Fused Silica Capillary GC column, Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas. The thermal program was set to increase from 170 to 260 ˚C at 2 ˚C min^{-1}. The temperature in the injection port and detector was both adjusted to 260 ˚C(Kabeya et al., 2014). FAME peaks were identified by comparing the retention time of each with that of the appropriate FAME standard.

5.2.5. **Total RNA extraction and cDNA synthesis**

Four fish per treatment (two fish per tank) was used for total RNA extraction and consequent gene expression analysis. Total RNA was extracted from 50 to 100 mg of liver tissues by the standard Trizol extraction method (Invitrogen, Carlsbad, CA, USA) and recovered in 100 µl of molecular biology grade water. Total RNA samples were pre-treated with RNase-free DNase (Promega, Madison, WI, USA) according to the manufacturer's instructions to remove possible genomic DNA contaminations. First strand cDNA was synthesized in 20 µL RT reactions with 1 µg total RNA template, MultiScribe™Reverse transcriptase, 10X RT buffer, 25X dNTP mix, 10X RT random primers (High Capacity cDNA Reverse Transcriptase Kit, Applied Biosystems, USA).

5.2.5.1. Quantitative real time PCR

The expression of insulin like growth factors (IGF-I and IGF-II), growth hormone receptor (GHR1) and fatty acid Δ6 desaturase (Fadsd6) and fatty acid elongase (elovl5) in liver tissue and IGF-I, IGF-II and growth hormone (GH) in gills of Nile tilapia fed FM diet or NFM diet under different salinity levels were studied by quantitative real time PCR (qRT-PCR). The expression of target gene was normalized using β-actin as a housekeeping gene. PCR primers for β-actin (*Oni-β-actin*), IGF-I (*Oni-IGF-I*),IGF-II (*Oni-IGF-II*), GHR1, fatty acid Δ6 desaturase (Fadsd6) and fatty acid elongase (elovl5) were designed according the Nile tilapia (Table 20). A qRT-PCR amplification was carried out in duplicate using SYBR green PCR master mix (Applied Biosystems, USA) and an ABI7300 quantitative PCR system (Applied Biosystems, USA) following the manufacturer's instructions. The conditions of quantitative PCR as follows: an initial denaturation step of 1 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and final step dissociation stage followed by 1 cycle 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, 60 °C for 15 s as described by Zhao et al. (2015).The β-actin was used as an internal control for sample normalization of the target primers. The specificity of the real time PCR reactions was confirmed by the agarose gel electrophoresis for all samples.

The comparative cycle threshold method (C_T method) was used for qRT-PCR data analysis, where C_T values refer to the number of cycles at which monitored fluorescence emissions in the qRT-PCR reactions exceed a manually set threshold. The relative gene expression model was used to evaluate the fold changes in mRNA expression between fish in different treatments using the$\Delta\Delta C_T$ method (Livak and Schmittgen, 2001). Relative gene expression ratios (R) between treated and control groups were calculated using the formula; $R = 2^{\wedge -\Delta CT}$ with $\Delta C_T = C_T$ (target gene)−C_T (β- actin) and with $\Delta\Delta C_T = \Delta C_T$ (treatment) - ΔC_T(control).

Fish reared in 0 ‰ was used as the respective control. Thus, dietary and salinity induced changes are presented as n-fold differences relative to the corresponding control set to 1.

5.2.6. **Statistical analysis**

Data were initially checked for normality and equal variance by using Levene's test. The main effects of dietary protein source and the salinity were tested using a two-way analysis of variance followed where pertinent by Tukey's multiple range tests. When dietary effects were significant for relative expression of particular gene, Student's t-test was used to compare expression level of that specific gene for two diets at a particular salinity level. Statistical significance was determined at 5%($p < 0.05$) for each set of comparisons. Statistical analysis of fatty acid composition was performed on arcsine transformed data. All statistical analyses were performed with SPSS version 16.0.

5.3. Results

5.3.1. **Growth performances and feed utilization efficiencies**

The weight gain, percentage specific growth rate (% SGR), feed intake and feed utilization efficiencies of fish fed two diets in different salinity levels are presented in Table 21. Growth performances of fish were not affected by diets while effects were significant for salinity. No interactive effects of diet and salinity on growth performances were detected. Significantly highest mean weight gain and feed intake was observed in 4‰ salinity irrespective of diets. The % SGR of fish in 4‰ of both diets was significantly higher than that of 8‰ and 12 ‰ and % SGR of fish reared in freshwater (0‰) was not different from other salinity levels. Significantly lowest and highest FCR was reported in 0‰ and 12 ‰ respectively and the effects were not significant for diets. The PER was affected by both factors.

Table 20.Sequences of PCR primers used in this study

Primer	GenBank Accession No	Sequence	PCR	Base pairs	Primer order
Oni-β-actin-F	EU88795	5'-TATCCTGACCCTGAAGTACC-3'	RT-PCR	194	Sense
Oni-β-actin-R	EU88795	5'-TGAAGGTCTCGAACATGATC-3'	RT-PCR	194	Antisense
Oni-β-actin-F1	EU88795	5'- CTCCCCTGAACCCCAAAGCC-3'	Real Time-PCR	113	Sense
Oni-β-actin-R1	EU88795	5'- AGAGGCGTACAGGGACAGCA-3'	Real Time-PCR	113	Antisense
Oni-IGF-I-F	NM_001279503	5'- AAGACTCCCAAGATTTCTCG-3'	RT-PCR	161	Sense
Oni-IGF-I-R	NM_001279503	5'- GAACTATGTCCAGGTGAAGG-3'	RT-PCR	161	Antisense
Oni-IGF-I-F1	NM_001279503	5'- CACCCTCTCACTACTGCTGT-3'	Real Time-PCR	118	Sense
Oni-IGF-I-R1	NM_001279503	5'- TCGCTCTCCACAGACAAACT-3'	Real Time-PCR	118	Antisense
Oni-IGF-II-F	EU272150	5'- TCGTAGAGGAGTGTTGTTTC-3'	RT-PCR	197	Sense
Oni- IGF-II -R	EU272150	5'- GTCACATGTTGCTTCTTCTG-3'	RT-PCR	197	Antisense
Oni- IGF-II -F1	EU272150	5'- CAAACCTGCCAAGTCCGAAA-3'	Real Time-PCR	110	Sense
Oni- IGF-II -R1	EU272150	5'- ACTTCACGGTCACATGTTGC-3'	Real Time-PCR	110	Antisense
Oni-GHR1-F1	EF052861	5'-CTCACTGACTGGGACCACAC-3	Real Time-PCR	126	Sense
Oni-GHR1-R1	EF052861	5'-GGAGGAGAGGTTGTGGAAGC-3	Real Time-PCR	126	Antisense
Oni-GH -F	HM565014	5'-ACATGTCATAGGAGAGCTCT-3	RT-PCR	221	Sense
Oni-GH-R	HM565014	5'-TCAGAAGATTATCGCATGGG-3	RT-PCR	221	Antisense
Oni-GH-F1	HM565014	5'-CTGGTTGAGTCCTGGGAGTT-3	Real Time-PCR	111	Sense
Oni-GH-R1	HM565014	5'-GATCAGCAGCAAGATTCCCG-3	Real Time-PCR	111	Antisense
Oni-Fadsd6-F	AB069727	5'- TGCTGTTTGAAACTTGTGAC-3'	RT-PCR	182	Sense
Oni-Fadsd6-R	AB069727	5'- ATGACCAGCCATTGATCATT-3'	RT-PCR	182	Antisense
Oni-Fadsd6-F1	AB069727	5'- TGCAGCCATCGTGCAGGATT-3'	Real Time-PCR	145	Sense
Oni-Fadsd6-R1	AB069727	5'- CCACATCCAGACGGTCAGCC-3'	Real Time-PCR	145	Antisense
Oni-Elov-F	AY170326	5'- CATGGACACCTTCTTCTTCA-3'	RT-PCR	243	Sense
Oni-Elov-R	AY170326	5'- AACTGCGTGATGTACTTCTT-3'	RT-PCR	243	Antisense
Oni-Elov -F1	AY170326	5'- ACACAGGCAGCCGTACTCCT-3'	Real Time-PCR	117	Sense
Oni-Elov -R1	AY170326	5'- TTGTAGCCTCCGTGCCATGC-3'	Real Time-PCR	117	Antisense

Table 21.Growth performances and feed utilization efficiencies of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (n=2)

