EFFECT OF COCONUT OIL LEVELS ON GROWTH AND REPRODUCTION PERFORMANCE OF FEMALE NILE TILAPIA (*Oreochromis niloticus* L.)

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บทคัดย่อ

เรื่อง : ผลของน้ำมันมะพร้าวต่อระบบสืบพันธุ์และการเติบโตของปลานิล (Nile tilapia, *Oreochromis niloticus* L.)

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อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ธนาทิพย์ แหลมคม

คำสำคัญ : Nile tilapia (*Oreochromis niloticus* L.), น้ำมันมะพร้าว, ระบบสืบพันธุ์, ประสิทธิภาพการเจริญเติบโต

การศึกษาระดับของน้ำมันมะพร้าว (Coconut Oil; CO) ต่อการเจริญเติบโตและระบบสืบพันธุ์ของลูกปลานิลเพศเมียที่อายุ 2 เดือน (Nile tilapia, *Oreochromis niloticus* L.) มีน้ำหนักเริ่มต้นเฉลี่ย 14.57 ± 0.96 กรัม ดำเนินการทดลอง 4 ชุดการทดลอง ประกอบด้วย ชุดการทดลองที่ผสมน้ำมันมะพร้าว 3 % (35 ml/Kg ของ CO) ชุดการทดลองที่ผสมน้ำมันมะพร้าว 6 % (70 ml/Kg ของ CO) ชุดการทดลองที่ผสมน้ำมันมะพร้าว 9 % (105 ml/Kg ของ CO) และ ชุดการทดลองที่ผสมน้ำมันมะพร้าว 12 % (140 ml/Kg ของ CO) มีการให้อาหารที่ระดับโปรตีน 40 % ทุกชุดการทดลอง อัตราการให้อาหาร 3 % ของน้ำหนักตัว เป็นเวลา 90 วัน จากผลการศึกษา พบว่า ชุดการทดลองที่ผสมน้ำมันมะพร้าว 6 % มีน้ำหนักที่ได้รับ (98.76 ± 9.50 กรัม) และการเติบโตต่อวันเฉลี่ย (ADG) (0.80 ± 0.04 กรัมต่อวัน) ที่สูงกว่าชุดการทดลองอื่น (P>0.05) การพัฒนาระบบสืบพันธุ์ของปลาแต่ละชุดการทดลองไม่มีความแตกต่างกัน ดัชนีความสมบูรณ์เพศในชุดการทดลองที่ผสมน้ำมันมะพร้าว 6 % (2.80 ± 1.76^{a} %) และ 12 % (2.55 ± 1.05^{ab} %) มีแนวโน้มสูงกว่าชุดการทดลองอื่น (P<0.05) เช่นเดียวกันกับเส้นผ่าศูนย์กลางของไข่ปลา (ED) มีค่าเท่ากับ 1.83 ± 0.51^{a} และ 1.63 ± 0.26^{ab} มิลลิเมตร ตามลำดับ ในขณะที่ ชุดการทดลองที่ผสมน้ำมันมะพร้าว 3 % มีค่าความดกของไข่ (AF) และความดกของไข่ที่เทียบกับน้ำหนักแม่ปลา (RF) มีค่าเท่ากับ 2284.88 ± 272.57^{a} ฟอง และ 27.33 ± 1.61^{a} ฟองต่อน้ำหนักแม่ปลา ตามลำดับ เมื่อวิเคราะห์สารอาหารปลาตัวอย่างแต่ละชุดการทดลอง และทดลองที่ 6 มีแนวโน้มระดับโปรตีนสูงกว่าชุดการทดลองอื่น (55.18 ± 0.98 %) ในขณะที่ชุดการทดลองที่ผสมน้ำมันมะพร้าว 6 (3.67 ± 0.26 %) และ 12 % (2.81 ± 0.08 %) มีแนวโน้มระดับไนโตรเจนอิสระที่สูงกว่าชุดการทดลองอื่น (P<0.05) จากผลการศึกษาสามารถสรุปได้ว่า มีความเป็นไปได้ในการใช้น้ำมันมะพร้าวผสมในอาหารในระดับที่เหมาะสมเพื่อเพิ่มการพัฒนาของระบบสืบพันธุ์ปลานิลเพศเมีย

ABSTRACT

TITLE : EFFECT OF COCONUT OIL LEVELS ON GROWTH AND REPRODUCTION PERFORMANCE OF FEMALE NILE TILAPIA (*Oreochromis niloticus* L.)
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Effects of coconut oil (CO) levels on growth and reproduction performance of female Nile tilapia (*Oreochromis niloticus* L.) (average weight = 14.57 ± 0.96 g) were investigated after preparation of four experimental diets containing different levels of coconut oil 3% (35 ml/kg of CO), 6% (70 ml/kg of CO), 9% (105 ml/kg of CO) and 12% (140 ml/kg of CO). Fish fry were acclimatized for two months in cement tanks and later were stocked in 80L fiber tanks connected to aeration system where were fed with 3% of body weight of the referred experiment diet for 90 days. Growth performance (P>0.05) was evident on treatment at 6% CO of coconut oil showing higher weight gain (98.76 ± 9.50 g) and average daily gain (0.80 ± 0.04 g/day). There is no significant difference of reproductive parameters (P>0.05). The higher gonadosomatic index and egg diameter of individuals fed on 6 % (2.80 ± 1.76^{a} % and 1.83 ± 0.51^{a} mm) and 12 % (2.55 ± 1.05^{ab} % and 1.63 ± 0.26^{ab} mm) coconut oil was found. The greater absolute and relative fecundity (2284.88 ± 272.57^{a} eggs and 27.33 ± 1.61^{a} eggs/g female) were found in individuals fed on 3 % coconut oil. Proximate analysis on dry matter basis of whole body was monitored (P>0.05). The greatest crude protein (55.18 ± 0.98 %) was found in treatment 6%. The higher nitrogen free extract was found in treatments fed on 6 and 12 % coconut oil (3.67 ± 0.26 and 2.81 ± 0.08 %) while the higher crude lipid exhibited in treatment fed on 9 % coconut oil (29.14 ± 1.70 %). It may possible that the suitable concentration of coconut oil in fish feed can promote the reproductive performances of Nile tilapia female.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Fish is the main source of protein that can supply to the local people largely. The trend of global fish production showed inconstantly from 2012 to 2016 while the aquaculture production (inland and marine) has grown continuously from 90.02 metric tons in 2012 to 110.20 metric tons in 2016 (FAO, 2017) (figure 1.1). Aquaculture products supplied to more than 50% world's fish consumption (Pettersson, 2010).

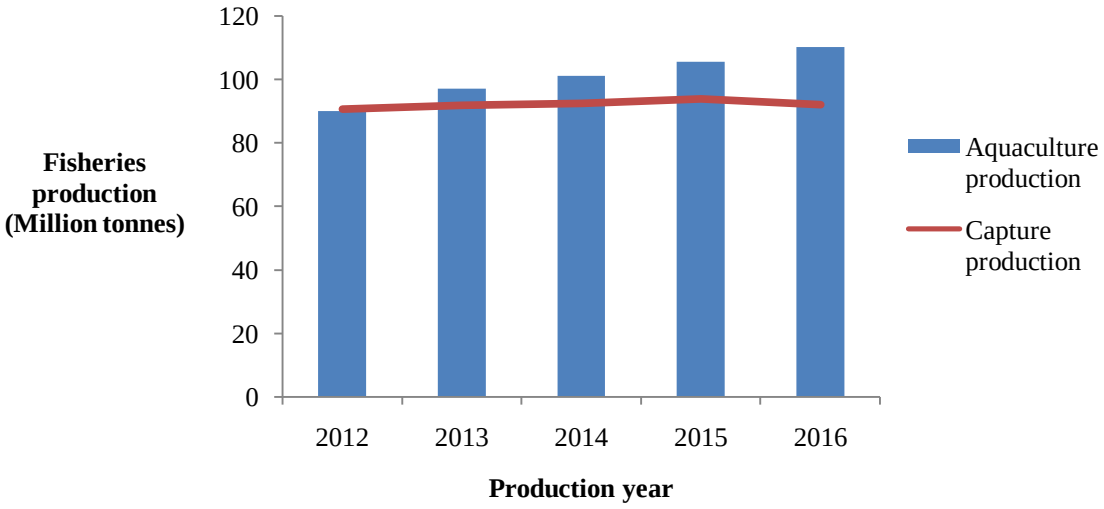

Figure 1.1 Aquaculture and capture production (Inland and marine) in 2012-2016
Source: Adapted from FAO (2017)

The growth of aquaculture industry depends on many factors e.g. sexual maturation broodstock, fry quality, hatchery management and nutrition of diet (Pettersson, 2010). Nile tilapia (*Oreochromis niloticus*) seed production is the second most demanded freshwater fish production after carp and ranged into 3.26 metric tons in 2012 and 3.93 metric tons in 2015 (FAO, 2017) (figure 1.2).

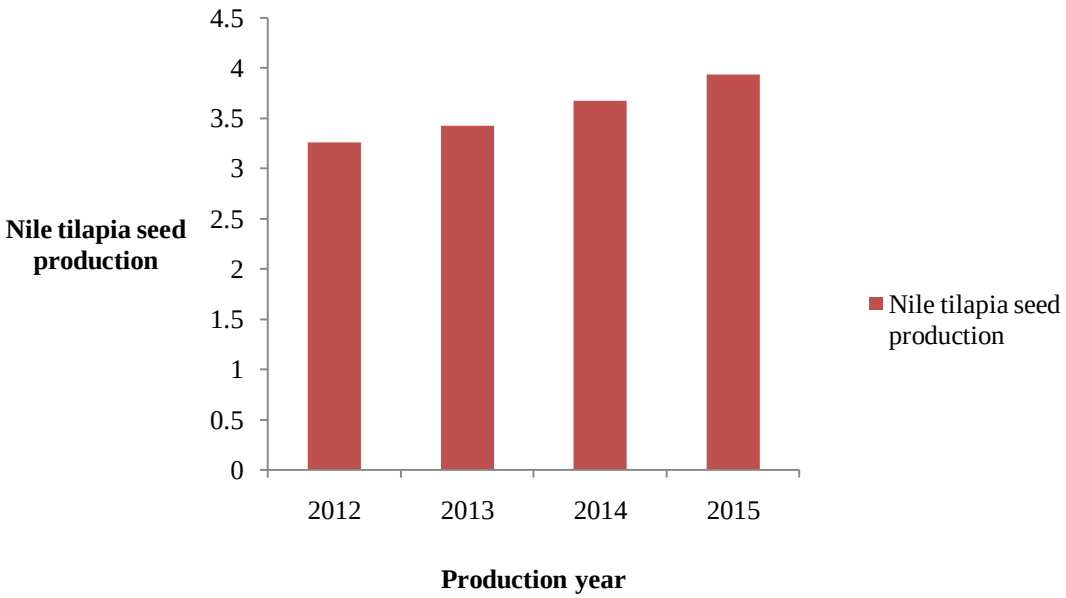

Figure 1.2 Nile tilapia world seed production in 2012-2015
Source: Adapt from FAO (2017)

Nile tilapia production has overcome a challenge to promote good quality of fingerlings by applying knowledge on management practices to improve broodstock reproductive efficiency e.g. nutrition, lipid source and ratio of fatty acid (Bhujel, 2000; Little & Edwards, 2003). Fish oil provided as lipid source in fish diet of Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*) and arctic charr (*Salvelinus alpinus*) promote the reproductive performances of broodstock (El-Sayed et al., 2005; Pettersson, 2010). With the scarcity and high price of fish oil, vegetable oil is the alternative source of oil (Bell & Sargent, 2002).

Several studies revealed the replacement of vegetable oil, palm oil, soybean oil and coconut oil in fish diet such as Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) (Santiago & Reyes, 1993; El-Sayed et al., 2005; Aderolu & Akinremi, 2009). Although composition of fatty acid in vegetable oil showed the different types and concentrations of saturated and unsaturated fatty acid, the mechanism of digestive and reproductive system can convert fatty acid to promote reproductive performances of broodstock. (Bell & Sargent, 2002; Pettersson, 2010).

Coconut oil is a cheap vegetable oil source which is available in Mozambique. The coconut oil composed of saturated fatty acids (lauric, mytistic, palmitic acids) and

less amount of unsaturated fatty acids (Rahman, 2000). Compared to other sources of vegetable oils, coconut oil has physic-chemical properties that make it a good candidate to replace crude lipid source from animal origin. Coconut oil is more stable from oxidative deterioration process and physic-chemical properties compared to other vegetables (Rahman, 2000).

1.2 Objectives

The present study was carried out to evaluate the effects of coconut oil on growth and reproduction performances of female Nile tilapia (*Oreochromis niloticus*) at different levels for development of the hatcheries productivity. Attention was focused on fish welfare and improvement of reproductive traits.