	FM Diet				NFM Diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
FMW	53.2 ± 3.0^{b}	59.4 ± 2.1^{a}	49.4 ± 1.2^{b}	49.2 ± 1.6^{b}	51.4 ± 1.1^{b}	58.3 ± 0.1^{a}	48.6 ± 0.3^{b}	49.7 ± 1.2^{b}	ns	*	ns
% SGR	2.5 ± 0.1^{ab}	2.6 ± 0.1^{a}	2.4 ± 0.1^{b}	2.4 ± 0.04^{b}	2.4 ± 0.1^{ab}	2.6 ± 0.1^{a}	2.4 ± 0.0^{b}	2.4 ± 0.0^{b}	ns	*	ns
MFI	46.5±2.5^{b}	53.7±1.6^{a}	45.8 ± 1.1^{b}	46.9 ± 2.1^{b}	45.0 ± 3.7^{b}	53.5 ± 0.9^{a}	45.8 ± 1.5^{b}	47.7 ± 2.3^{b}	ns	*	ns
FCR	1.00 ± 0.01^{c}	1.02±0.01^{bc}	1.04±0.01^{ab}	1.09 ±0.03^{a}	1.00 ±0.05^{c}	1.04±0.02^{bc}	1.08 ± 0.02^{ab}	1.11 ± 0.02^{a}	ns	*	ns
PER	2.98 ± 0.04^{a}	2.92 ± 0.04^{ab}	2.83± 0.01^{bc}	2.71± 0.02^{c}	2.89 ±0.16^{a}	2.81±0.06^{ab}	2.66 ± 0.06^{b}	2.62 ± 0.06^{b}	*	*	ns

Results are given as mean ± standard deviation. n=2 for each factor combination. Different letters in same row denote significant differences (Tukey's test, $P<0.05$) between salinity treatment within each dietary group. D, diet; S, salinity; ns, not significant ($p > 0.005$).

5.3.2. **Proximate composition of whole body and fillet**

Whole body and fillet proximate composition of Nile tilapia fed FM and NFM diets under four salinity levels is presented in Table 22. Results revealed that there was no apparent effect of diet on the whole body composition of fish. In both diets, whole body ash content was the only factor that was influenced by water salinity and it was highest in 12‰ compared to other salinity levels. However, there were no interactive effects of diets and salinity was observed either in whole body or fillet proximate composition. Analytical data of fish fillet showed that, impacts of dietary protein source is limited to fillet protein content. However, salinity does not affect on fillet proximate composition of Nile tilapia.

5.3.2.1. Fatty acid composition of fillet and liver tissue

Nineteen different fatty acids were identified in fillet of Nile tilapia. The percentage of individual fatty acids, saturated fatty acids (SFA), monounsaturated fatty acids and polyunsaturated fatty acids (PUFA) in fillet and liver expressed as a percent of the total fatty acid pool is presented in Table 23 and Table 24 respectively. In fish fillet, oleic acid (18:1n-9) was the dominant fatty acid in all treatments followed by palmitic acid (16:0) and linoleic acid (LA, 18:2n-6) (Table 26). Those three fatty acids collectively account to two third of the total fatty acid pool of each treatment. Except, fillet fatty acid composition of 18:0 and 18:1n-7, significant dietary impacts were observed for other fatty acids. Except arachidonic acid (ARA, 20:4n-6), none of the HUFA of fish fillet was able to show statistical differences for salinity and effects were mostly observed in C16 and C18 fatty acids. However, salinity had significant impact on the mono unsaturated fatty acids in fish fillet. The LA, α-linolenic acid (LNA, 18:3n-3), and ARA were statistically significant for main effects as well as for interactive effect of two factors, diet and salinity.

Table 22:Proximate composition (% wet basis) of whole body and fillet of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (n=3)

%	FM Diet				NFM Diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
Whole body											
Moisture	71.9 ± 0.2	72.1 ± 0.4	71.4 ± 0.1	71.9 ± 0.7	72.6 ± 0.4	71.6 ± 0.3	71.8 ± 0.3	71.9 ± 0.1	ns	ns	ns
Protein	15.3 ± 0.3	15.9 ± 0.5	15.6 ± 0.3	15.8 ± 0.1	15.6 ± 0.2	16.2 ± 1.1	15.5 ± 0.2	15.5 ± 0.5	ns	ns	ns
Lipid	9.1 ± 1.2	8.6 ± 0.3	9.4 ± 0.4	8.0 ± 0.7	8.4 ± 0.8	9.7 ± 0.8	10.0 ± 0.2	9.1 ± 0.5	ns	ns	ns
Ash	3.1 ± 0.3^{bc}	2.9 ± 0.2 ^{c}	3.5 ± 0.1^{b}	3.8 ± 0.1 ^{a}	2.6 ± 0.2^{b}	3.2 ± 0.3^{b}	2.8 ± 0.1^{b}	3.8 ± 0.2 ^{a}	ns	*	ns
Fillet											
Moisture	77.4 ± 0.4	77.1 ± 0.2	76.7 ± 0.2	77.0 ± 0.4	77.4 ± 0.1	77.5 ± 0.4	77.4 ± 0.1	77.2 ± 0.2	ns	ns	ns
Protein	20.1 ± 0.2	20.1 ± 0.2	20.6 ± 0.1	20.8 ± 0.3	20.0 ± 0.2	19.5 ± 0.3	19.4 ± 0.4	19.5 ± 0.2	*	ns	ns
Lipid	1.4 ± 0.0	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.7 ± 0.3	1.7 ± 0.1	ns	ns	ns
Ash	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	ns	ns	ns

Results are given as mean ± standard deviation. n=2 for each factor combination. Different letters in same row denote significant differences (Tukey's test, $P<0.05$) between salinity treatment within each dietary group

D, diet; S, salinity; ns, not significant ($p > 0.005$)

Table 23: Percentage of individual fatty acids ± SE (% of all fatty acids in total lipids) together with total saturates, total monoenes, polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA and n-3 to n-6 ratio in the fillet of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (area %, n=3).

	FM diet				NFM diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
14:0	1.8 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	1.8 ± 0.1	1.3 ± 0.0	1.6 ± 0.3	1.3 ± 0.1	1.4 ± 0.1	*	ns	ns
16:0	24.9 ± 0.1	24.3 ± 0.4	25.3 ± 1.5	24.3 ± 0.1	24.1 ± 0.4	23.8 ± 2.0	22.7 ± 1.2	23.3 ± 0.7	*	ns	ns
16:1*n*-7	3.0 ± 0.3	3.4 ± 0.2	3.0 ± 0.2	3.3 ± 0.1	2.4 ± 0.1^{bc}	3.0 ± 0.4^{a}	2.6 ± 0.2^{ab}	2.5 ± 0.1^{bc}	*	*	ns
18:0	7.3 ± 0.4	6.9 ± 0.2	7.7 ± 0.0	6.9 ± 0.1	7.6 ± 0.1^{a}	6.5 ± 0.3^{b}	7.3 ± 0.2^{a}	7.2 ± 0.0^{a}	ns	*	ns
18:1*n*-9	17.3 ± 0.4^{b}	19.8 ± 0.6^{a}	17.9 ±0.1^{b}	19.8 ± 0.1^{a}	19.1 ± 0.5^{c}	23.1 ± 0.0^{a}	22.3 ±0.1^{ab}	21.5 ± 0.1^{b}	*	*	ns
18:1*n*-7	3.3 ± 0.1^{ab}	3.2 ± 0.1^{b}	3.6 ± 0.0^{a}	3.2 ± 0.0^{b}	3.2 ± 0.0^{a}	2.8 ± 0.0^{b}	3.1 ± 0.1^{a}	3.2 ± 0.1^{a}	ns	*	ns
18:2*n*-6	19.1 ± 0.4^{ab}	17.3 ± 0.7^{c}	18.5 ±0.1^{bc}	18.3± 0.1^{bc}	20.2 ± 0.1	20.3 ± 0.4	19.9 ± 0.2	20.0 ± 0.1	*	*	*
18:3*n*-3	1.1 ± 0.0^{a}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	0.8 ± 0.0^{b}	0.9 ± 0.1^{a}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}	*	*	*
18:4*n*-3	0.2 ± 0.0^{ab}	0.3 ± 0.1^{a}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	ND	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	*	*	ns
20:1*n*-11	0.8 ± 0.0^{ab}	1.1 ± 0.0^{a}	0.7 ± 0.1^{c}	0.9 ± 0.0^{b}	0.9 ± 0.1	1.0 ± 0.2	1.1± 0.1	1.0 ± 0.0	*	*	ns
20:4*n*-6	2.5 ± 0.2	2.4 ± 0.1	2.8 ± 0.2	2.4 ± 0.1	4.0 ± 0.1^{a}	3.1 ± 0.3^{b}	3.5 ± 0.2^{ab}	3.8 ± 0.2^{ab}	*	*	*
20:4*n*-3	ND	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	ND	ND	ND	ND	-	ns	-
20:5*n*-3	0.8 ± 0.0	1.0 ± 0.2	0.8 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	*	ns	*
22:4n-6	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.0	1.2 ± 0.0	1.1 ± 0.3	1.4 ± 0.2	1.3 ± 0.1	*	ns	ns
22:5n-6	1.2 ± 0.1	1.1 ± 0.0	1.0 ± 0.1	1.2 ± 0.0	3.1 ± 0.1	2.6 ± 0.7	2.8 ± 0.3	2.9 ± 0.2	*	ns	ns
22:5*n*-3	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	*	ns	ns
22:6*n*-3	7.5 ± 0.6	7.0 ±0.2	7.5 ± 0.9	7.5 ± 0.2	2.9± 0.4	2.1 ± 0.5	2.2 ± 0.2	2.1 ± 0.1	*	ns	ns
Σ SFA	34.0 ± 0.1	33.3 ± 0.5	35.0 ± 1.7	33.0 ± 0.2	32.9 ± 0.5	31.8 ± 2.0	31.3 ± 1.2	31.9 ± 0.8	*	ns	ns
Σ Monoenes	24.8 ± 0.2^{c}	28.1 ±0.2^{a}	25.2 ± 0.3^{bc}	27.2 ± 0.1^{b}	25.6 ± 0.6^{c}	30.0 ± 0.2^{a}	29.1± 0.1^{ab}	28.2 ±0.1^{b}	*	*	*
ΣPUFA	34.2 ± 1.6	32.2± 0.4	33.7 ± 1.4	33.5 ± 0.1	33.0 ± 0.1	30.1 ± 1.4	31.4 ± 0.6	31.7 ± 0.7	*	ns	ns
n-3	10.9 ± 0.8	10.6 ± 0.4	10.8 ± 1.1	10.8 ± 0.2	4.5 ± 0.1	3.7 ± 0.6	3.9 ± 0.2	3.8 ± 0.1	*	ns	ns
n-6	23.3 ± 0.8	21.6 ± 0.8	22.9 ± 0.3	22.7 ± 0.1	28.4 ± 0.2	27.1 ± 0.8	27.6 ± 0.4	28.0 ± 0.6	*	ns	ns
n-3/*n*-6	0.46	0.49	0.47	0.48	0.16	0.14	0.17	0.16			
PUFA/SFA	1.00	0.97	0.96	1.01	1.00	0.95	1.00	0.99			