The specifics objectives are:

(1) Analyse the effects of coconut oil on female Nile tilapia on body composition

(2) Analyse the reproductive performance under effect of coconut oil in experiment diet of female Nile tilapia

(3) Analyse the growth parameters of female Nile tilapia when submitted to coconut oil as lipid source of experiment diet

CHAPTER 2
LITERATURE REVIEW

This chapter discusses indepth aspects related to Nile tilapia management, reproduction, feeding management, nutrition, various sources of alternative dietary lipid. It is organized as follows:

2.1 Nile tilapia production

2.2 Breeding of Nile tilapia

2.3 Nursing of Nile tilapia

2.4 Culture of Nile tilapia

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2.1 Nile tilapia production

Genus *Oreochromis* belongs to Cichild group as the native fish in African continentwhich has potential for aquaculture (Nwachi & Esa, 2016). Currently, many tilapia species such as Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*O. mossambicus*) and Blue tilapia (*O. aureus*) and their hybrid strains have been introduced and cultured in worldwide (Nwachi & Esa, 2016). Nile tilapia is the higher growth rate, sexual maturation within 3-4 months, tolerance to disease and higher survival rate in a wide range of environmental conditions (Hajizadeh & Shinn, 2015). Nile tilapia was improved genetically from the native strain to many varieties of strains such as GIFT (Genetically Improved Farmed Tilapia) and Chitralada strains (Furuya et al., 2004; Hajizadeh & Shinn, 2015; Nwachi & Esa, 2016).

The global production of Nile tilapia reached 4,500,000 tonnes in 2013(FAO, 2015) which showed the highest order in Republic of China (1600000 tonnes) followed by Egypt, Indonesia and Brazil (Fitzsimmons et al., 2014). In Republic of Mozambique, the production cycle of Nile tilapia ranged 6-8 months and obtained production around 838-1,179 tonnes per year in 2014-2016 (figure 2.1). The fingerlings have been provided by the private sector and the distribution is subsidised by the government. The stocking density of 5 fingerlings per square meter is ideal for extensive small scale farmers which fed on natural food (algae) and leftovers food stuffs. The limitation of water and feed management affects on fish growth and production (Chirindza, 2009).

Figure 2.1 Nile tilapia production in Republic of Mozambique.

Source: IDEPA (2016)

2.2 Breeding of Nile tilapia

Selective breeding of Nile tilapia it is been taken in account as one alternative to improve growth and reproductive traits since it has short generations periods. The breeding program of Nile tilapia aims to vary coloration in fish, to make it more tolerant to cold temperature and salinity, resistance to disease, and make all-male to have high growth rate. However, it is necessary to avoid potential hazards of inbreeding that may reduce quality of genetic traits. Another issue to take in consideration is hybridization because it is responsible for reduction in number of eggs due to low compatibility between the species (Bhujel, 2000). This reason let to hatchery farmers find new strategies to cope with reduction in number of eggs such as use broodstock to a certain younger age before absolute and relative fecundity declines and to avoid spawn frequency to decrease as tilapia female gets older (Bhujel, 2000). In tilapia, females maturing earlier at smaller size produces smaller eggs but relatively more eggs than a larger fish per unit body weight. Females that grow and mature fast tend to stop spawning earlier. Relative fecundity decreases with maternal age, weight and length. Nile tilapia females of larger size were found to produce more and bigger eggs and more fry per female, but smaller females spawn

more frequently. Hierarchy social behaviour of male tilapia ends up controlling most of the spawning, resulting in many females not spawning. To break this hierarchy it is been provided artificial nests and in this way more females make contact with more males and more spawn frequency. Hierarchy can also be minimized by spawning females with smaller and uniformed-sized males (Bhujel, 2000).

2.3 Nursing of Nile tilapia

Hatcheries farms face a challenge for nursing Nile tilapia as it requires labour workforce, specialized water recirculation incubator for eggs and nearly hatched fry and depend on the seasonal demand for Nile tilapia seed. This high demand of Nile tilapia in the beginning of the monsoon season increases the need to nurse fries in great quantities in happas for long periods when water low temperature reduce spawning of Nile tilapia. 30 days after hatching Nile tilapia fry are fed fish meal impregnated with 17 α-methyl testosterone to become all-male Nile tilapia by doing this method of sex reversal in a decreasing feeding rate of 20%, 15% and 10% of biomass/day during days intervals of 0 to 10, 11 to 20 and 21 to 30, respectively before transfer to happas in earthen ponds. After nursing fry for 30 days, Nile tilapia is transferred and classified according to size and transferred to different happas in earthen ponds or tanks. In this new environment it is necessary to protect the floating cages from predatory fish main species, snakehead (*Channa striata*) and climbing perch (*Anabas testudineus*) to guarantee high survival rate of Nile tilapia fingerlings (Little & Edwards, 2003).

2.4 Culture of Nile tilapia

Fingerling production depends on the culture system and the degree of intensification. The culture system can be a earthen pond, cage in ponds and tanks managed according to the degree of intensification (semi-intensive, intensive and extensive). Cage farming in ponds it is a cheaper and convenient way to operate large numbers of broodstock compared to earthen ponds and tanks although the latter it is more efficient. In earthen ponds are normally fertilised with manure that allows the production of natural food to culture fingerlings up to juveniles and adult Nile tilapia. The levels of recruitment in mono-sex and mixed-sex fish for answer demand of

market makes the production cycle to raise from fingerlings to adult stage Nile tilapia for a period of 5 months, as harvested individuals achieves the mean size and yield demanded by needs of local market. Limiting factors of culturing Nile tilapia are presence of predatory fish and method of seed production which determines the quantity and quality of Nile tilapia individuals from mono-sex and mixed-sex production after hormonal sex reversal (Little & Edwards, 2003).

2.5 Factors of Nile tilapia production

2.5.1 Broodstock

Broodstock strain and management are the crucial factors on in several steps production e.g. breeding, nursing and culture (Hajizadeh & Shinn, 2015). The optimal environment of Nile tilapia culture composed of the range of water temperature at 26-29^{0}C. The water temperature is lower than 26^{0}C can retrieve fry production of Nile tilapia as it triggers low spawning frequency among Nile tilapia broodstock depending on strain (Soltan et al., 2011).

2.5.2 Strains

There are many strains of Nile tilapia: Chiltralada, GIFT. These and other strains were genetically improved to promote growth and reproduction performance, cold and disease resistance, salinity tolerance. According to needs of hatchery or grow-out farm each strain were obtained by mass selection, within family selection for many generations until get the superior individuals by exploiting the addictive genetic effects. Nile tilapia fish breeds throughout the year which is an advantage for genetic improvement manipulation in short time since hybridization is found to be not efficient in terms of seed production outcome due incompatibility of broodfish. Chiltralada strain is considered to be more pure in terms of high fecundity and spawning frequency which is not significantly different from GIFT strain. Therefore, selective breeding of stains improves growth and reproductive traits (Bhujel, 2000).

2.5.3 Fish broodstock nutrition

Several fish species have different protein requirements for proper growth performance. Nile tilapia optimum protein level range from 20 to 35% to improve fecundity, promote earlier spawning, for broostock growth. However, continuous

supply of protein to Nile tilapia can reduce spawn frequency, number of eggs per spawning and increases spawning interval.

Lipids are important in fish diets especially during embryonic development when significant changes and mobilization of lipids take place. An experiment found that females fish when submitted to squid meal provided good quality of eggs during the spawning period. The optimum lipid level depends on the content of fatty acids in its composition. Nile tilapia submitted with 5% of soybean oil in fish feed showed high production of seed whereas when administrated 5% of cod oil reported poor reproductive performance and good weight gain (Bhujel, 2000). Bendhack et al. (2014) conducted an experiment to evaluate the replacement of fish oil by soybean oil on juveniles fat snook (*Centropomus parallelus*) and concluded that lipid source substitution does not affect the performance and body composition of fat snook juveniles because body composition, growth rate, total fat deposition was not significantly different from each case possibly because depending on the diet supplementation of EPA and DHA levels might not affect juveniles fat snook (*Centropomus parallelus*).

2.5.4 Broodstock management

Many studies refer to the optimum stocking density to provide great fish production in any culture system. It was found that in cage farming in ponds 5 fish/m^2 it is more productive than 10 fish/m^2 for grow-out system and in hatchery system. Moreover, increasing stocking densities may cause deterioration of water quality causing to reduction of water quality leading to stress conditions that make fish not report great feed utilization efficiency, feed conversion rate, social hierarchy and competition for food (El-Sayed et al., 2002). In Mozambique Nile tilapia has been stocked in a density of 5 fish/m^2 in earthen pond (Chirindza, 2010).

In hatchery farms the best production of fingerlings were found in breeding happas that stock sex ratio was 2:1 (male:female) compared to 1:1 and 1:2 ratios due to high spawning frequency of females Nile tilapia even though fecundity in not affected (Bhujel, 2000). Moreover, it is necessary to select the broodstock according to the size preferably larger females and smaller males to increase the courtship rate, reduce male aggression to female Nile tilapia due to social hierarchy (Bhujel, 2000).

Nile tilapia is a mouthbreeders fish that incubate their eggs in mouth until it achieve swim free fry stage. Harvesting eggs from female Nile tilapia every 5 days is the appropriate procedure to ensure reduction of inter spawning interval so that it may increase egg production. Nonetheless, the harvesting period can vary from 5 to 15 days but still provides more fry output than if Nile tilapia would incubate eggs naturally after spawn (Bhujel, 2000).

2.6 Environmental factors

Currently, the environmental condition fluctuating by seasonal and climate changes can limit the activities of fish production e.g. flooding and drought (Bhujel, 2000). In the summer season, the river, lagoons and small lakes occur severe drought and no water supply for aquaculture activities in Republic of Mozambique (IDEPA, 2016).

2.6.1 Physical parameters

Environment fluctuation can be hazardous for fish in any culture system. The variation of day light duration and intensity can undermine fish growth, locomotor activities, metabolic rate, skin pigmentation, sexual maturation and reproduction development (Vera et al., 2013). An experiment was conducted by Campos-Mendonza et al. (2003) to evaluate reproduction response to photoperiod manipulation on Nile tilapia. The luminosity fluctuation was set to four photoperiods: short day (6L:18D), normal day (12L:12D), long day (18L:6D), and full day (24L:0D). Campos-Mendonza et al. (2003) found that larger eggs (egg size) were produced significantly under normal daylight (12L:12D) compared to other photoperiods treatments. Nile tilapia reared under long daylight (18L:6D) showed significant higher absolute and relative fecundity, and reduction of inter spawning interval in comparison with other treatments. This experiment reached the conclusion that a long daylight in tropics it is essential to trigger dynamics of ovarian development in Nile tilapia by affecting the brain–pituitary–gonadal axis that result in changes in gonadotropin releasing hormone (GnRH), and pituitary, FSH, LH. Moreover, day light duration and intensity influences the release of melatonin, a hormone that is strongly correlated with photoperiod manipulation in salmonids resulting in the advance or delay of spawning time, working as a regulator in

reproductive behaviour. In this way, the regulation of light duration and intensity in addition with increasing water temperature may help Nile tilapia to reach optimum synchronised spawning frequency in early season of production cycle. The results of this experiment concludes that regulation the luminosity environment in culture system alongside with increase water temperature might influence the reproductive endocrinology of tilapia, and thus affect the dynamics of ovarian development (Campos-Mendonza et al., 2003)

El-Sayed et al. (2005) conducted an investigation of effects dietary lipid source on spawning performance of Nile tilapia broodstock reared at different levels of salinity and found that in brackish water Nile tilapia reduce hatchability and absolute fecundity, increase of spawning interval, take more time for egg to hatch and yolk-sac to be absorbed compared to Nile tilapia reared in freshwater. This variation of environment from freshwater to brackish water affects reproduction performance in Nile tilapia. This study concluded that may be necessary to provide additional lipid source containing omega 3 highly unsaturated fatty acids (HUFA) for the broodstock reared in brackish water whereas on Nile tilapia reared in freshwater soybean oil it is ideal lipid source (El-Sayed et al., 2005).

In the tropics, the change from dry to raining season affects spawning performance and consequently reproduction. Many authors have reported increase of spawning intensity and egg output during the fresh raining season compared to warm dry season in tropics. This seed output increase have been reported due to reduced cool temperatures, increased water levels of the culture systems and natural systems (rivers and lakes), dilution of hormones or chemicals inhibitors, and waste metabolites. On other way, heavy rain may not be beneficial for tilapia since it is been found that it reduced spawning intensity. In this way, in culture systems it is been used water sprinkling to make artificial rain during the warm and dry seasons with a certain raining frequency (Bhujel, 2000).

Water temperature influences greatly fish metabolism especially reproduction. Nile tilapia and *Oreochromis mossambicus* can tolerate from 8 to 42^{o}C temperature range. Normally tilapia stops feeding below 16^{o}C but reproduction occurs above that 20^{o}C. Water temperature between 28 to 31^{o}C it is known to improve seed production. Higher temperatures can halt reproduction, affect viability of eggs and fry

as well as broodfish productivity. However, higher water temperature characteristic of tropics can be surpassed by making deep earthen ponds, pond shading, water sprinkling and improving nutrition (add vitamin E to broodstock diet) (Bhujel, 2000).

2.6.2 Chemical parameters

Dissolved oxygen it is one of many parameters that can determine survival of Nile tilapia in any culture system. The low level of dissolved oxygen (DO) can make fish start gasping for atmospheric air as a mean of survival in such environment. Therefore, low DO it is considered a stressful condition for fish development and it might indicate reduction on feed intake, appearance of black melanin pigments in the skin (morphology) and behaviour (gasping for air). Moreover, as fish becomes sick seed production and quality halts as indication that spawning was inhibited, and decrease of fecundity and hatchability. In this way, it is necessary to ensure constant aeration especially in green water systems such as earthen ponds that lack of DO range recommended for aquaculture (>5mg/L) is not a limiting factor for reproduction, or establish re-circulating systems in hatchery farms (Bhujel, 2000; Shoko et al., 2014).

The principal metabolic waste product of fish is ammonia in pond water. Ammonia is the most important water quality parameter that affects fish growth and production after dissolved oxygen. Ammonia occurs in two forms in water, and the sum of it is known as total ammonia nitrogen (TAN). TAN is composed of ionised ammonia (NH_4^+) and unionised ammonia (NH_3–N). Unionised ammonia is the most toxic form of ammonia to fish and its levels should always be zero. Ammonia range of 0.05–0.1 mg/l is recommended for aquaculture pond waters. It causes stress and damages gills and other tissues, even in small amounts. Exposure of fish to even low levels of ammonia over time makes them susceptible to bacterial infections, have poor growth and will not tolerate daily management of culture system. Poor fish pond water quality management can lead to lower fish production yields (Shoko et al., 2014).

pH is an important water quality parameter in fish farming systems as it affects the toxicity of other compounds to fish such as ammonia and chlorine. Controlling pH levels are related with aquatic animals respiration, photosynthesis, carbon dioxide (CO_2) and the bicarbonate (HCO_3^-) buffering system. pH levels are

recommended to be in range of 6.5–9.0 for aquaculture pond waters. Lower and upper than 4 and 11 pH levels respectively it is considered lethal for tilapia and freshwater fish in general as it prefers neutral or slightly alkaline water environments (Bhujel, 2000; Shoko et al., 2014).

2.6.3 Biological parameters

The biotic environment of pond water it is a biological parameter that is necessary to control to ensure that enough natural food is available for fish culture in particular of Nile tilapia. The biotic environment can be composed of macroinvertebrates and microinvertebrates. Most commonly is the presence of plankton in pond water that serve as natural food for Nile tilapia fry. However, selection and filtration of plankton depends on size of fish (Turker et al., 2003). The most common representative groups of phytoplankton species are Chlorophytes (*Pediastrum* sp., *Scenedesmus* sp., *Spirogyra* sp., *Tetraedron* sp., *Phacus* sp.), Cyanobateria (*Coelosphaerium* sp., *Cyanosarcina* sp., *Oxillatoria* sp., *Chroococcus* sp., *Pseudanabaena* sp. and *Microcystis* sp.), Euglenophytes (*Euglena* sp., *Strombomonas* sp., *Trachelomonas* sp., *Chroococcus* sp., *Coelospharium* sp.), Bacillariophyceae (cladocerans, diatoms), that are feed for zooplankton copepodes and rotifers in pond water. Benthic macroinvertebrates such as snails *Melanoides tuberculata*, *Biomphalaria pfeifferi, Lymnaea natalensis, Bulinus truncatus* and, oligochaetes, chironomid larvae (Diptera), *Microvela* sp., *Zygomyx* sp. insects are also beneficial for *Oreochromis niloticus* diet (Jean-Renaud et al., 2015; Moura e Silva et al., 2015).

In the pond water there are microorganisms such as bacteria that are essential for the ecology of the pond. Ammonia plays an important role within the nitrogen cycle of any aquatic environment. The nitrogen cycle of the aquatic environment is conducted by bacteria that run the oxidative process in which ammonia is first converted into nitrite (NO_2^-) by naturally occurring *Nitrosospira* sp. and *Nitrosomonas* sp. bacteria in the water, before further bacterial species *Nitrospira* sp. and *Nitrobacter* sp. convert the nitrite into nitrate (NO_3^-). This nitrification process occurs either on the surface of the mud substrate and plants or within the biofilter of a tank based system. The nitrite (NO_2^-) is still toxic to fish species but encourages the

growth and colonisation of *Nitrobacter* sp. to convert it to the less toxic nitrate form (Shoko et al., 2014).

2.7 Feed quality

The nutrition in fish feed can promote the quality of Nile tilapia fingerlings (Izquierdo et al., 2001). The variable types and levels of fatty acid can increase reproductive development of fish e.g. absolute fecundity, fertility, hatching success and sex cell quality through biosynthesis of highly unsaturated fatty acid (HUFA) (Izquierdo et al., 2001; El-Sayed et al., 2005).

2.7.1 Growth of Nile tilapia

Growth of Nile tilapia is related to water environment conditions, fish feed quality, stocking density and type of culture system. Water quality parameters out of the range recommended for aquaculture practice may cause loss in production yield in fish culture system. One factor that may affect growth is deterioration of water quality parameters increasing density of fish in culture system leads to social stress causing chronic stress response. Therefore, growth is not effective since metabolic energy is mobilized by physiological alterations provoked by the stress response. Therefore, increasing stocking densities may trigger deterioration of water quality causing to reduction of water quality leading to stress conditions that make fish not report great feed utilization efficiency, feed conversion rate, social hierarchy and competition for food (El-Sayed et al., 2002). In the extensive culture system fish are stocked in high density and still the productivity yield is high thanks to high technology applied to help monitor water quality parameters, amount of feed administrated compared to semi-intensive culture system. The fish feed quality also plays a role growth of Nile tilapia when the nutrients are in the right proportion to meet requirements for physiological process to provide metabolic energy (Bhujel, 2000).

2.7.2 Feeding rate and regime

In fish farms broodstock management involves determine the appropriate feeding rate and regime for any particular fish species. Nile tilapia broodstock appropriate feeding rate is 1% of biomass or body weight per day results in great number of seed production. For the case of female Nile tilapia mouthbreeder it is convenient to prove higher energy supplements with lower feeding rate since female

Nile tilapia have fry in mouth. Alternation of feeding rate from high immediately to lower or with natural feed in the pond water is more appropriate to reduce cost of production (Bhujel, 2000). In nature Nile tilapia is a constant grazer meaning that lesser feeding interval the better for seed production such as feeding regime of two times per day is commonly applied in hatcheries. The best time in day to feed Nile tilapia broodfish it a few hours after sunrise and before sunset because at night and early morning fish don't eat because lack of dissolved oxygen in aquatic environment (Bhujel, 2000).

2.7.3 Growth parameters

The growth parameters of average daily gain (ADG), feed conversion rate (FCR), Specific growth rate (SGR) reflects fish response to culture system condition. Many experiments use these growth parameters to measure the performance of fish in terms of amount and efficiency of feed was used to convert in body mass per day, and to estimate the weight gain over a certain production period. It is important for the fish farmer because it allows for the estimation of how much feed is required for the growing cycle. The rate of growth in fish depends on different factors such as species, age, water temperature, quality and quantity of food. Young fish are capable of doubling their weight in a much shorter time than when they are older due to fast growth rate. Therefore it is useful to know specific growth rate in different age of the fish. An experiment was conducted to evaluate the effects of different feeding levels on larval performance. Growth parameters such as weight gain, specific growth rate and feed conversion rate were calculated. The increase of feeding rates did not result in significant increase of growth parameters. On contrary, the lower feeding rate of 10% of biomass per day was the most efficient for FCR than other feeding levels, while SGR and weight gain were increased at higher feeding levels. The growth rate is accordance with previous studies and for better fry growth it is recommended to provide 30% to 45% of feeding level per day to Nile tilapia fry (El-Sayed et al., 2002).

2.8 Nutrition for Nile tilapia

2.8.1 Protein

Nutrition is an important factor that must be considered in effective aquaculture management otherwise the fish would be unable to get enough energy for their rapid growth (Ye et al., 2015).

2.8.2 Particularities of protein in Nile tilapia nutrition

Proteins are one of many essential nutrients that influence reproduction performance (number of eggs per spawning and spawning interval). Moreover, proteins are present in the eggs of fish, such as lipoproteins, hormones, and enzymes, responsible for determine egg quality and consequently the production of fry and fingerlings on a large scale. Therefore, it is necessary to ensure that nutritious status of female is guaranteed because it can influence gonad development and amount and quality of the eggs (Coldebella et al., 2011).

2.8.3 Optimal protein level in fish feed

There is an optimum dietary protein level ideal for high seed production yield in Nile tilapia that ranges from 27% to 35% (Bhujel, 2000). This goes in accordance with Gunasekera et al. (1996) that stated that the level of dietary protein influences the viability of offspring and gave the example of very low levels (10–20%) resulted in low fertilization rates of eggs and a large percentage of deformed larvae.

2.8.4 Results from previous experiment on protein levels in fish feed for several fish species

Bhujel (2000) revealed a study that shows evidence of this fact that *Oreochromis niloticus* fed with 35% produced eggs with more protein content than individuals fed with 20% and 10%. Moreover, 35% and 20% of crude protein in Nile tilapia feed showed high egg production per spawning than individuals fed with 10% same result found by El-Sayed et al. (2003) when submitted Nile tilapia to 40% of protein in feed in freshwater environment. In other study report, spawning interval reduced when Nile tilapia broodstock was fed with medium and higher levels of protein (27.5, 35, 42.5 and 50%) (less than 49 days) compared to individuals that were fed with 20% of protein (more than 58 days). On the other hand, fecundity parameters values were higher in the fish fed with medium dietary protein (27.6 and 35%) than

those fed with higher protein levels (42.6 and 50.1%), as high protein diets produced heavier and larger eggs at longer spawning intervals OR lower spawning frequency (Bhujel, 2000).

2.9 Lipid

2.9.1 Particularities of lipid in Nile tilapia nutrition

Broodstock nutrition is one of the most important factors limiting fish fry production and larval quality (Izquierdo et al., 2001). Apart of that, lipid composition of broodstock fish plays a crucial rule in spawning performance and larval survival and growth (El-Sayed et al., 2005). Highly unsaturated fatty acid (HUFA) content of broodfish feed significantly affects fecundity, fertility, hatching and viability of fish eggs and larval growth (Izquierdo et al., 2001). Depending on habitat fish requires a certain type of fatty acids because marine fish require n-3 fatty acids whereas freshwater fish requires n-6 fatty acids (El-Sayed et al., 2005).

2.9.2 Optimal lipid level in fish feed

The optimum lipid rate in fish feed varies since it depends on properties of dietary lipid source and fish species. Ghaedi et al. (2014) mentions Snakehead fish fry (*Channa striatus*) lipid requirement for better growth is 90g/Kg, 180g/Kg for rainbow traut, 280g/Kg gilthead seabream (*Sparus auratus*), whereas lipid requirement for sexual maturity and reproductive performance (fecundity, hatching rate, egg diameter and larvae length) is 180g/Kg for broodstock of Snakehead fish (*Channa striatus*).

2.9.3 Results from previous experiment on lipid levels in fish feed for several fish species

Many crude lipid sources have been tested in Nile tilapia and other broodfish species such as cod liver oil, corn oil, soybean oil, coconut oil (based cooking oil), combination of cod liver oil and corn oil, palm oil, fish oil or flaxseed oil, rapeseed oil and herring oil (Santiago & Reyes, 1993; Shearer & Swanson, 2000; El-Sayed et al., 2005; Rennie et al., 2005; Anido et al., 2015). Santiago & Reyes (1993) tested the effect of these crude lipid sources at 5% level on Nile tilapia reproductive performance and its results found out that cod liver oil (rich in n-3 high unsaturated fatty acid HUFA) resulted in poor reproductive performance, while the

highest fry production was obtained from fish fed a diet supplemented with soybean oil (rich in n-6 fatty acids).

2.10 Carbohydrate

2.10.1 Particularities of carbohydrate in Nile tilapia nutrition

Starch is a cheap source of energy and its introduction in the fish feed influences faeces stability (Amirkolaie, 2005). Besides this, dietary starch also increases gut microbial activity in tilapia and this may have a negative effect on faeces stability in Nile tilapia (Amirkolaie, 2005). The use of carbohydrates by fish is less efficient than land animals possibly due to low digestibility of carbohydrates associated with the availability of the enzyme α-amylase (Zainuddin et al., 2014).

2.10.2 Optimal carbohydrate level in fish feed

Most of broodfish fish diet contains carbohydrate that ranges between 29% to 32% not only to provide energy source during the reproduction process but also to improve spawning performance (El-Sayed et al., 2003; El-Sayed et al., 2005).

2.10.3 Results from previous experiment on carbohydrate levels in fish feed for several fish species

Hemre et al. (1995) studied the effects of dietary carbohydrate on gonad development of broostock cod (*Gadus morhua*) by submitting it to increasing levels of potato and corn starch. The results pointed to no significant effects on feed conversion rate, broodstock growth and gonodal development. Regarding biochemical test, plasma glucose remained on normal range indicating that no negative effects on high levels of dietary carbohydrate in broodfish. Glycogen, dry matter, protein and lipid levels in eggs didn’t vary significantly among different dietary treatments.