Table 24: Percentage of individual fatty acids (% of all fatty acids in total lipids) together with total saturates, total monoenes, polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA and *n-3* to *n-6* ratio in the liver of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (area %, n=3).

	FM diet				NFM diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
14:0	3.5 ± 0.1	3.5 ± 0.1	4.2 ± 0.2	3.9 ± 0.7	3.9 ± 0.1	3.8 ± 0.1	3.7 ± 0.2	3.8 ± 0.7	ns	ns	ns
16:0	24.7 ± 0.2	26.7 ± 0.6	25.6 ± 0.6	27.4 ± 2.6	23.5 ± 0.3 ^{c}	27.6 ± 0.2^{a}	26.1 ± 0.4 ^{b}	26.2 ± 1.0 ^{b}	ns	*	ns
16:1*n*-7	7.1 ± 0.0 ^{b}	7.1 ± 0.2 ^{b}	8.5 ± 0.4 ^{a}	7.9 ± 0.9 ^{ab}	6.7 ± 0.2	6.8 ± 0.2	7.1 ± 0.1	7.0 ± 0.4	*	*	ns
18:0	6.6 ± 0.1	6.9 ± 0.5	6.3 ± 0.1	6.2 ± 0.5	5.6 ± 0.1 ^{b}	7.0 ± 0.1 ^{a}	5.6 ± 0.2^{b}	5.9 ± 0.1 ^{b}	*	*	ns
18:1*n*-9	36.7 ± 0.4	36.9 ± 1.6	35.5 ± 0.1	33.5 ± 1.7	35.6 ± 0.3	36.0 ± 0.1	35.9 ± 1.3	35.6 ± 1.0	ns	ns	ns
18:1*n*-7	3.4 ± 0.1	3.3 ± 0.2	3.0 ± 0.1	3.2 ± 0.2	2.9 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	*	ns	ns
18:2*n*-6	10.5 ±0.3 ^{a}	7.9 ± 1.2 ^{b}	8.5 ± 0.4 ^{b}	8.4 ± 0.3 ^{b}	13.2 ± 0.0 ^{a}	9.1 ± 0.6 ^{b}	11.4 ± 0.9 ^{b}	11.0 ± 1.5 ^{b}	*	*	ns
18:3*n*-3	0.4 ± 0.0	0.3 ± 0.1	0.8 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	ns	ns	ns
20:1n-11	1.4 ± 0.1 ^{a}	1.4 ± 0.3 ^{a}	0.8 ± 0.1 ^{b}	1.2 ± 0.4 ^{ab}	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	ns	*	ns
20:4*n*-6	0.5 ± 0.0 ^{b}	0.6 ± 0.1 ^{a}	0.4 ± 0.1 ^{b}	0.6 ± 0.1 ^{ab}	0.8 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	*	*	ns
20:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	ND	ND	ND	ND	-	ns	-
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	*	ns	ns
22:5n-6	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	*	ns	ns
22:5n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	ND	ND	ND	ND	-	ns	-
22:6*n*-3	0.8 ± 0.1	0.9 ±0.1	1.0 ± 0.2	1.0 ± 0.3	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3± 0.1	*	ns	ns
Σ Saturates	34.7 ± 1.4	37.0 ± 0.4	36.1 ± 0.6	37.5 ± 2.8	33.0 ± 0.2 ^{c}	38.3 ± 0.4 ^{a}	35.5 ± 0.5 ^{b}	35.9 ± 0.2 ^{b}	ns	*	ns
Σ Monoenes	48.7 ± 0.5	49.0 ± 1.5	47.9 ± 0.3	47.9 ± 1.4	46.3 ± 0.5	47.0 ± 0.2	47.1± 1.6	46.6 ±1.7	ns	ns	ns
Σ PUFA	12.4 ± 0.3 ^{a}	10.3 ± 1.2 ^{b}	11.0 ± 0.3 ^{b}	10.5 ± 0.8 ^{b}	15.4 ± 0.1 ^{a}	12.1 ± 0.6 ^{b}	13.4 ± 1.0 ^{b}	13.5 ± 1.1 ^{b}	*	*	ns
n-3	1.4 ± 0.0	1.5 ± 0.1	1.9 ± 0.3	1.5 ± 0.4	0.8 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	*	ns	ns
n-6	11.3 ±0.2 ^{a}	8.9 ± 1.1 ^{b}	9.9 ± 0.1 ^{b}	9.3 ± 0.6 ^{b}	14.6 ± 0.1 ^{a}	11.5 ± 0.6 ^{b}	12.6 ± 0.1 ^{b}	12.8 ± 0.4 ^{b}	*	*	ns
n-3/*n*-6	0.13	0.17	0.15	0.17	0.05	0.06	0.06	0.06			

Hepatic fatty acid composition expressed as a percentage of total fatty acid pool is shown in Table 7. In all treatments, only 16 fatty acids were identified in fish liver tissue. A one third of the fatty acid pool was represented by oleic acid in all treatments and it was independent from dietary treatments and salinity. All the*n-6* fatty acids were influences by the diet and among those fatty acids only LA and ARA showed significant effects for salinity. Decosahexaenoic acid (DHA, 22:6n-3) was the single n-3 HUFA detectable in NFM group and DHA in FM group was almost three times greater than that in NFM group. Similar to fillet fatty acid composition, none of the HUFA except ARA was affected by the salinity. However, interactive effects of diets and salinity were not observed in the liver fatty acids.

5.3.3. **Relative gene expression**

5.3.3.1. Relative gene expression of hepatic IGF-I,IGF-II and GHR1 of Nile tilapia

Relative expression of hepatic IGF-I and IGF-II was significantly influenced by the salinity. Two-way ANOVA showed that there were no dietary effects or interactive effects of diets and salinity on gene expression of IGF-I and IGF-II (Fig.1). The IGF-I expression of different salinity levels were not different from that of fish in 0‰ while hepatic IGF-I expression in 8‰ and 12‰ was down regulated in compared to fish reared at 4‰. Apparently IGF-II expression progressively decreased with increasing salinity. However, the drop of IGF-II expression between 0‰, 4‰ and 8‰ was not statistically different and down regulation was statistically significant at 12‰. The statistical analysis showed that there were no dietary impacts on expression level of hepatic IGF-II in Nile tilapia However, expression level of 4‰ and 8‰was not different either from 0‰ or 12‰. Gene expression of hepaticGHR1 of Nile tilapia was not affected by diets and salinity (Figure 5).

Table 25**.**Proximate composition (% wet basis) of whole body and fillet of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (n=3).