2.11 Vitamins and mineral traces

2.11.1 Particularities of vitamins and mineral traces

Vitamin E is important to vitelogenesis process that occurs in liver because it is transporter of lipoproteins (vitellogenin) from liver to the gonads allowing the oocytes to store enough lipids sufficient for embryo development in Nile tilapia fry (Bhujel, 2000; Izquierdo et al., 2001; El-Sayed et al., 2005; Aryani et al., 2014).

2.11.2 Optimal level of vitamins and mineral traces in fish feed

Nile tilapia broodfish feed as it garantees high fecundity rate and larval survival rate especially when combined 600 mg/Kg of Vitamin E, 1250 mg/Kg of Vitamin C and 120mg/Kg Zinc (Gammapila et al., 2007).

2.11.3 Results from previous experiment on vitamins and mineral levels in fish feed for several fish species

James et al. (2008) conducted an experiment to evaluate the effects of different levels of dietary vitamin E (0, 100, 200, 300, and 600 mg/kg diet) on growth, gonad weight, fecundity, and leukocyte count on in goldfish (*Carassius auratus*) for 120 days, and results shown that fish fed the 300 mg vitamin E/kg diet had the best feeding rate, weight gain, and specific growth rate. Also in same level of dietary vitamin E, James et al. (2008) reported great reproduction performance based on high gonad weight, high absolute fecundity and great hatchability rate, reduced spawning interval after 40 days, as well egg weight and diameter, and larvae weight and length compared to control group. Apparently 300 mg vitamin E/kg diet was ideal level to improve immune and reproductive system in goldfish (*Carassius auratus*). These results are in accordance with previous studies related to effects of vitamin E on gonadal maturity in the common carp (*Cyprinus carpio*), red seabream (*Pagrus major*), rainbow trout, Atlantic salmon (*Salmo salar*), which resulted in a higher gonadosomatic index, larger eggs, and more eggs with higher hatchability, and shorter spawning interval, as well as improved immune system of channel catfish (*Ictalurus punctatus*) (James et al., 2008).

2.12 Fatty acid from crude animals oil source

2.12.1 Type of fatty acid and composition

Animals have in their proximate body composition lipids that can be harvested to obtain fatty acids used in fish feed. Most commonly it is been used fish oil (e.g cod oil) since it is a source n-3 high unsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid.

2.12.2 Effect of fatty acid on growth and reproductive performances

Marine fish requires crude lipid sources containing omega 3 highly unsaturated fatty acids (HUFA) for optimum growth and reproduction performance

whereas freshwater fish requires broodstock diets containing omega 6 HUFA or an appropriate n-6 HUFA/n-3 HUFA ratio (Craig & Helfrich, 2009).

2.12.3 Limitation of fatty acid from animals

The natural freshwater food provides substantial nutrients that can improve growth, spawning and reproduction performance in fish. Primary feed is available in water column in great diversity and it is important for natural freshwater food for several fish (Musa et al., 2012). So far, it has been identified brine shrimp (*Artemia* spp.) and rotifer (*Brachionus plicatilis*) as an important live foods to be supplied thanks to their nutritive suitability (Hasan, 2001). *Artemia nauplii* and rotifers contain protein (74%), carbohydrate (3%) and lipids (18%) that more than sufficient compared to composition of commercial feed provided to fish (Musa et al., 2012). Even though *Artemia* spp. is low in the essential fatty acids eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), it is sufficient to grand good growth and survival rate to fish but not sufficient for total fecundity and larval quality, therefore it is necessary to have a formulated reproduction diet that can meet requirements for good reproduction performance (Hasan, 2001).

2.13 Fatty acid from crude vegetable oil source

2.13.1 Type of fatty acid and composition

Vegetable oil (e.g soybean oil, palm oil, coconut oil, linseed oil) that are a source of omega 6 highly unsaturated fatty acids like arachidonic acid. Vegetable oils can be different from one to another depending on its chemical composition. Generally, vegetable oils are rich in C18 polyunsaturated fatty acids (PUFA), but lack of highly unsaturated fatty acids (HUFA) (Bell & Sargent, 2002). Specifically vegetable oils are rich in omega 6 and omega 9 fatty acids, mainly linoleic acid (18:2n-6) and oleic acid (18:1n-9), and with moderate or low levels of omega 3 (except linseed oil), mainly α-linolenic acid (18:3n-3) (Pettersson, 2010).

2.13.2 Properties of coconut oil

Crude coconut oil is composed by 90% of saturated fatty acids predominantly by lauric (47.5%), followed by mytistic, palmitic acid and less percentage of linoleic acid 18:2n-6 (1.6%) (table 2.1). Most characteristics found in coconut oil that distinguish from other oils are the high content of short and moderate

content of long chain fatty acids. At cold temperatures coconut oil is solidified (<5^{o}C) but melting point ranges between 24 – 27^{o}C. This melting point range is consequence of triacylglycerol (lower molecular weight of glycerol) and fatty acids composition (Rahman, 2000). Table 2.1 expresses the fatty acid profile of coconut oil according to Rahman (2000).

Table 2.1 Fatty acid profile of coconut oil

Fatty acid composition	Molecular structure	Weight percentage (Wt%)
Caproic acid	C6:0	0.5
Caprylic acid	C8:0	7.8
Capric acid	C10:0	6.7
Lauric acid	C12:0	47.5
Myristic acid	C16:0	18.1
Palmitic acid	C14:0	8.8
Stearic acid	C18:0	2.6
Oleic acid	C18:1	6.2
Linoleic acid	C18:2	1.6

Source: Rahman (2000)

Compared to other vegetable oils coconut oil has advantage of being more stable in terms of oxidative deterioration when exposed to atmospheric oxygen due to high concentration of saturated fatty acids, to the way of processing it, and the presence of phospholipids and tocopherols since it is known to grant some degree of stability on oils and fats (Rahman, 2000). Coconut oil contains low content of phospholipids (0.2%) when compared to other vegetable oils (1-3%). According to Rahman (2000), contains low concentration of total tocopherol of 50 ppm in which 10% is composed by alpha-tocopherol. Tocopherol are natural antioxidants responsible for oxidative protection in oils when in contact with air. Therefore, the low composition of tocopherols in coconut oil may reflect the low necessity of oxidative protection due to the fact that there is low amount of unsaturated oils (10% of total fatty acids) in its fatty acid profile (Rahman, 2000).

The results of dietary crude coconut oil of lipid source at 5% level in Nile tilapia broodfish diet expressed that there was no significant difference in number of fry produced per spawn compared with soybean oil due to increased levels of crude fat deposition in liver and gonads (Santiago & Reyes, 1993). However, the ratio of n-6/n-3 HUFA in coconut oil and soybean oil were significant different, having soybean oil showing higher ratio that are responsible for enhancing reproduction (Santiago & Reyes, 1993). Coconut oil was selected to be used in the present study because it contains a certain amount of arachidonic acid (omega 6 HUFA) that is responsible for sexual maturation in fish (Bell & Sargent, 2002).

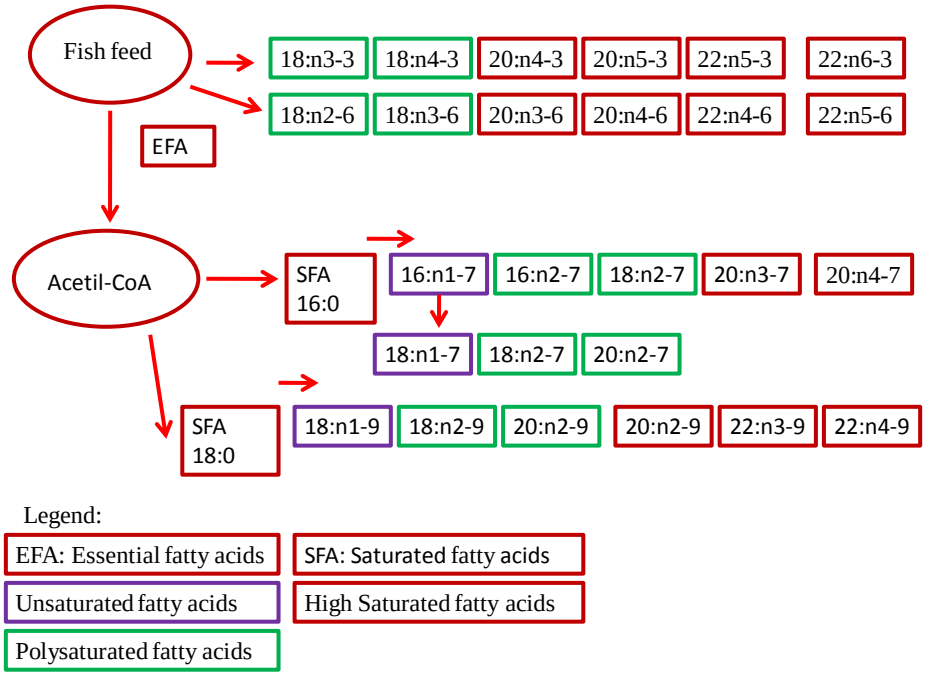

Figure 2.2 Metabolism of fatty acids present in coconut oil lipid composition

Source: Adapted from Tocher (2003)

Since coconut oil contains 1.6% of linoleic acid (18:2n-6) (Rahman, 2000), once it added to fish feed and consumed by Nile tilapia broodfish it can be further elongated and desaturated into HUFA such as 20:4n- 6 (arachidonic acid). Since fish lack δ-9 desaturases, therefore 18:2n-6 cannot be synthesized internally (Pettersson, 2010). Despite that fish has other desaturases (delta 5 and delta 6 desaturases) that are

directly involved in the synthesis of these essential fatty acids (HUFA) such as 18:3n-3 and 18:2n-6 that occurs in the microsomal fraction of the liver of freshwater fish. This ability for acyl elongation and desaturation is more effective in freshwater fish than in marine fish because marine fish already can obtain these essential fatty acids from natural diet (figure 2.2) (Tocher, 2003; Pettersson, 2010).

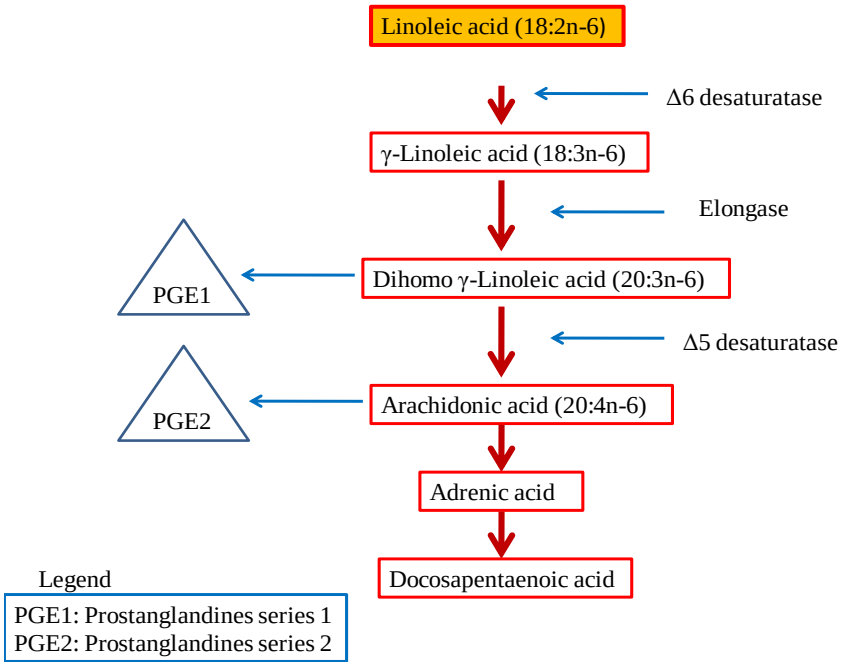

Figure 2.3 Conversion of polyunsaturated fatty acids into highly unsaturated fatty acids

Source: Adapted from Tocher (2003)

2.13.3 Results from experiment on effects of several sources of vegetable oil on growth and reproductive performances in several fish species

The benefit roles of crude lipid in broodstock diet in reproduction performance have been reported in recent years. El-Sayed et al. (2005) mentioned that fish diets rich in protein fed to the fish makes impossible gonad to mature but fatty acids diet like soybean oil, fish oil mixed with soybean oil can influence in maturation and spawning of Nile tilapia. Apraku et al. (2017) found that coconut oil alone in a concentration of 30g/Kg fed to Nile tilapia (*Oreochromis niloticus*) grew better and

showed reduced mortality rate compared to those fed with blended coconut oil and fish oil and therefore these growth parameters values were significant different.

2.14 Replacement of vegetable oil

2.14.1 Cause of replacement

In present days there is a tendency of using vegetable oil rather than fish oil sources in broodstock diets because supplies of fish oils for aquaculture production are becoming critical as it was predicted by Bell & Sargent (2002). The use of vegetable oils in broodstock diets in substitution of fish oils or in combination with fish oils may not show significant difference to growth performance (Bendhack et al., 2014) as it could have an effect upon egg and fry quality when the broodstock diets contains only vegetable oils (Rennie et al., 2005).

2.14.2 Results from experiment on effects fatty acids from vegetable oil sources on growth and reproductive performances in Nile tilapia

Based on several studies the best lipid source of Nile tilapia for sexual maturation must contain some trace of arachidonic acid (ARA, 20:4 n-6). Arachidonic acid is a long chain highly unsaturated fatty acid (HUFA) and is responsible for ensure reproductive success in many fish species (Anido et al., 2015) and promotes gonad maturation and egg quality (Izquierdo et al., 2001), and growth (Lim et al., 2011). However, some vegetable oil substitutes including rapeseed, linseed, soya, palm and olive oils, didn't contain ARA even though it contained linoleic acid (18:2 n-6), allowing to accumulate it while ARA will be reduced in the fish (Bell & Sargent, 2002). Recent papers refer that the combination of fish oil and vegetable oil such as soybean oil can improve reproductive traits in fish. Ghaedi et al. (2014) mentioned that high levels (180 g/Kg) of fish oil and soybean oil increased HUFA (EPA, DHA, ARA), total PUFA, n-3 and n-6 series in ovary and liver of female *Channa striatus* that consequently resulted the best reproductive performance such as fecundity, hatching rate, egg diameter and larvae length. Hajizadeh et al. (2008) reported great reproduction performance (Larval quality, hatchability and fecundity) in treatments that Nile tilapia (*Oreochromis niloticus*) were fed with combination with palm oil and cod oil ratio of (9:1) even though there was no significant differences compared with palm oil only treatment (100g/Kg).

2.15 Effect of fatty acid on reproductive performances

2.15.1 Reproductive performances

Fatty acids guarantee reproductive performance depending on its type and quantity in dietary lipid source. EPA, DHA and ARA are important fatty acids supplemental fatty acids to the lower gut (primarily linoleic acid, EPA and DHA) may target reproductive tissues to improve reproductive function and fertility. Improvement in embryo survival may be associated with suppression of uterine prostaglandin secretion via linoleic acid or other longer chain unsaturated fatty acids (Staples et al., 2015). Fecundity and spawning frequency are improved in fish diet that contains high amount n-3 fatty acids such as EPA and DHA (El-Sayed et al., 2005).

2.15.2 Biochemical mechanism of fatty acids on reproductive performances

In relation to lipid metabolism it is important to make reference to biosynthesis, elongation and desaturation processes. The synthesis is occurred in the cytoplasm where Acetyl-CoA is used to start the pathway mediated by fatty acids synthase. Fish are able to produce endogenously saturated fatty acids (SFA) with 16 and 18 carbons molecules more exactly 16:1n-9 and 18:1n-9 when this reaction is catalyzed by desaturases. However, essential fatty acid molecules such as omega 6 (18:2n-6) and omega 3 (18:3n-3) cannot be synthesized endogenously in fish. In order to fish to obtain these essential fatty acids it is necessary to enrich it in fish feed so that later the fish can elongate and desaturate it into polyunsaturated fatty acids like arachidonic acid (ARA) (20:4n-6), EPA (20:5n-3), and DHA (20:6n-3) when mediated by elongase and desaturase in the liver. It is considered that this ability of converting saturated fatty acids into the polyunsaturated fatty acids common in freshwater fish rather than in marine fish it is because in the later there is avaibility of polyunsaturated fatty acids in natural feed in ocean (Pettersson, 2010). β oxidation occurs in the mitochondria matrix and perixissomes, and it is responsible for reducing the long carbon chain in certain polyunsaturated fatty acids. It consists in sequential removal of 2 carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule. Every cycle of the β-oxidation generates 1 NADH, 1 $FADH_2$ and one acetyl-CoA. The result of continuous oxidation of the acetyl-CoA to CO_2 in the

tricarboxylic acid cycle is the production of 3 molecules of NADH, 1 molecule of $FADH_2$ and 1 molecule of ATP (Pettersson, 2010).

The Physiological role of omega 3 and omega 6 in fish it is related with environment adaptation (DHA), energy source (through β-oxidation), structure composition of cell membrane, precursors of hormone synthesis, mediators of many physiological processes, role in reproduction function, stress control, and hormone release (ARA and EPA).

Vegetable oils by its fatty acid composition can work in fish grow-out production depending on freshwater fish ability to desaturate and elongate the C18 fatty acids into C20 and C22 fatty acids in which is associated with their ability for fatty acyl desaturation and elongation (figure 2.3) (Tocher, 2003). On other hand, vegetable oil is an important due to its capability to provide sufficient energy in the form of saturated and monounsaturated fatty acids to maintain high growth rates. In addition, it should contain moderate levels of 18:3n-3, the fatty acid precursor for the endogenous conversion into eicosopentaenoic acid (EPA) and docosahexaenoic acid (DHA), and low amounts of 18:2n-6 since it is poorly oxidized such as rapeseed oil and palm oil (Pettersson, 2010). Moreover, eicosopentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) are essential polyunsaturated fatty acids responsible for great reproduction performance by stimulation of gonads to mature after vitellogenisis process catalysed by vitamin E and vitamin C in liver to produce vitellogenin to guarantee larval development (hatchability) and survival (Bhujel, 2000; Izquierdo et al., 2001; El-Sayed et al., 2005)

The importance of studying the effect of dietary lipid source on spawning performance of tilapias has been increasing according to the number of published articles and papers about this matter. As matter of fact, Santiago & Reyes (1993) studied the effects of dietary lipid source on reproductive performance and tissue lipids of Nile tilapia. Their results found that cod liver oil (rich in n−3 HUFA) has poor reproductive performance, while highest fry production was obtained from fish fed a diet supplement with soybean oil (rich in n-6 fatty acids). In another similar study, El-Sayed et al. (2005) conducted a research on effect of dietary lipid source on spawning performance at different salinities and concluded that tilapia need fish oil

for better reproductive performance in brackish water whereas plant oil (soybean oil) is appropriate for freshwater rearing.

CHAPTER 3
MATERIALS AND METHODS

3.1 Experimental diet

The experiment was carried out into completely randomized design (CRD) composed of 4 treatments and 3 replications. Four diets were formulated (Table 3.1). The fish feed was prepared to be isonitrogeneous (29.44 % protein) and isocaloric (15.47 KJ Gross energy/Kg) containing four different lipid levels (Table 3.1). The different levels of coconut oil were fed on the fish samples at rate of 3 % body weight.

Table 3.1 Composition and proximate analysis of experimental diets (on dry matter basis).

Ingredients	Treatments (Coconut oil levels)			
	T1 (3%)	T2 (6%)	T3 (9%)	T4 (12%)
Fish meal	10	10	10	10
Soybean meal	50	50	50	50
Cassava meal	21	18	15	12
Rice bran	15	15	15	15
Coconut oil	3	6	9	12
Premix	1	1	1	1
Total (%)	100	100	100	100
Proximate analysis (%)				
Crude protein	29.45	29.44	29.43	29.42
Crude lipid	4.98^{d}	8.02^{c}	11.05^{b}	14.09^{a}
NFE	51.7^{a}	48.72^{b}	45.72^{c}	42.73^{d}
Crude fiber	7.63	7.60	7.58	7.55
Crude ash	6.22	6.22	6.21	6.20
Gross energy (KJ/Kg)	15.46	15.47	15.47	15.48

Remark: 3% CO = 30 g of coconut oil/Kg; 6% CO = 60 g of coconut oil/Kg; 9% CO = 90 g of coconut oil/Kg; 12% CO = 120 g of coconut oil/Kg.

*All proximate analysis values are on dry matter basis. NFE: Nitrogen free extract.

3.2 Experimental fish and feeding trial

The 2 months old fingerlings of Nile tilapia (average weight = 14.57±0.96g and total length = 7.59 ±0.13cm) were transferred from UbonRatchathani Inland Fisheries Research and Development Center and acclimatized in the hatchery of Faculty of Agriculture, UbonRatchathani University. After the fingerlings accepted the pellet feed for 7 days, all fish samples was visually sexed and raised in cement tank (2x4x1.5 m) for 2 months (stockingdensity = 100 fish/m^2) until experiment. The pellet feed was fed on twice per day (9.00 and 16.00) at 3 % body weight.The dechlorinated and aerated water was added 80 litres per experimental tank. Prior to the feeding trial, all female individuals (initial body weight 27.57 ± 0.96g g and standard length=11.80 ±0.13cm) were randomly stocked in fiber tanks with 20 fish per tank.

Fish samples were fed with the experimental diets at 3 % body weight twice per day at 9.00 and 16.00 for a period of 90 days. Body weight and standard length of samples were measured twice a month. Uneaten diets and faeces were removed from the tank after feeding twice a day. The renewable water was added into tank up to 80 L. Water quality was monitored twice a month for dissolved oxygen, pH, water temperature, total ammonia, unionized ammonia and total suspended solid (Table 1 in AppendixI).

3.3 Fish feed preparations

All the ingredients mentioned on table 2 were mixed using rotating machine (see appendix II figure 2a) as distilled water were added gradually until it was obtained a soft dough consistency followed by its submission to heat and pressure in extruder machine to make pellets dough. The dough were pelleted using a 5mm mincer and air-dried under shade and overnight (see appendix II figure 2c). The pellets dough were grinded to 5mm diameter using grinding machine according to the size of Nile

tilapia broodfish to be fed (Gunasekera et al., 1995) (see appendix II figure 2b). After air dry in indoors facility to avoid the lipid and vitamin content in the experiment diets to be degraded or peroxidised that consequently would be harmful to the fish samples, experimental fish feed were stored in fridge at 4^{o}C (Bhujel, 2000; El-Sayed et al., 2005; Gammapila et al., 2007). The test of ability of Nile tilapia fingerlings to eat fish feed pellets were conducted and it sunk but fish ate it.

3.4 Proximate analysis

The composition of nutrition in fish samples was investigated with method of proximate analysis at initial and final phase according to AOAC (1990).The 100 g muscle samples were collected from fresh specimens followed by dryingovernight in oven at 105^{o}C. After cooling the individuals in desiccator, the samples were ground and stored in refrigerator at 4^{o}C.

3.4.1 Crude protein

The Kjeldahl method was used to extract crude protein by digestion of 0.5g of fish sample in presence of H_2SO_4 96%, followed by distillation by steam condensation (with distilled water and NaOH 45%) and lastly titration (H_2SO_4 0.1N). The volume of titration was recorded when solution changed from light green to purple colour in presence of indicator of bromophenol blue (Karanth et al., 2009). (See Appendix II figure 3e)

$$\textbf{Crude protein (\%)} = \textbf{[(Vtb)*N*V*100\%] / W} \qquad (3.1)$$

Where: Vtb= volume of titration of blank;

N: Normality of acid;

V: volume of titration;

W: sample weight;

3.4.2 Crude lipid

Crude lipid extract was obtained from immersing 3g of fish sample from each treatment group in the cellulose paper beaker into the thimble filled with petroleum ether attached to the IPS machine. Automatically the IPS machine

conducted all steps until recover crude lipid extract at 100^{o}C. The crude lipid extract weight was measured after thimble. (See Appendix II figure 3d)

Crude lipid (%) = (FW/W) * 100% (3.2)

Where: FW= final weight;

W: sample weight;

3.4.3 Crudefiber

The extraction of crude fiber from fish samples was composed by digestion of 2g of fish sample in 200ml of H_2SO_4 1.25% and NaOH 1.25% respectively in digester oven for 30 minutes. During the intervals of each digestion, the remaining product of digestion was filtered by white cloth and placed back in 1L beaker for the following digestion. After the last digestion, remaining precipitate were placed in crucibles to burn in muffle at 550 ^{o}C for 30 minutes. This incineration was necessary to obtain the fiber from fish sample after cooling the crucibles in desiccators prior to weight measurement. (See Appendix II figure 3c).

Crude fiber (%) = (FW/W) * 100**%** (3.3)

Where: FW= final weight;

W: sample weight;

3.4.4 Total ash

Crude ash extraction consisted in pre-heating crucibles in muffle at 550^{o}C followed by measurement of 2g of fish sample correspondent to each treatment group and burn for 2 hours in muffle at 550^{o}C. After 2 hours burned ash sample were cooled in desiccators and weighted (Karanth et al., 2009). (See Appendix II figure 3b).

Crude ash (%) = (WA/W) * 100% (3.4)

Where: WA= Weight of ash;

W: Weight of dry sample;

3.4.5 Moisture

The differential of initial and final weight in crucibles were possible to estimate crude moisture values (%) on sample and also the dry matter content of that sample after remove it from oven at 105^{o}C for 6 hours (Karanth et al., 2009) (See Appendix II figure 3a).