%	FM Diet				NFM Diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
Whole body											
Moisture	71.9 ± 0.2	72.1 ± 0.4	71.4 ± 0.1	71.9 ± 0.7	72.6 ± 0.4	71.6 ± 0.3	71.8 ± 0.3	71.9 ± 0.1	ns	ns	ns
Protein	15.3 ± 0.3	15.9 ± 0.5	15.6 ± 0.3	15.8 ± 0.1	15.6 ± 0.2	16.2 ± 1.1	15.5 ± 0.2	15.5 ± 0.5	ns	ns	ns
Lipid	9.1 ± 1.2	8.6 ± 0.3	9.4 ± 0.4	8.0 ± 0.7	8.4 ± 0.8	9.7 ± 0.8	10.0 ± 0.2	9.1 ± 0.5	ns	ns	ns
Ash	3.1 ± 0.3^{bc}	2.9 ± 0.2 ^{c}	3.5 ± 0.1^{b}	3.8 ± 0.1 ^{a}	2.6 ± 0.2^{b}	3.2 ± 0.3^{b}	2.8 ± 0.1^{b}	3.8 ± 0.2 ^{a}	ns	*	ns
Fillet											
Moisture	77.4 ± 0.4	77.1 ± 0.2	76.7 ± 0.2	77.0 ± 0.4	77.4 ± 0.1	77.5 ± 0.4	77.4 ± 0.1	77.2 ± 0.2	ns	ns	ns
Protein	20.1 ± 0.2	20.1 ± 0.2	20.6 ± 0.1	20.8 ± 0.3	20.0 ± 0.2	19.5 ± 0.3	19.4 ± 0.4	19.5 ± 0.2	*	ns	ns
Lipid	1.4 ± 0.0	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.7 ± 0.3	1.7 ± 0.1	ns	ns	ns
Ash	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	ns	ns	ns

Results are given as mean ± standard deviation. n=2 for each factor combination. Different letters in same row denote significant differences (Tukey's test, $P<0.05$) between salinity treatment within each dietary group D, diet; S, salinity; ns, not significant ($p > 0.005$)

Table 26. Percentage of individual fatty acids ± SE (% of all fatty acids in total lipids) together with total saturates, total monoenes, polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA and n-3 to n-6 ratio in the fillet of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (area %, n=3).

	FM diet				NFM diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
14:0	1.8 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	1.8 ± 0.1	1.3 ± 0.0	1.6 ± 0.3	1.3 ± 0.1	1.4 ± 0.1	*	ns	ns
16:0	24.9 ± 0.1	24.3 ± 0.4	25.3 ± 1.5	24.3 ± 0.1	24.1 ± 0.4	23.8 ± 2.0	22.7 ± 1.2	23.3 ± 0.7	*	ns	ns
16:1*n*-7	3.0 ± 0.3	3.4 ± 0.2	3.0 ± 0.2	3.3 ± 0.1	2.4 ± 0.1^{bc}	3.0 ± 0.4^{a}	2.6 ± 0.2^{ab}	2.5 ± 0.1^{bc}	*	*	ns
18:0	7.3 ± 0.4	6.9 ± 0.2	7.7 ± 0.0	6.9 ± 0.1	7.6 ± 0.1^{a}	6.5 ± 0.3^{b}	7.3 ± 0.2^{a}	7.2 ± 0.0^{a}	ns	*	ns
18:1*n*-9	17.3 ± 0.4^{b}	19.8 ± 0.6^{a}	17.9 ±0.1^{b}	19.8 ± 0.1^{a}	19.1 ± 0.5^{c}	23.1 ± 0.0^{a}	22.3 ±0.1^{ab}	21.5 ± 0.1^{b}	*	*	ns
18:1*n*-7	3.3 ± 0.1^{ab}	3.2 ± 0.1^{b}	3.6 ± 0.0^{a}	3.2 ± 0.0^{b}	3.2 ± 0.0^{a}	2.8 ± 0.0^{b}	3.1 ± 0.1^{a}	3.2 ± 0.1^{a}	ns	*	ns
18:2*n*-6	19.1 ± 0.4^{ab}	17.3 ± 0.7^{c}	18.5 ±0.1^{bc}	18.3± 0.1^{bc}	20.2 ± 0.1	20.3 ± 0.4	19.9 ± 0.2	20.0 ± 0.1	*	*	*
18:3*n*-3	1.1 ± 0.0^{a}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	0.8 ± 0.0^{b}	0.9 ± 0.1^{a}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}	*	*	*
18:4*n*-3	0.2 ± 0.0^{ab}	0.3 ± 0.1^{a}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	ND	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	*	*	ns
20:1*n*-11	0.8 ± 0.0^{ab}	1.1 ± 0.0^{a}	0.7 ± 0.1^{c}	0.9 ± 0.0^{b}	0.9 ± 0.1	1.0 ± 0.2	1.1± 0.1	1.0 ± 0.0	*	*	ns
20:4*n*-6	2.5 ± 0.2	2.4 ± 0.1	2.8 ± 0.2	2.4 ± 0.1	4.0 ± 0.1^{a}	3.1 ± 0.3^{b}	3.5 ± 0.2^{ab}	3.8 ± 0.2^{ab}	*	*	*
20:4*n*-3	ND	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	ND	ND	ND	ND	-	ns	-
20:5*n*-3	0.8 ± 0.0	1.0 ± 0.2	0.8 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	*	ns	*
22:4n-6	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.0	1.2 ± 0.0	1.1 ± 0.3	1.4 ± 0.2	1.3 ± 0.1	*	ns	ns
22:5n-6	1.2 ± 0.1	1.1 ± 0.0	1.0 ± 0.1	1.2 ± 0.0	3.1 ± 0.1	2.6 ± 0.7	2.8 ± 0.3	2.9 ± 0.2	*	ns	ns
22:5*n*-3	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	*	ns	ns
22:6*n*-3	7.5 ± 0.6	7.0 ±0.2	7.5 ± 0.9	7.5 ± 0.2	2.9± 0.4	2.1 ± 0.5	2.2 ± 0.2	2.1 ± 0.1	*	ns	ns
Σ SFA	34.0 ± 0.1	33.3 ± 0.5	35.0 ± 1.7	33.0 ± 0.2	32.9 ± 0.5	31.8 ± 2.0	31.3 ± 1.2	31.9 ± 0.8	*	ns	ns
Σ Monoenes	24.8 ± 0.2^{c}	28.1 ±0.2^{a}	25.2 ± 0.3^{bc}	27.2 ± 0.1^{b}	25.6 ± 0.6^{c}	30.0 ± 0.2^{a}	29.1± 0.1^{ab}	28.2 ±0.1^{b}	*	*	*
ΣPUFA	34.2 ± 1.6	32.2± 0.4	33.7 ± 1.4	33.5 ± 0.1	33.0 ± 0.1	30.1 ± 1.4	31.4 ± 0.6	31.7 ± 0.7	*	ns	ns
n-3	10.9 ± 0.8	10.6 ± 0.4	10.8 ± 1.1	10.8 ± 0.2	4.5 ± 0.1	3.7 ± 0.6	3.9 ± 0.2	3.8 ± 0.1	*	ns	ns
n-6	23.3 ± 0.8	21.6 ± 0.8	22.9 ± 0.3	22.7 ± 0.1	28.4 ± 0.2	27.1 ± 0.8	27.6 ± 0.4	28.0 ± 0.6	*	ns	ns
n-3/*n*-6	0.46	0.49	0.47	0.48	0.16	0.14	0.17	0.16			
PUFA/SFA	1.00	0.97	0.96	1.01	1.00	0.95	1.00	0.99			

Table 27. Percentage of individual fatty acids (% of all fatty acids in total lipids) together with total saturates, total monoenes, polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA and *n-3* to *n-6* ratio in the liver of Nile tilapia fed FM diet or NFM diet in different salinity levels over 12 weeks (area %, n=3).