DM=100 – moisture (%) (3.5)

Where: DM = Dry matter

Crude moisture (%) = [(FW - IW)/(FW)]*100% (3.6)

Where: FW = Final Weight
IW = Initial Weight

3.5 Experimental analysis

3.5.1 Growth performances

SGR = 100 * [(ln W2 – ln W1) / T] (3.7)

WhereSGR = Specific growth rate (%/day)
W1 = the initial body weight
W2 = the final body weight
T = number of days in the feeding period

ADG = (W2 – W1) / T (3.8)

WhereADG = Average daily gain (g/day)
W1 = the initial body weight
W2 = the final body weight
T = number of days

FCR = (F / WG) * 100 (3.9)

Where: FCR = Feed conversion ratio (%)

F = Feed intake

WG = Weight gain

WG = FW – IW (3.10)

Where: WG = Weight gain (g)

FW = Final Weight

IW = Initial Weight

SR = (S/SF) * 100 (3.11)

Where SR = Survival rate (%)

S = Number of fish at end of experiment

SF = Number of fish initially stocked

3.5.2 Reproductive performances

GSI (%) = [W/BW] *100(Soltan et al., 2011) (3.12)

Where: GSI = Gonadosomatic index (%)

W = weight of ovary (g)

BW = Body weight (g)

HSI =[W/BW] * 100 (3.13)

Where: HSI = Hepatosomatic index (%)

W = Weight of liver (g)

BW = Body weight (g)

AF = NO (3.14)

Where: AF = Absolute fecundity

NO = number of oocytes (eggs)

RF = NO/ BW (3.15)

Where: RF = Relative fecundity(eggs/g)

NO = number of oocytes (eggs)

BW = Body weight (g)

3.6 Data analysis

The growth parameters (body weight, standard length, specific growth rate, average daily gain and feed conversion ratio), reproductive parameters (gonadosomatic index, hepatosomatic index, absolute fecundity, relative fecundity), survival rate and water quality were presented as mean ± standard error of the mean (or standard deviation). Differences in growth and reproductive parameters, survival rate and water quality between different groups of samples were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (R-package). Significance results were considered if $P < 0.05$. Regression analysis was conducted to find correlation between water quality parameters and growth and reproduction parameters at level of confidence of 95%.

CHAPTER 4
RESULTS

4.1 Growth performances of Nile tilapia (*Oreochromis niloticus* L.) with the different levels of coconut oil

The body weight, specific growth rate, average daily gain and feed conversion ratio of *Oreochromis niloticus* fed different levels of coconut oil was monthly measured (Table 4.1).The growth parameters reflect no significant differences among the treatment groups of 3%, 6%, 9% and 12% of crude coconut oil. The final weight (FW) of the experimental fish fed 6% CO diets (126.98±9.52 g) was greater than those of Nile tilapia fed 3% CO, 9% CO and 12% CO (118.57±10.12, 116.31±7.21 and 122.95±13.3 g; $P< 0.05$). Most of fish samples grew in average more than 13 centimetres of standard length and it didn't vary among treatments groups. The average daily gain of the fish fed expressed no significant differences in all treatment groups and fish grew in average less than 1g/day during the experiment period. Among treatments groups, there was no significant difference in the survival rate of Nile tilapia samples ($P> 0.05$) and treatment 3 (9%) and treatment 4 (12%) showed up to 98.33 ± 1.52% and 91.25 ± 6.95% of survival rate respectively. Feed conversion rate(FCR) highest value were 1.80 ± 0.10% recorded on treatment 12% CO even thou there were no significant differences on other treatments. The same trend was observed on specific growth rate that despite no mean variation treatment 2 (6%) obtained highest mean value of 1.67 ± 0.09%/day. Weight gain (WG) was insignificantly different from other treatments but nonetheless higher on treatment 2 (6%)98.76±9.50g compared to other coconut oil treatment groups.

Table 4.1 Growth parameters results from dietary coconut oil fed to Nile tilapia (mean ± SD)

Parameters	Treatments (mean ±SD)			
	Treatment 1 (3%)	**Treatment 2 (6%)**	**Treatment 3 (9%)**	**Treatment 4 (12%)**
Mean IW	27.79 ± 0.46	28.22 ± 0.16	27.48 ± 0.29	27.57 ± 0.96
Mean FW	118.57 ± 10.12	126.98 ± 9.52	116.31± 7.21	122.95 ± 13.38
Mean ISL	9.81 ±0.09	9.97 ±0.12	9.87 ±0.10	9.87 ±0.10
Mean FSL	15.06 ±0.46	15.74 ±0.50	15.38 ±0.46	15.45 ±0.43
WG	90.78±10.44	98.76 ± 9.50	88.83 ± 6.95	95.38 ± 12.69
FCR	1.94 ± 0.34	2.03 ± 0.25	2.00 ± 0.23	1.80 ± 0.10
ADG	0.80 ± 0.22	0.80 ± 0.04	0.75 ± 0.13	0.87 ± 0.13
SGR	1.61± 0.11	1.67 ± 0.09	1.60 ± 0.06	1.66 ± 0.09
SR	95.00 ± 5.00	96.66 ± 2.88	93.33 ± 5.77	75.00 ± 18.00

Remarks:IW= Initial weight; FW= Final weight; ISL= initial standard length (cm); FSL= Final standard length (cm); WG=weight gain (g); ADG=average daily gain (g/day); SGR=specific growth rate (%/day);SR= survival rate (%);FCR (%);3% coconut oil (CO)=30 g of coconut oil/Kg; 6% CO=60 g of coconut oil/Kg; 9% CO=90 g of coconut oil/Kg; 12% CO=120 g of coconut oil/Kg.

4.2 Proximate analysis of Nile tilapia (*Oreochromis niloticus* L.) fed on the different levels of coconut oil

The proximate analysis of Nile tilapia muscle fed on the different levels of coconut oil was measured (Table 4.2).The moisture, ash, crude fiber, crude lipid, crude protein, nitrogen free extract of fish samples fed the variable concentrations of coconut oil were not significantly different (P> 0.05).The crude lipid values reported no significant differences among coconut oil (CO) treatments groups (P> 0.05) even though treatment 9% CO (29.14±1.70%) showed highest value compared to other treatments groups. The crude protein of individuals fed on 6% of coconut oil treatment (55.18±0.98 %) was greater than those of Nile tilapia fed on the fish feed

containing coconut oil at 3%, 9% and 12% (54.88±0.25, 54.38±1.75 and 54.66±1.97 %, respectively; *P*> 0.05) even thou there was no significant difference among these results. The crude fiber of fish samples fed on 12% CO diets (0.06±0.01 %) was greater than those of individuals fed on 3% CO, 6% CO and 9% CO (0.03±0.00, 0.02±0.00 and 0.04±0.03 %, respectively; *P*> 0.05). The significant nitrogen free extract data result showed no significant difference found in all treatments fed specifically on 3% CO (2.29±0.58 %), 6% CO (3.67±0.26 %), 9% CO (2.53±0.36 %) and 12% CO (2.81±0.028 %) (*P*> 0.05)

Table 4.2 Proximate analysis of Nile tilapia (*Oreochromis niloticus*L.) females that fed on the different levels of coconut oil

Dietary coconut oil levels	Proximate analysis (%)					
	Crude Moisture	Crude Protein	Crude Lipid	Nitrogen free extract	Crude Ash	Crude Fiber
Initial	19.46±0.93	59.66±1.30	20.28±0.11	0.56±0.03	19.46±0.93	0.04±0.02
3%	29.70±0.89	54.88±0.25	26.63±0.50	2.29±0.58	16.17±2.65	0.03±0.00
6%	29.07±0.78	55.18±0.98	23.54±1.04	3.67±0.26	17.59±1.03	0.02±0.00
9%	29.50±1.49	54.38±1.75	29.14±1.70	2.53±0.36	13.90±3.05	0.04±0.03
12%	29.71±1.66	54.66±1.97	26.52±1.24	2.81±0.08	15.83±2.66	0.06±0.01

Note:3% coconut oil (CO)=30 g of coconut oil/Kg; 6% CO=60 g of coconut oil/Kg; 9% CO=90 g of coconut oil/Kg; 12% CO=120 g of coconut oil/Kg.

*All proximate analysis values are on dry matter.

4.3 Reproductive performances of Nile tilapia (*Oreochromis niloticus* L.) fed on the different levels of coconut oil

During three month experiment gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated and analysed. Periodic and treatment variation of hepatosomatic and gonadosomatic indices are shown in table 4.3 respectively representing the time changes of these indices values (mean ± standard deviation) of Nile tilapia. Results from one-way ANOVA liver analysis show that hepatosomatic index values were not significantly different (P>0.05) among treatment groups. Treatment groups in which Nile tilapia were fed with 12% of dietary coconut oil reported the highest results (1.55 ± 0.20%) compared to other treatment groups.

Significant differences (P<0.05) were observed for GSI values of coconut oil treatments during the tree month experiment. At the end of the experiment, treatment 6% CO showed higher GSI value (2.80 ± 1.76^{a} %) andnot significant different from treatment 12% CO (2.55 ± 1.05^{ab} %), but significant different from treatments 3% CO and 9% COresults(0.77 ± 0.10^{b}%, 0.70 ± 0.11^{b}%respectively;P<0.05).

Table 4.3 Reproductive parameters of Nile tilapia (*Oreochromisniloticus*L.)

Parameter	Dietary coconut oil treatment levels			
	3% CO	6% CO	9% CO	12% CO
GSI	0.77 ± 0.10^{b}	2.80 ± 1.76^{a}	0.70 ± 0.11^{b}	2.55 ± 1.05^{ab}
HSI	1.48 ± 0.37	1.41 ± 0.27	1.12 ± 0.23	1.55 ± 0.20
AF	2284.88 ± 272.57^{a}	1506.53 ± 153.70^{b}	1725.85 ± 268.60^{ab}	1363.44 ± 265.67^{b}
RF	27.33 ± 1.61^{a}	15.16 ± 3.49^{b}	16.21 ± 5.71^{b}	15.31 ± 3.99^{b}
ED	0.63 ± 0.32^{c}	1.83 ± 0.51^{a}	0.73 ± 0.30^{bc}	1.63 ± 0.26^{ab}

Abbreviation:GSI=gonadosomatic index (%); HSI=hepatosomatic index (%); AF=Absolute fecundity (eggs); RF=Relative fecundity (eggs/ g body weight); ED=Egg diameter (mm).

Note: 3% coconut oil (CO)=30 g of coconut oil/Kg; 6% CO=60 g of coconut oil/Kg; 9% CO=90 g of coconut oil/Kg; 12% CO=120 g of coconut oil/Kg.

Fecundity and egg diameter were analysed using one-way ANOVA. As result, significant differences (P<0.05) were registered in absolute fecundity values among different coconut oil level treatments. Therefore, there werehigherabsolute fecundity values on treatment 3% CO (2284.88 ± 272.57[a]eggs) compared to other treatment groups. Mean absolute fecundity results showed tendency for a slightly reduction of its values as dietary coconut oil levels were increased(*P*=0.25) (figure 4.1).

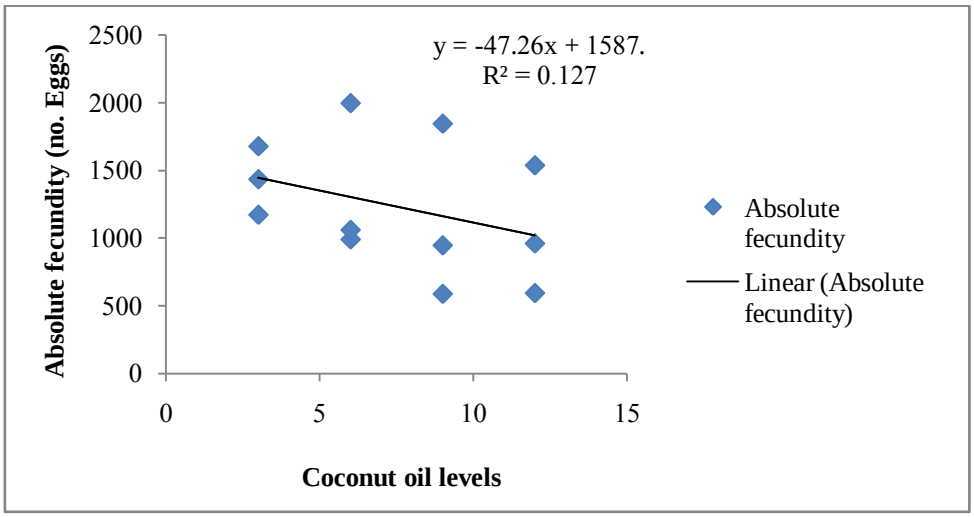

Figure 4.1 Relationship between Absolute fecundity and dietary coconut oil levels assessed using simple regression.

Mean absolute fecundity values ranged from 1363.44 ± 265.67[b]eggs to 2284.88 ± 272.57[a]eggs (table 4.3). Mean relative fecundity valueswere significant different among coconut oil treatment groups (P<0.05).These mean relative fecundity values ranged from 15.16 ± 3.49[b]to 27.33 ± 1.61[a]eggs/g body weights(table 4.3).This highest relative fecundity (27.33 ± 1.61[a]eggs/g) was registered on treatment that fish were fed with 3% of coconut oil (table 4.3). Relative fecundity showed a negative weak correlation with dietary coconut oil levels (*P*=0.14) (figure 4.2).

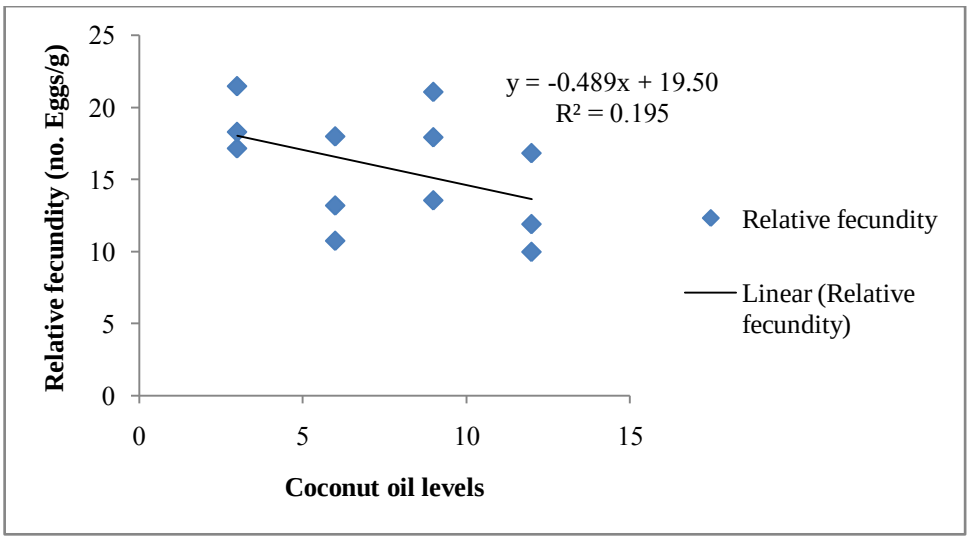

Figure 4.2 Relationship between relative fecundity and dietary coconut oil levels assessed using simple regression.

Effect of different coconut oil levels of Nile tilapia on egg diameter were significantly visible ($P<0.05$). Despite this fact, egg diameter highest value was reported on treatment 6% of coconut oil (1.83 ± 0.51^{a} mm). The increase of coconut oil levels didn't show proportional increase of egg diameter. The mean egg diameter from treatment 3% CO, 9% CO and 12% CO results (0.63 ± 0.32^{c} mm, 0.73 ± 0.30^{bc} mm and 1.63 ± 0.26^{ab} mm respectively; $P<0.05$) (table 4.3). On treatments 3% CO and 9% CO it was observed eggs contained less amount of yolk with white and light yellow colour although in great numbers in gonads whereas on treatments 6% CO and 12% CO eggs were showing yellow and green colour and more yolk but in lesser numbers per gonad. Table 4.4 expresses egg diameter in clusters predominant in each coconut oil level treatments and its respective descriptive characteristics.

Table 4.4 Main clusters of egg diameter (mm) values of different coconut oil levels treatments and respective descriptive observations.

Egg diameter cluster (mm)	Treatments				Descriptive observations
	3% CO	6% CO	9% CO	12% CO	
0.0 – 0.5					Mainly very small size eggs, no colour, white and yellow colour
0.6 – 1.0	X		X		Mainly small size eggs, white and yellow colour
1.1 – 1.5			X		Mainly medium size eggs, light yellow colour
1.6 – 2.0	X	X	X	X	Mainly medium size eggs, green and yellow colour
2.1 – 2.5	X			X	Mainly large size eggs, green and yellow colour
2.6 – 3.0					Mainly large size eggs, green colour only

Note:3% coconut oil (CO)=30 g of coconut oil/Kg; 6% CO=60 g of coconut oil/Kg; 9% CO=90 g of coconut oil/Kg; 12% CO=120 g of coconut oil/Kg.

4.4. Environment parameters of *Oreochromis niloticus* with the different levels of coconut oil

Water quality parameters including dissolved oxygen (mg/L), Total ammonia (mg/L), Total unionised ammonia (mg/L), Total suspended solid (mg/L), pH, water temperature were monitored during the experiment period. The mean values of dissolved oxygen ranged between 6.89± 0.11 mg/L and 7.28± 0.17 mg/L and showed no significant differences among treatment groups. On contrary, total ammonia varied

significantly among treatment groups and highest mean value were reported on water samples of fish fed with 6% of coconut oil (0.081 ± 0.0022^{a} mg/L). Unionised ammonia mean values were not significantly different and lowest values were observed on treatment group of fish fed with 12% of coconut oil ($0.00080\pm 3.58*10^{-05}$ mg/L). Results of total suspended solids appoint to significant differences among treatment groups and these mean values ranged from 0.023 ± 0.0095^{b} mg/L to 0.058 ± 0.0121^{a} mg/L. Water temperature (25.98 ± 0.12^{o}C) and pH (7.20 ± 0.021) values were not significantly different in their respective treatment groups (table II: Appendix I).

Table 4.5 expressed results of simple regression between water quality parameters and reproductive parameters. The weak correlation and level of significance confirmed that water quality parameters did not affect reproductive parameters.

Table 4.5 Relationship between water quality parameters and reproductive parameters assessed by regression analysis at 95% level of confidence.

Water quality parameters	Regression (R^2) and P values									
	GSI	**P**	**HSI**	**P**	**AF**	**P**	**RF**	**P**	**ED**	**P**
Total unionised ammonia	0.10	0.58	0.20	0.54	0.25	0.77	0.09	0.69	0.08	0.70
Total ammonia	0.09	0.77	0.02	0.84	0.03	0.95	0.02	0.83	0.09	0.80
Dissolved Oxygen	0.53	0.25	0.99	0.002	0.17	0.67	0.07	0.58	0.57	0.22
Total suspended solid	0.44	0.94	0.04	0.79	0.08	0.71	0.07	0.72	0.46	0.31
Temperature	0.01	0.86	0.15	0.60	0.22	0.51	0.28	0.46	0.007	0.90
pH	0.99	0.002	0.24	0.50	0.61	0.22	0.40	0.36	0.99	0.002

Abbreviation: GSI=gonadosomatic index (%); HSI=hepatosomatic index (%); AF=Absolute fecundity (eggs); RF=Relative fecundity (eggs/ g body weight); ED=Egg diameter (mm).

According to regression analysis and p value between water quality parameters and growth parameters leads to conclude that growth parameters were not affected by water quality parameters (table 4.6).

Table 4.6 Relationship between water quality parameters and growth parameters assessed by regression analysis at 95% level of confidence.

WQP	Regression (R^2) and P values									
	WG	**P**	**FCR**	**P**	**ADG**	**P**	**SGR**	**P**	**SR**	**P**
TUA	0.009	0.90	0.89	0.051	0.62	0.21	0.08	0.70	0.99	0.002
TA	0.29	0.45	0.43	0.33	0.04	0.79	0.12	0.64	0.50	0.29
DO	0.73	0.14	0.22	0.52	0.002	0.95	0.55	0.25	0.11	0.66
TSS	0.68	0.17	0.25	0.49	0.0005	0.97	0.48	0.30	0.17	0.58
TPT	0.14	0.61	0.18	0.57	0.0025	0.94	0.98	0.007	0.11	0.66
pH	0.94	0.028	0.02	0.83	0.36	0.39	0.04	0.78	0.37	0.38

Abbreviation: WQP = Water quality parameters; TUA=Total unionised ammonia (mg/L); TA=Total ammonia (mg/L); DO=Dissolved Oxygen (mg/L); TSS=Total suspended solid (mg/L); TPT=Temperature (^oC); pH; WG=weight gain (g); ADG=average daily gain (g/day); SGR=specific growth rate (%/day); SR= survival rate (%); FCR (%) = Feed conversion rate.

CHAPTER 5
DISCUSSION

5.1 Growth performance of Nile tilapia (*Oreochromis niloticus* L.) fed on the different levels of coconut oil

The coconut oil is one of vegetable oil composed of linoleic fatty acid which has a potential to be substrate in mechanism of highly unsaturated fatty acid (HUFA) which can promote sexual maturation (Izquierdo et al., 2001; Anido et al., 2015). The growth performances (WG, ADG, SGR and FCR) of treatments containing coconut oil showed no significantly differences. The increased levels of coconut oil resulted in not great variation of growth parameters. Nile tilapia has an optimum dietary coconut oil level and above the optimum dietary lipid level it has the tendency to cause reduction of growth because it might compromise ability of the fish to digest, absorb food and also interfere with metabolic activities. This fact was not evident since there was no significant difference of growth parameters on coconut oil treatments (Ayisi et al., 2017).

This higher growth performance in coconut oil treatments gain may be because coconut oil provides flavour to increase feed intake as it were reported after African catfish (*Clarias gariepinus*) been submitted to coconut oil fish feed (Aderolu and Akinremi, 2009). The lack of effect of dietary coconut oil on growth parameters in the present study may be attributed to same level of digestibility of this vegetable oil included in diet composition that made this experiment fish feed more palatable since it is known for not showing negative effects on growth performance of Nile tilapia. (Daudpota et al., 2016; Ayisi et al., 2017).

The optimal concentration of coconut oil may affect on the growth of Nile tilapia. The treatments 3% coconut oil (CO), 6% CO, 9% CO, 12% CO coconut oil containing in feed of Nile tilapia promoted growth. The feeding behaviour possibly is the reason for good growth performance in all coconut oil treatments. Aderolu & Akinremi (2009) confirmed that fish with omnivorous feeding behaviour were more prone to have better growth performance than carnivorous fish since the amount of

protein in fish feed was 29% recommended for Nile tilapia or omnivorous fish. Highest growth of *Clarias gariepinus* was found after fed on diet containing 50 and 100 g/Kg coconut oil for 6 weeks (Aderolu & Akinremi, 2009). This phenomenon can be explained by Apraku et al. (2017) reported that digestibility and absorption processes depend on fish species and feed intake capacity. Moreover, the results from treatment confirm that linoleic acid in coconut oil is required for maximum growth performance of Nile tilapia which is easily absorbed for metabolic activities (Aderolu & Akinremi, 2009; Apraku et al., 2017). FCR values were not significantly different among all treatments, it were slightly better on treatment 1 (3% CO) and treatment 4 (12% CO) than treatment 2 (6% CO) and treatment 3 (9% CO) probably because fish needed to fulfil its energy requirements by eating more fish feed. The protein sparing effect, where administration of dietary coconut oil improved fish performance, were not evident in the present study since increased levels of coconut oil had no beneficial effects on growth performance possibly because the protein and energy ratio was not adequate to reduce the catabolism of protein for energy (Ayisi et al., 2017).

The change of feeding behaviour in fish can occur in the stage of development including the digestive process, enzyme and metabolism (Tawwab & El-Marakby, 2004). Nile tilapia fingerling, herbivores can digest the phyto-materials potentially while the adults becomes feeding behavior to be omnivores which digestive mechanism can response to animal materials increasingly. In this studies, the 4 months old fish individuals which physiological system can be changed from fingerling to be adult. Additionally, Nile tilapia female can develop maturing oocyte within 3-4 months. It may be possible that the variable adaptation of Nile tilapia can affect on the growth and sexual maturation (Hajizadeh et al., 2008).

The different coconut oil levels did not affect significantly survival rate of female Nile tilapia although treatment 12% CO that showed lower survival rate compared to other coconut oil treatment groups. Thus, this result does go according previous findings that juvenile Nile tilapia is more sensitive to higher levels of crude lipid. It was found by Apraku et al. (2017) that Nile tilapia fed with 3% CO of coconut oil concentration had reduced mortality rate by (26.67±1.76 %). According to Apraku et al., (2017) coconut oil has antibacterial, antiprotozoal and antiviral properties that provide great survival rate in Nile tilapia. Lauric acid it is part of coconut oil fatty

acid profile (Rahman, 2000) that has the ability to destroy gram-negative bacteria enhancing in this way immune system without compromising growth performance of Nile tilapia (Apraku et al., 2017). Moreover, higher levels of coconut oil can compromise steady growth in culture system even though it has anti-oxidative properties that avoid deterioration (Aderolu & Akinremi, 2009). On the other hand, genetic composition of Nile tilapia strain played a role in growth and survival performance since it determines the activeness of proper feed intake as it grants higher resistance to cope with the environment of culture system (Daudpota et al., 2016).

5.2 Proximate analysis of Nile tilapia (*Oreochromis niloticus* L.) fed on the different levels of coconut oil

The proximate analysis of Nile tilapia muscles revealed that the experimental diets composed of crude protein 54.38 - 55.18 %. The crude protein of control experiment contained the highest level (55.18 %), while great growth performances (final weight, weight gain, average daily gain and specific growth rate) were found. The optimal protein should be considered for stimulating growth is 32% (Bhujel, 2000; El-Sayed et al., 2003). The protein requirement of Nile tilapia ranged from 27% to 35% (Bhujel, 2000) and the experimental fish feed isonitrogeneous level was set at 29%. The presence of fish meal as ingredients in experimental fish feed was not crucial to improve crude protein values of body proximate composition of female Nile tilapia besides improving palatability. Possibly the fish samples may used more energy to excrete excess nitrogen and remain the less energy for the growth. El-Sayed & Teshima (1992) reported that the present study revealed that growth were significantly low despite the fact that in body composition tests (muscle tissue) show evidence high rate of crude protein which indicates that protein in fish feed may have been used for energy (by deamination process and breakdown of carbon skeleton) as it was demonstrated by El-Sayed & Teshima (1992) when high dietary protein source and energy level in fish feed did not improve growth and feed utilization.