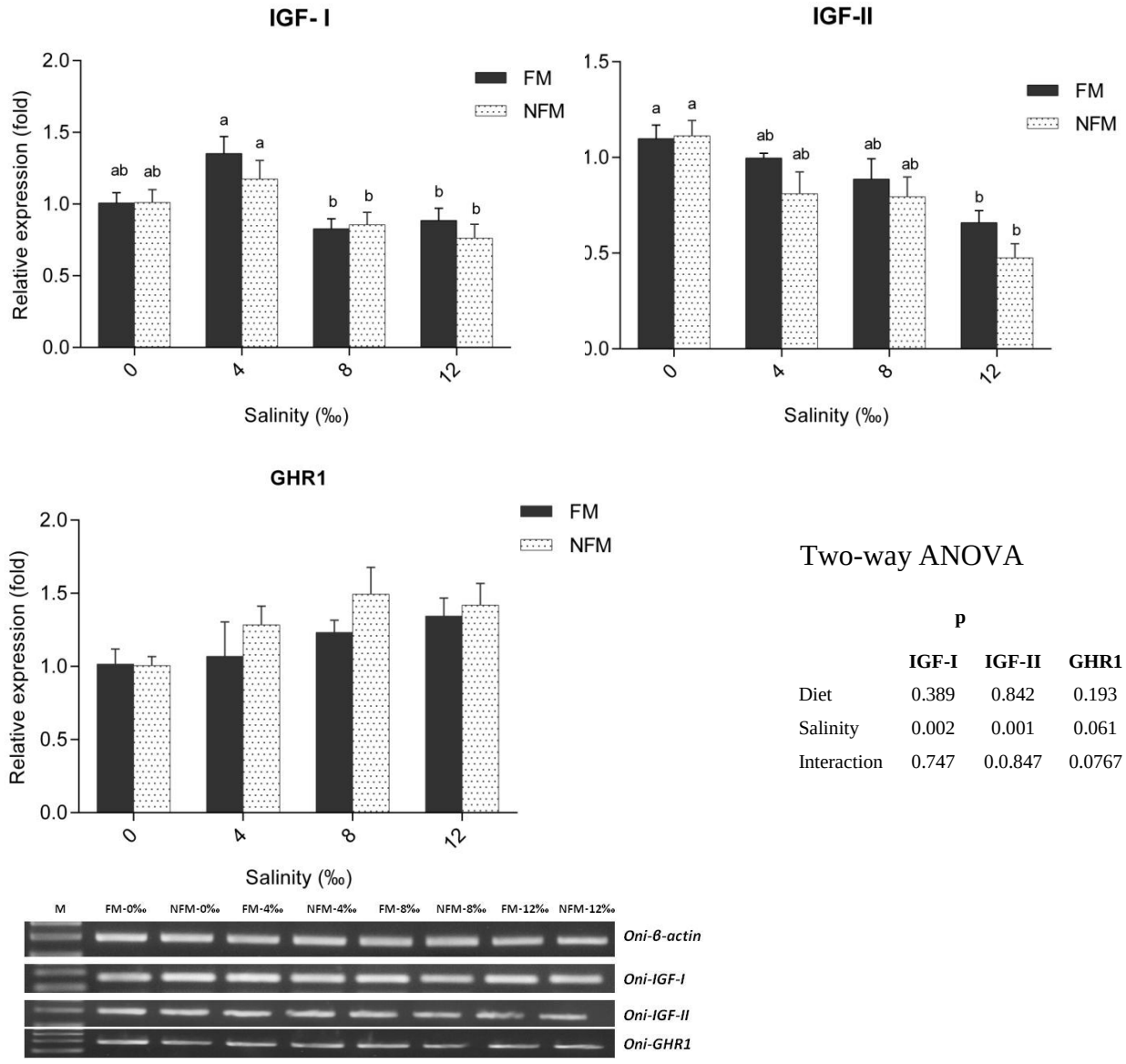
	FM diet				NFM diet				p		
	0 ‰	4 ‰	8 ‰	12 ‰	0 ‰	4 ‰	8 ‰	12 ‰	D	S	D x S
14:0	3.5 ± 0.1	3.5 ± 0.1	4.2 ± 0.2	3.9 ± 0.7	3.9 ± 0.1	3.8 ± 0.1	3.7 ± 0.2	3.8 ± 0.7	ns	ns	ns
16:0	24.7 ± 0.2	26.7 ± 0.6	25.6 ± 0.6	27.4 ± 2.6	23.5 ± 0.3 [c]	27.6 ± 0.2 [a]	26.1 ± 0.4 [b]	26.2 ± 1.0 [b]	ns	*	ns
16:1*n*-7	7.1 ± 0.0 [b]	7.1 ± 0.2 [b]	8.5 ± 0.4 [a]	7.9 ± 0.9 [ab]	6.7 ± 0.2	6.8 ± 0.2	7.1 ± 0.1	7.0 ± 0.4	*	*	ns
18:0	6.6 ± 0.1	6.9 ± 0.5	6.3 ± 0.1	6.2 ± 0.5	5.6 ± 0.1 [b]	7.0 ± 0.1 [a]	5.6 ± 0.2 [b]	5.9 ± 0.1 [b]	*	*	ns
18:1*n*-9	36.7 ± 0.4	36.9 ± 1.6	35.5 ± 0.1	33.5 ± 1.7	35.6 ± 0.3	36.0 ± 0.1	35.9 ± 1.3	35.6 ± 1.0	ns	ns	ns
18:1*n*-7	3.4 ± 0.1	3.3 ± 0.2	3.0 ± 0.1	3.2 ± 0.2	2.9 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	*	ns	ns
18:2*n*-6	10.5 ±0.3 [a]	7.9 ± 1.2 [b]	8.5 ± 0.4 [b]	8.4 ± 0.3 [b]	13.2 ± 0.0 [a]	9.1 ± 0.6 [b]	11.4 ± 0.9 [b]	11.0 ± 1.5 [b]	*	*	ns
18:3*n*-3	0.4 ± 0.0	0.3 ± 0.1	0.8 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	ns	ns	ns
20:1n-11	1.4 ± 0.1 [a]	1.4 ± 0.3 [a]	0.8 ± 0.1 [b]	1.2 ± 0.4 [ab]	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	ns	*	ns
20:4*n*-6	0.5 ± 0.0 [b]	0.6 ± 0.1 [a]	0.4 ± 0.1 [b]	0.6 ± 0.1 [ab]	0.8 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	*	*	ns
20:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	ND	ND	ND	ND	-	ns	-
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	*	ns	ns
22:5n-6	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	*	ns	ns
22:5n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	ND	ND	ND	ND	-	ns	-
22:6*n*-3	0.8 ± 0.1	0.9 ±0.1	1.0 ± 0.2	1.0 ± 0.3	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3± 0.1	*	ns	ns
Σ Saturates	34.7 ± 1.4	37.0 ± 0.4	36.1 ± 0.6	37.5 ± 2.8	33.0 ± 0.2 [c]	38.3 ± 0.4 [a]	35.5 ± 0.5 [b]	35.9 ± 0.2 [b]	ns	*	ns
Σ Monoenes	48.7 ± 0.5	49.0 ± 1.5	47.9 ± 0.3	47.9 ± 1.4	46.3 ± 0.5	47.0 ± 0.2	47.1± 1.6	46.6 ±1.7	ns	ns	ns
Σ PUFA	12.4 ± 0.3 [a]	10.3 ± 1.2 [b]	11.0 ± 0.3 [b]	10.5 ± 0.8 [b]	15.4 ± 0.1 [a]	12.1 ± 0.6 [b]	13.4 ± 1.0 [b]	13.5 ± 1.1 [b]	*	*	ns
n-3	1.4 ± 0.0	1.5 ± 0.1	1.9 ± 0.3	1.5 ± 0.4	0.8 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	*	ns	ns
n-6	11.3 ±0.2 [a]	8.9 ± 1.1 [b]	9.9 ± 0.1 [b]	9.3 ± 0.6 [b]	14.6 ± 0.1 [a]	11.5 ± 0.6 [b]	12.6 ± 0.1 [b]	12.8 ± 0.4 [b]	*	*	ns
n-3/*n*-6	0.13	0.17	0.15	0.17	0.05	0.06	0.06	0.06			

5.3.3.2. Relative gene expression of hepatic fadsd6 and elovl5 of Nile tilapia

Gene expression of hepatic fatty acid Δ6 desaturase of Nile tilapia was affected by salinity while fatty acid elongase expression was affected by the diets (Figure 6). Student's t-test showed that up-regulation of hepatic fatty acid elongase expression of fish fed NFM compared to that of fish fed FM diet is limited only to 8 ‰ and 12 ‰ salinity levels. This result suggests that dietary effects on the regulation of elongation gene expression are further moderated by the salinity.

5.3.3.3. Relative gene expression of IGF-I,IGF-II and GH of gills

In gills, relative gene expression of IGF-I and GH was not affected by both factors and their interaction (Figure 7). However, similar to liver, expression of IGF-II was down-regulated with increasing salinity and changes were not significant among 4‰, 8‰ and 12‰ salinity levels in both diets.



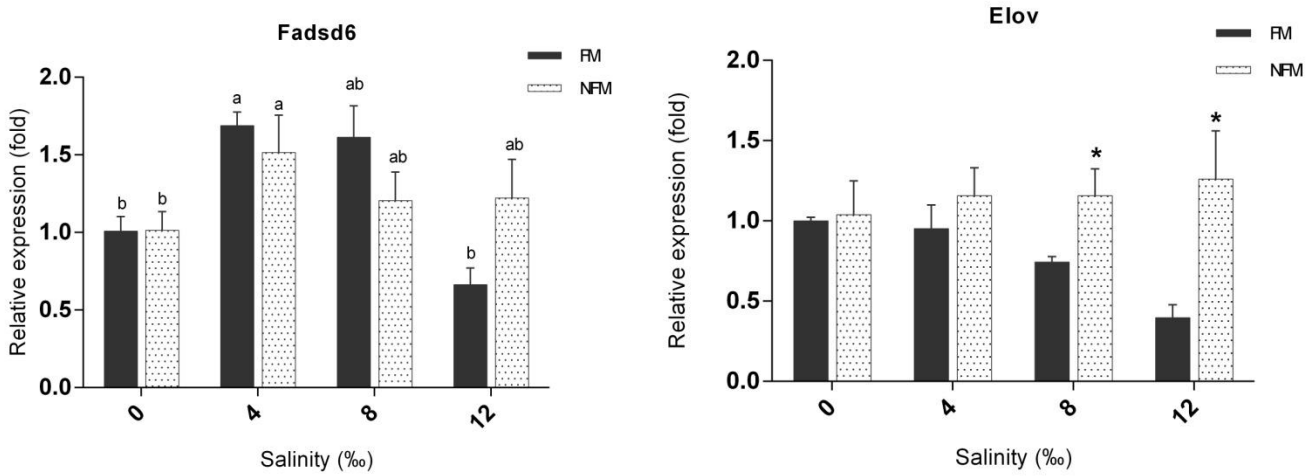
Two-way ANOVA

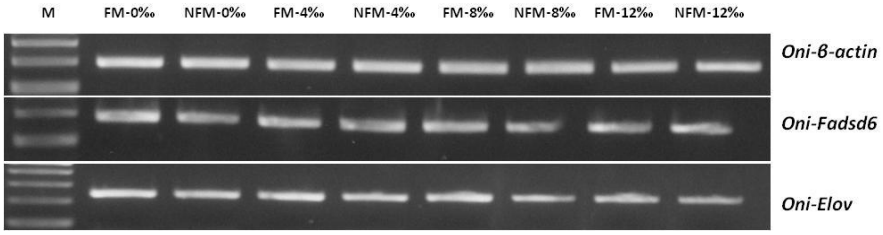
p

	IGF-I	IGF-II	GHR1
Diet	0.389	0.842	0.193
Salinity	0.002	0.001	0.061
Interaction	0.747	0.0.847	0.0767

Figure 5.Effects of FM and NFM diet and salinity on the expression of hepatic IGF-I, IGF-II, and GHR1 genes in Nile tilapia normalized to β-actin.

The values are mean ± SEM from four samples after duplicate PCR analysis. Different letters denotes significant differences ($p < 0.05$) between salinity levels within each dietary group (Tukey's test).The PCR products were verified by agarose gel electrophoresis and are shown. M ; 100 bp molecular marker

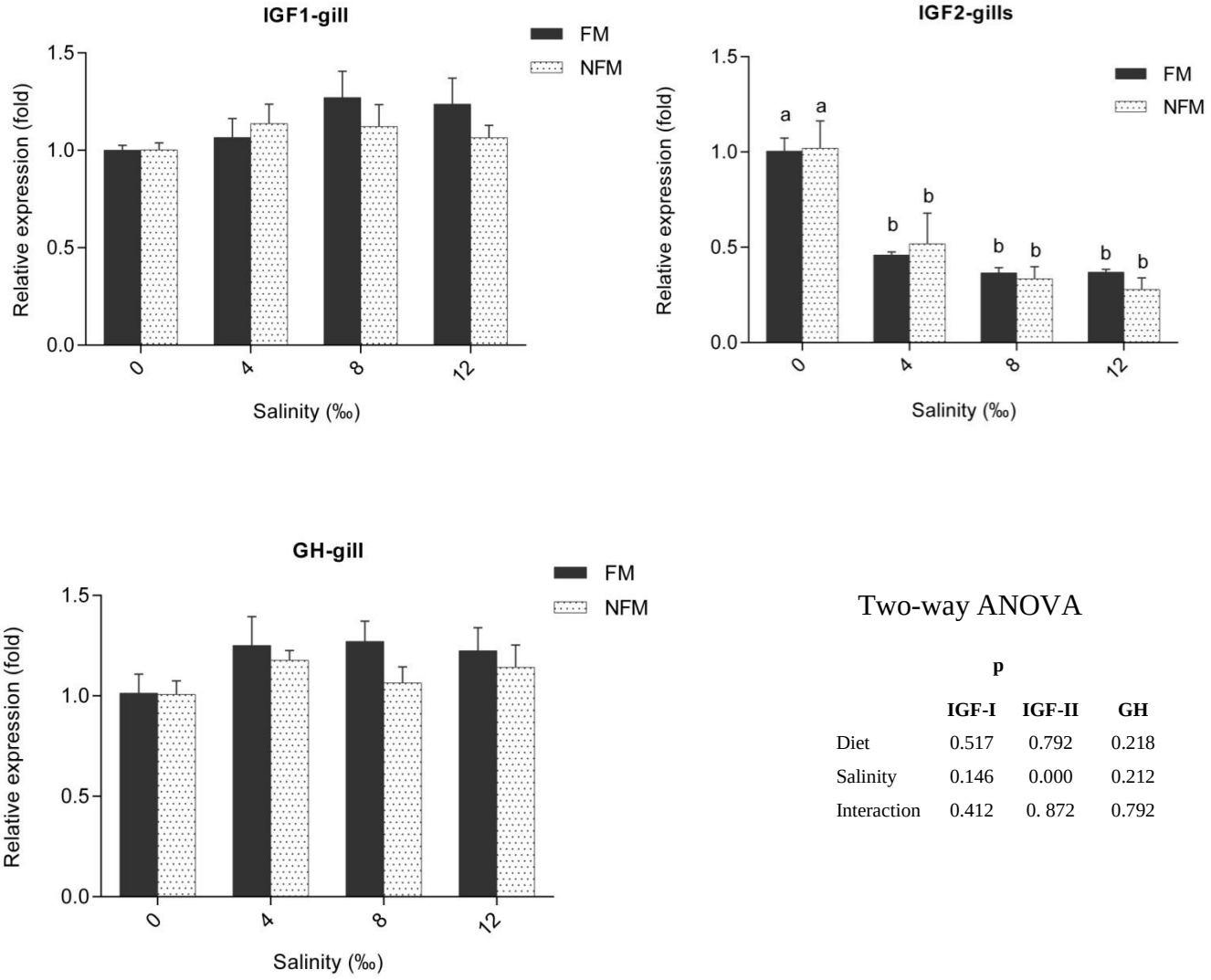




	p	
	Fadsd6	**Elov**
Diet	0.974	0.009
Salinity	0.004	0.517
Interaction	0.069	0.105

Figure 6.Effects of FM and NFM diet and salinity on the expression of fatty acid Δ6 desaturase and putative PUFA elongase genes in liver of Nile tilapia normalized to β-actin.

The values are mean ± SEM from four samples after duplicate PCR analysis. Probability values (p value) of main effects (diet and salinity) and interactive effects determined by two-way ANOVA are shown. Different letters denotes significant differences ($p < 0.05$) between salinity levels within each dietary group (Tukey's test). An asterisk indicates that the expression of a specific gene at a particular salinity for fish fed NFM was significantly different ($p < 0.05$) to the gene expression in fish fed FM (Student's t-test). The PCR products were verified by agarose gel electrophoresis and are shown



Two-way ANOVA

	p		
	IGF-I	**IGF-II**	**GH**
Diet	0.517	0.792	0.218
Salinity	0.146	0.000	0.212
Interaction	0.412	0. 872	0.792

Figure 7**.** Effects of FM and NFM diet and salinity on the expression of IGF-I, IGF-II, and GH genes in gills of Nile tilapia normalized to β-actin.

The values are mean ± SEM from four samples after duplicate PCR analysis. Different letters denotes significant differences ($p < 0.05$) between salinity levels within each dietary group (Tukey's test). The PCR products were verified by agarose gel electrophoresis and are shown. M ; 100 bp molecular marker

5.4. Discussion

Nile tilapia is a freshwater fish and results of this study suggest that fishmeal free diets could be applied to tilapia cultured in saline waters up to 12‰ without negative effects on growth performances compared to fish reared in freshwater (0‰). This was evident by the equal or higher FMW and % SGR reported for three salinity levels tested in compared to 0‰ in the present study for both diets and comparable to values previously reported for same species in freshwater (Likongwe et al., 1996, El‐Sayed, 1998, Fontainhas-Fernandes et al., 1999, Stadtlander et al., 2013, Plaipetch and Yakupitiyage, 2014). Fish reared in 4‰ showed higher % SGR compared to that of fish in 8‰ and 12‰ and this could be partly associated to the influence of salinity on feed intake and feed utilization efficiencies. However, from our result, it is difficult to explain whether the higher feed intake observed in 4‰ was due to salinity or other poorly defined factors. Therefore further studies are required to fully explore feeding regimes of juvenile Nile tilapia in saline water.

Effects of salinity on feed intake have previously been reported for many species (Imsland et al., 2001, Rubio et al., 2005, Wang et al., 1997).Growth and feed conversion efficiency of common carp was high in 0‰ to 2.5‰ salinity range and they found that this higher growth rate is correlated with the feed intake which observed to be diminished with increasing salinity (Wang et al., 1997).The present study also showed that increasing salinity increases the FCR and decrease the PER and can therefore be increased the feed cost in aquaculture of this species in saline water. However, the feed utilization parameters observed in this study for different salinity levels are in agreement with previous studies of same species in freshwater (Twibell and Brown, 1998, Takeuchi et al., 2002, Coyle et al., 2004, He et al., 2014, Plaipetch and Yakupitiyage, 2014).

Although, significant changes in growth performances were not observed among two dietary treatments, we found that dietary inclusion of fishmeal as a single source of marine derived feedstuff in the diet made the significant impact on the fatty acid composition of whole body and fillet. However, the overall fatty acid profile we observed in this study for Nile tilapia is almost similar to previously reported values (de Souza et al., 2007, Justi et al., 2003, Ng et al., 2013). Similar to other fish species, fatty acid composition of tilapia is closely influenced by the dietary fatty acid inputs (Justi et al., 2003, Ng et al., 2013, Tadesse et al., 2003, Teoh et al., 2011) and in this study, fatty acids deposited in flesh of fish in all salinity level were closely correlated with that of their respective diets. In general, HUFA such as 20:4n-6, 22:5n-3 and 22:6n-3 showed the selective deposition in fish fillet as concentration of fillet is always higher than that of dietary concentration. Interestingly, selective utilization of 18:2n-6 and 18:3n-3 was also observed with lower concentration of those two fatty acids in the fillet than the diets. This result suggests that a high degree of metabolism of 18:2n-6 and 18:3n-3 for β-oxidation and/ or desturation and elongation to higher homolog(Francis et al., 2007).

When individual products of the various enzymatic stages of fatty acid desaturation and elongation pathway of 18:2n-6 were summed up, fish fed NFM diet had higher amount of n-6 products of all the steps in the pathway compared to fish fed FM diet. Opposite of this was observed in the pathway of 18:3n-3 desaturation and elongation. The dietary 18:2n-6 input is almost similar in two diets (1 to 1.1 ratio of FM to NFM) while 18:3n-3 was in 3:2 ratios for FM to NFM diet. Thedietary n-3HUFA inputs of FM diet were 40 times greater in compared to NFM diet which had only 0.1% of n-3 HUFA. At the end of the 12 week feeding trial, desaturation and elongation enzymes of Nile tilapia were able to reduce this ratio to 2:1 in whole body, 3:1 in fillet and 7:2 in liver.The capability of tilapia to bio-convert 18 carbon PUFA to

their long chain homologous has previously been reported (Henderson and Tocher, 1987, Olsen et al., 1990, Teoh et al., 2011, Tocher et al., 2002)and this result is also aclear indication of the effectiveness of fatty acid desaturase and elongase enzyme activity of Nile tilapia when diets were not supplemented with marine derived feedstuff.This observation confirmed the nutritional regulation of fatty acid desaturation and elongation of tilapia (Tocher et al., 2002).

However, individual fatty acid composition of whole body, fillet and liver of Nile tilapia in this study showed that desaturation and elongation of 18:3n-3 to its long chain homologous in fish fed NFM diet was unlikely to contribute the similar proportion of EPA and DHA to that of fish fed FM diet as previously reported (Tocher et al., 2002). Since fish oil was not used in this study, fishmeal was the single dietary ingredient represent marine derived feedstuff and NFM diet does not contain fish meal. Hence these results explain the consequence of fishmeal and fish oil free tilapia diets in commercial culture on the fatty acid composition of resulting fish.

According to the fatty acid composition of fillet and liver tissue, impacts of salinity was limited to C16, C18 and C20 fatty acids. This result suggests that changes in water salinity from 0 ‰ to 12 ‰ are unlikely to change the metabolic pathway of C22 HUFAs. This is further supported by the gene expression results. Relative expression of hepatic fatty acid Δ6 desaturase gene of Nile tilapia showed that its expression is not regulated by the dietary composition as well as dietary salinity interactions.However, the expression of fatty acid elongase gene demonstrated that it is under nutritional regulation and pair wise comparison of FM and NFM at each salinity level confirmed that this regulation is limited only to 8‰ and 12‰ salinity levels. Nutritional regulation of fatty acid desaturase and elongase genes has been reported for fish including Nile tilapia (Tocher et al., 2002, Zheng et al., 2004, Ren et al., 2012). Failure to observe nutritional regulation of desaturase gene in this study could be related with the dietary fatty acid

composition and source. A correlation of dietary fatty acid source and gene expression of Stearoyl CoA desaturase of tilapia has been reported (Hsieh et al., 2007).Therefore it is meaningful to explain this resultas differences in dietary fatty acid composition were not strong enough to show significant difference in expression of hepatic fatty acid Δ6 desaturase gene in two dietary groups. Further this may also be attributed to the wide variation of relative gene expression across individual animals. Even if fatty acid elongase gene was significantly up-regulated in NFM group, statistical differences in tissue fatty acid composition was not observed among dietary groups of particular salinity levels. This results suggests that Δ6 desaturase of fish was a rate limiting factor in bio-conversion pathway of C18 PUFA to their long chain homologous when diets were totally free from marine derived feedstuff in moderately high salinity. However, nutritional and environmental regulatory mechanisms of these two genes are needed to be clarified with further studies.

Relative expression of hepatic IGF-I was almost overlapped with the %SGR and indicated its role in growth. The IGF-II gene of Nile tilapia showed that its expression is regulated by the salinity in both liver and gill tissues. Similar to this study, down-regulation of hepatic IGF-II was observed in black-chinned tilapia (Link et al., 2010). Growth hormone or growth hormone receptor gene expression in this study was not affected by the diet or salinity and it might be due to the adaptation of fish to that saline condition as gene expression was measured after 12 weeks.

5.5. Conclusion

This study revealed the possibility of totally replacing fishmeal in Nile tilapia juveniles in saline water(up to 12‰) salinity without compromising growth performances.Further growth performance and feed intake of Nile tilapia juveniles is comparably higher in moderately low

salinity (4‰).However feed utilization efficiencies of non fishmeal diets are not comparable (poorer than fishmeal) with the fishmeal based diets in saline water. Results of gene expression study showed that there is no dietary and salinity interactive effects on expression level of the genes evaluated.

References

AOAC (2005) Official methods of analysis of AOAC International, AOAC International.

Baysoy, E., Atli, G. & Canli, M. (2013) The effects of salinity and salinity+ metal (Chromium and Lead) exposure on ATPase activity in the gill and intestine of tilapia Oreochromis niloticus. Archives of environmental contamination and toxicology, 64, 291-300.

Bœuf, G. & Payan, P. (2001) How should salinity influence fish growth? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 130, 411-423.

Carter, C., Houlihan, D., Kiessling, A., Médale, F. & Jobling, M. (2001) Physiological effects of feeding.Food intake in fish, 297-331.

Coyle, S.D., Mengel, G.J., Tidwell, J.H. & Webster, C.D. (2004) Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research, 35, 365-370.

Cruz, E.M.V., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B. & Borski, R.J. (2006) Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, Oreochromis *niloticus*. Aquaculture, 251, 585-595.

de Souza, N.E., Matsushita, M., de Oliveira, C.C., Franco, M.R.B. & Visentainer, J.V. (2007) Manipulation of fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fillets with flaxseed oil. Journal of the Science of Food and Agriculture, 87, 1677-1681.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2003) Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 220, 619-632.

El-Sayed, A.-F.M., Mansour, C.R. & Ezzat, A.A. (2005) Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture, 248, 187-196.

El‐Saidy, D.M. & Gaber, M. (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‐Saidy, D.M. & Gaber, M. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research, 34, 1119-1127.

El‐Sayed, A.F. (1998) Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. Aquaculture Research, 29, 275-280.

FAO, U. (2014) FAOstat. Retrieved Feb, 2014.

Folch, J., Lees, M. & Sloane-Stanley, G. (1957) A simple method for the isolation and purification of total lipids from animal tissues.J biol chem, 226, 497-509.

Fontainhas-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.

Francis, D., Turchini, G., Jones, P. & De Silva, S. (2007) Growth performance, feed efficiency and fatty acid composition of juvenile Murray cod, *Maccullochella peelii peelii*, fed graded levels of canola and linseed oil. Aquaculture nutrition, 13, 335-350.

He, J.-y., Han, B., Tian, L.-x., Yang, H.-j., Zeng, S.-l.& Liu, Y.-j. (2014) The sparing effect of cystine on methionine at a constant TSAA level in practical diets of juvenile Nile tilapia *Oreochromis niloticus*. Aquaculture Research, n/a-n/a.

Henderson, R.J. & Tocher, D.R. (1987) The lipid composition and biochemistry of freshwater fish. Progress in lipid research, 26, 281-347.

Imsland, A.K., Foss, A., Gunnarsson, S., Berntssen, M.H., FitzGerald, R., Bonga, S.W., v Ham, E., Nævdal, G. & Stefansson, S.O. (2001) The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). Aquaculture, 198, 353-367.

Justi, K., Hayashi, C., Visentainer, J., De Souza, N. & Matsushita, M. (2003) The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. Food Chemistry, 80, 489-493.

Kabeya, N., Takeuchi, Y., Yamamoto, Y., Yazawa, R., Haga, Y., Satoh, S. & Yoshizaki, G. (2014) Modification of the n-3 HUFA biosynthetic pathway by transgenesis in a marine teleost, nibe croaker. Journal of Biotechnology, 172, 46-54.

Kajimura, S., Uchida, K., Yada, T., Riley, L., Byatt, J., Collier, R., Aida, K., Hirano, T. & Grau, E. (2001) Stimulation of insulin-like growth factor-I production by recombinant bovine growth hormone in Mozambique tilapia, *Oreochromis mossambicus*. Fish Physiology and Biochemistry, 25, 221-230.

Kamal, A.H.M.M. & Mair, G.C. (2005) Salinity tolerance in superior genotypes of tilapia, Oreochromis niloticus, Oreochromis mossambicus and their hybrids. Aquaculture, 247, 189-201.

Kilambi, R.V. (1980) Food consumption, growth and survival of grass carp *Ctenopharyngodon idella* Val at four salinities. Journal of Fish Biology, 17, 613-618.

Lambert, Y., Dutil, J.-D.& Munro, J. (1994) Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 51, 1569-1576.

Likongwe, J.S., Stecko, T.D., Stauffer Jr, J.R. & Carline, R.F. (1996) Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture, 146, 37-46.

Link, K., Berishvili, G., Shved, N., D'Cotta, H., Baroiller, J.-F., Reinecke, M. & Eppler, E. (2010) Seawater and freshwater challenges affect the insulin-like growth factors IGF-I and IGF-II in liver and osmoregulatory organs of the tilapia. Molecular and cellular endocrinology, 327, 40-46.

Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method.methods, 25, 402-408.

Magdeldin, S., Uchida, K., Hirano, T., GRAU, E.G., Abdelfattah, A. & Nozaki, M. (2007) Effects of environmental salinity on somatic growth and growth hormone/insulin‐like growth factor‐I axis in juvenile tilapia *Oreochromis mossambicus*. Fisheries Science, 73, 1025-1034.

Mancera, J.M. & McCormick, S.D. (1998) Evidence for growth hormone/insulin-like growth factor I axis regulation of seawater acclimation in the euryhaline teleost*Fundulus heteroclitus*. General and comparative endocrinology, 111, 103-112.

Moutou, K.A., Panagiotaki, P. & Mamuris, Z. (2004) Effects of salinity on digestive protease activity in the euryhaline sparid *Sparus aurata* L.: a preliminary study. Aquaculture Research, 35, 912-914.

Ng, W.-K., Chong, C.-Y., Wang, Y. & Romano, N. (2013) Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. Aquaculture, 372, 97-110.

Nordrum, S., Bakke-McKellep, A., Krogdahl, Å. & Buddington, R. (2000) Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmosalar* L.) and rainbow trout (*Oncorhynchusmykiss*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 125, 317-335.

Olsen, R.E., Henderson, R.J. & McAndrew, B. (1990) The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by *Tilapia* (*Oreochromis*) *nilotica* in vivo. Fish Physiology and Biochemistry, 8, 261-270.

Plaipetch, P. & Yakupitiyage, A. (2014) Effect of replacing soybean meal with yeast ‐ fermented canola meal on growth and nutrient retention of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). Aquaculture Research, 45, 1744-1753.

Prunet, P. & Bornancin, M. (1989) Physiology of salinity tolerance in tilapia: an update of basic and applied aspects. Aquatic Living Resources, 2, 91-97.

Pullin, R.S. & Lowe-McConnell, R.H. (1982) The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy, WorldFish.

Reinecke, M., Bjornsson, B.T., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D.M. & Gutierrez, J. (2005) Growth hormone and insulin-like growth factors in fish: Where we are and where to go. General and Comparative Endocrinology, 142.

Rubio, V., Sánchez-Vázquez, F. & Madrid, J. (2005) Effects of salinity on food intake and macronutrient selection in European sea bass.Physiology & behavior, 85, 333-339.

Santiago, C.B. & Lovell, R.T. (1988) Amino acid requirements for growth of Nile tilapia.The journal of nutrition, 118, 1540-1546.

Schaeffer, T.W., Brown, M.L., Rosentrater, K.A. & Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co ‐ products by Nile tilapia.Journal of animal physiology and animal nutrition, 94, e348-e354.

Stadtlander, T., Khalil, W., Focken, U. & Becker, K. (2013) Effects of low and medium levels of red alga Nori (*Porphyra yezoensis Ueda*) in the diets on growth, feed utilization and metabolism in intensively fed Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture Nutrition, 19, 64-73.

Tadesse, Z., Boberg, M., Sonesten, L. & Ahlgren, G. (2003) Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish *Oreochromis niloticus* L.–A laboratory study. Aquatic Ecology, 37, 169-182.

Takeuchi, T., Lu, J., Yoshizaki, G. & Satoh, S. (2002) Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. Fisheries science, 68, 34-40.

Tandler, A., Anav, F.A. & Choshniak, I. (1995) The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, *Sparus aurata*, larvae. Aquaculture, 135, 343-353.

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.

Tocher, D.R., Agaba, M., Hastings, N., Bell, J.G., Dick, J.R. & Teale, A.J. (2002) Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). Fish Physiol Biochem, 24.

Tsuzuki, M.Y., Sugai, J.K., Maciel, J.C., Francisco, C.J. & Cerqueira, V.R. (2007) Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. Aquaculture, 271, 319-325.

Twibell, R.G. & Brown, P.B. (1998) Optimal Dietary Protein Concentration for Hybrid Tilapia *Oreochromis niloticus* × *O. aureus* Fed All‐Plant Diets. Journal of the world aquaculture Society, 29, 9-16.

Ueda, A. (2005) Assessment of growth and its regulation through insulin-like growth factor-I in southern flounder, Paralichthys lethostigma.

Wang, J.-Q., Lui, H., Po, H. & Fan, L. (1997) Influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. Aquaculture, 148, 115-124.

Watanabe, W., Ellingson, L., Wicklund, R. & Olla, B. (1988) The effects of salinity on growth, food consumption and conversion in juvenile, monosex male Florida red tilapia In The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings, Vol. 15, pp. 515-523.

Watanabe, W.O., French, K.E., Ernst, D.H., Olla, B.L. & Wicklund, R.I. (1989) Salinity during early development influences growth and survival of Florida red tilapia in brackish and seawater. Journal of the World Aquaculture Society, 20, 134-142.

Yan, B., Wang, Z.-H.& Zhao, J.-L. (2013) Mechanism of osmoregulatory adaptation in tilapia. Molecular biology reports, 40, 925-931.

Zhao, B., Katagiri, T., Kondo, H. & Hirono, I. (2015) Comparative analysis of two types of CXCL8 from Japanese flounder (*Paralichthys olivaceus*). Developmental & Comparative Immunology, 52, 37-47.

CHAPTER 6

Chapter 6. General Conclusion

According to the recent FAO statistics, tilapia aquaculture consumes 7% of fishmeal total fishmeal usage in aquaculture. Therefore, complete fishmeal replacement with corn co-product is important for reducing production cost as well as reducing the reliance on marine derived feedstuff.

These three experimental results concluded that, HPDDG and DDGS can be effectively utilize as complete fishmeal replacer with amino acid supplementation in Nile tilapia diets without compromising growth or feed utilization parameters in freshwater or saline water. Further there is no possibility of changing the fillet color of fish due to the yellow pigments (Xanthophylls) present in the corn co-products.

Although HPDDG and DDGS are suitable for zero fishmeal diets, the relatively poor n-3 highly unsaturated fatty acid profiles of the resulting fish fillets are a potential drawback for these diets from the perspective of human nutrition. To mitigate this problem and to optimize the balance between cost-effectiveness of feed and the human-health protective properties of fish, a fish oil–based finishing diet could be applied. Further studies are required to demonstrate the effectiveness of this approach.