Coconut oil, vegetable oil composed of saturated fatty acids in greater amount compared to less amount of unsaturated fatty acids. Moreover, coconut oil contains less amount of phospholipids (0.2%) and higher amount of triacylglycerols (95%)

(Rahman, 2000). This less amount of phospholipids present in dietary coconut oil affected lipid digestibility, absorption and transport than lipids with great amount of triacylglycerols (Babalola et al., 2012). In this way, saturated and monosaturated lipids in dietary coconut oil composition were not sufficient to produce enhanced energy through β-oxidation in order to improve growth of fish by sparing protein for fish growth.

Results of present study are in accordance with El-Sayed & Teshima (1992) that have stated that optimum requirement level of crude protein in body composition is between 20% to 56% in several species of tilapia.

Results of crude lipid body composition ranged from 23.54±1.04% to 29.14±1.70%. These results are in accordance with Ahmad et al. (2005) that found 28.5% of crude lipid body composition of Nile tilapia. Nonetheless, crude lipid in fish fed with coconut oil different levels were significant higher than crude lipid contained in experimental fish diet possibly because tilapias are able to store lipid from fish feed in carcass and viscera than to use it for growth (Chowdhury 2011). Whole-body crude lipid, crude moisture and, crude ash were not affected by increasing different levels of coconut oil in fish feed. This suggests that at this coconut oil concentration is optimum for obtain whole body nutrients composition as market demands possibly because there was good transfer of nutrients from experiment fish feed diet to the fish (Ali et al., 2000).

5.3 Reproduction performances of Nile tilapia (*Oreochromis niloticus* L.) females fed on the different levels of coconut oil

Gonadosomatic index is one of parameter that indicate development of gonad and sexual maturation in fish (Bhujel, 2000; Ballestrazzi et al., 2003). GSI values of Nile tilapia female ranged 1.2-1.6 % (Bhujel, 2000). However, in this study GSI values ranged from 1.86 to 3.07 %. The sexual maturation of Nile tilapia occurs within 3-4 months old. These concentrations of coconut oil are attributed to the ability of this experimental diet to provide the necessary energy and essential fatty acids for optimal GSI (Izquierdo et al., 2001; Ghaedi et al., 2014). Ghaedi et al. (2014) found that the optimum lipid level for significant better GSI (11.9%) and fecundity varied between 140 g/Kg to 180g/kg while using combination of soybean oil and fish oil in fish feed

composition of snakehead catfish (*Channa striatus*) which is attributed to the ability of the experiment diet to provide sufficient energy and essential fatty acids. On the other hand, Ballestrazzi et al. (2003) reported optimum level of coconut oil to be 130g/Kg to obtain significant better GSI value of 15.6% in rainbow trout (*Oncorhynchus mykiss*) which were higher that results from present study GSI at levels of 6% CO (2.80 ± 1.76%) and 12% CO (2.55 ± 1.05%) of coconut oil. This implies that there is an optimum crude lipid level for development of reproduction traits depending on its level and fish species (Izquierdo et al., 2001).

Results of hepatosomatic index (HSI) from present study appoint that it ranged from 1.12 ± 0.23% to 1.55 ± 0.20% and were not significantly different among treatments (Apraku et. al. (2017). The increase of dietary crude coconut oil levels did not influence increase of HSI. The significant low hepatosomatic index of the present study are in accordance with Cordebella et al. (2011) since female Jundia catfish (*Rhamdia quelen*) similarly to female Nile tilapia (*Oreochromis niloticus*) may had high demand of energy by female Nile tilapia during the vitellogenesis stage when the protein, lipid and glycogen from external source (experiment diet) and internal source (somatic tissues and cells) were synthesized by the liver and absorption by oocytes for future embryonic development. Therefore, there was no opportunity for the liver to increase its weight since the energy was transformed for the vitellogenesis process (Coldebella et al., 2011). Freccia et al. (2014) reported reduced hepatosomatic index values in sex reversed Nile tilapia after ingestion of fish feed supplemented probiotic addititives and later attributed this effect to the nutritional aspect of the fish and their growth rate. Similarly in the case of present study, female Nile tilapia used their metabolic reserves like glycogen and lipid to enhance immunity system against adverse environment in fiber tanks in between every scheduled cleaning due to excess uneaten feed in tank.

The results showed that absolute fecundity of fish fed with dietary coconut oil feed were not significantly different among the treatments. This fact could be due to the ratio n-6 and n-3. Previous studies showed that Nile tilapia requires maximum amounts of n-6 for growth and reproduction enhancement and minimum amounts of n-3. Watanabe (1982) when fed Nile tilapia with soybean oil that contains high n-6 HUFA, found that absolute fecundity was significantly higher. Moreover, El-Sayed &

Garling (1988) reported that n-6 HUFA is required for optimum growth in *Tilapia zilli*. This may be due to fact that in coconut oil composition there is less or no amount n-6 HUFA based on lack of linoleic acid (C18:2n-6) (Rahman, 2000; Staples et al. 2002). Linoleic acid (C18:2n-6) is saturated fatty acids and need to be converted into polyunsaturated fatty acids like arachidonic acid (ARA) (20:4n-6), EPA (20:5n-3), and DHA (20:6n-3) to boost reproduction performance in freshwater fish (Pettersson, 2010). The effects of arachidonic acid did not manifest significant reproduction performance possibly that Nile tilapia was accumulating linoleic acid (C18:2n-6) before ARA was being slowly reduced or converted in the fish in small amounts (Bell & Sargent, 2003). Another possible reason for absolute fecundity results might be related to inhibitory effects of linoleic acids by competing with arachidonic acid for the same biding enzyme prostaglandins hormone synthethase (PGHS) resulting in reduction and inhibition of prostaglandins synthesis (Staples et al., 2002).

There were no significant differences on relative fecundity and egg diameter values of Nile tilapia females. Mean relative fecundity of fish individuals fed on 3% of coconut oil (18.87 ± 4.23 eggs/g body weight) was greater than other treatments. This result from relative fecundity expresses the fact that Nile tilapia requires minimum concentration of lipid source in order to enhance growth and reproductive performance (Hajizadeh & Shinn, 2015).

Mean egg diameter of Nile tilapia fed on 9% CO showed lower values compared to but not significant different other treatments ($P>0.05$). Among the treatments, the differences in egg diameter may be explained that female Nile tilapia shared different nutritional state during the ovarian development, different concentration of essential fatty acids during maturation of ovary and also the different size of the female Nile tilapia broodstock (Ghaedi et al., 2014). Coconut oil contains very low amount of alpha-tocopherols (vitamin E) (Rahman, 2000). The vitellogenesis process that occurs in liver, triggers the production of vitellogenin and it accumulates in the oocytes causing gonads to increase weight and maturation (Agnette et al., 2014). In this same moment vitamin E (alpha-tocopherol) is also mobilized from peripheral tissues to the gonads without altering plasma vitellogenin content suggesting that vitamin E may be involved in the transport of lipoproteins during this period (Aryani et al., 2014).

Nekoubin et al. (2012) reported that food and vitamin shortages can lead to suspension of vitellogenesis, resorption of oocytes and decrease fecundity in goldfish (*Carassius auratus*). Furiuta (1998) mentions that eggs production, hatching rate and larval survival are negatively affected by deficiency of certain nutrients as well as vitamin E. Arayani & Suharman (2015) mention that green catfish (*Hemibagrus nemurus*) fed with enriched vitamin E ranged from 150 to 450 mg/kg feed resulted in fecundity ranged from 23,750 to 30, 000 eggs/kg body weight. The range of vitamin E for Nile tilapia can be from 100mg/Kg to 600mg/Kg of fish feed resulted in 1.86% to 3.47% of GSI (Tacon, 1987; Grammapila et al., 2007).

5.4 Environment parameters of Nile tilapia *(Oreochromis niloticus L.)* with the different levels of coconut oil

Water quality parameters levels were under the standards for fish aquaculture. Water quality parameters remained favourable standards for rearing fish in aquaculture system and were not significantly affected by coconut oil levels except for total ammonia and total suspended solid. Water temperature of 25.98±0.12^{o}C was in range for Nile tilapia aquaculture. Water temperature above 20^{o}C triggers reproduction in Nile tilapia and water temperature between 28 to 31^{o}C it is known to improve seed production. During the experiment period water temperature values did not affect growth and reproduction.

CHAPTER 6
CONCLUSION

This thesis investigated the effects of different coconut oil levels on growth and reproduction performance of female Nile tilapia (*Oreochromis niloticus* L.). The main focus was to evaluate which of the different coconut oil (CO) levels was optimum for growth and reproduction performance followed by specific objective of searching the effects of these coconut oil levels on several growth and reproduction parameters.

In general, the results show that growth parameters were not significant different ($P>0.05$). The results appoint that growth increased slightly in all treatment groups. Survival rate of fish treatment groups at different dietary coconut oil levels were not significantly different and most values closer to 100% except for treatment 4 (12% CO).

Fish fed with 3% of body weight was able to increase its initial weight and standard length among all treatment groups especially on that where female Nile tilapia were fed with 6% CO of coconut oil. Overall, coconut oil levels treatments groups showed lower growth possibly because formulated fish feed contained soybean meal known to reduce palatability of fish feed even though coconut oil provides flavour to increase feed intake. Best FCR (Feed conversion rate) value was observed in treatment 12% CO and this is showing evidence of digestibility and ability to absorb of lipid source at this concentration. Moreover, linoleic acid in coconut oil is required for maximum growth performance of Nile tilapia which is easily absorbed for metabolic activities. Besides that, coconut oil contains less amount of phospholipids (0.2%) and higher amount of triacylglycerols (95%) in its composition. The presence of phospholipids affects lipid digestibility, absorption and transport than lipids with great amount of triacylglycerols. In this way, saturated and monosaturated lipids in dietary coconut oil composition were insufficient to produce enhanced energy through β-oxidation in order to improve growth of fish by sparing protein for fish growth. Acceptable results from ADG and SGR from present study of growth performance implies that coconut oil can be used as alternative vegetable oil

to provide good source of lipid to improve growth and to replace with lipid sources. The different coconut oil levels did not affect significantly survival rate of female Nile tilapia in any of the treatments possibly because coconut oil has antibacterial, antiprotozoal and antiviral properties that provide great survival rate in Nile tilapia due to presence of lauric acid in the coconut oil fatty acid profile that has the ability to destroy gram-negative bacteria enhancing in this way immune system of Nile tilapia. However, treatment 4 (12% CO) showed lower survival rate possibly as concentration of crude lipid increases it decreases survival rate because it can compromise steady fish growth. Results from proximate analysis on dry matter basis of body composition of Nile tilapia fed with different dietary coconut oil levels appoint that all proximate composition parameters were not significantly different. Body composition was changed by different coconut oil levels compared to initial data. The low crude lipid in fish muscle tissue is justified possibly due transformation of metabolised lipid by β-oxidation in liver into metabolic energy by sparing protein for fish growth as is reported by high values of protein in muscle. Nonetheless, crude lipid in fish fed with coconut oil different levels were significant higher than initial data possibly because tilapias are able to store lipid from fish feed in carcass and viscera than to use it for growth. Moreover, essential fatty acids such as linoleic acid were possibly mobilized from muscle and liver during ovary maturation since it can be useful for gonad development. Crude protein was significantly affected by different coconut oil levels but did not show negative trend as the coconut oil different levels increased gradually. This result can be explained by the possible high concentration of crude protein in fish feed that was directly proportional to crude protein in body composition which is supported by previous studies. Overall, among all coconut oil different levels treatments, fish fed with coconut oil level of 6% CO showed better body composition in fish carcass which suggests that at this coconut oil concentration is optimum for obtain whole body nutrients composition for market demands.

Reproduction parameter were affected by different coconut oil levels and showed same trend during the three month experiment trial. The reproductive parameters in general were significantly affected by different coconut oil levels except for hepatosomatic index (HSI). Therefore, the GSI didn’t increase gradually as coconut oil levels increased and throughout the experiment period among the treatments as

fish grew even though Nile tilapia is a multi-spawner. Apart from that, GSI values were higher compared to other previous studies possibly because the Nile tilapia strain used as sample was chiltrada stain which is a train that were developed to promote more growth than reproduction performance compared to other strains such as GIFT strain. The results showed that absolute and relative fecundity of fish fed were significantly affected by dietary coconut oil feed and this could be due to the ratio n-6 and n-3 since in coconut oil composition there is less or no amount omega 6 highly unsaturated fatty acid (HUFA) based on lack of linoleic acid (C18:2n-6) that needs to be converted into polyunsaturated fatty acids like arachidonic acid (ARA) (20:4n-6), EPA (20:5n-3), and DHA (20:6n-3) to boost reproduction performance in freshwater fish. The higher egg diameter in treatments groups fish fed with dietary coconut oil especially treatment 6% CO during the experiment period. Since coconut oil contains very low amount of alpha-tocopherols (vitamin E) possibly the vitellogenin produced in liver would not be well mobilized from peripheral tissues to oocytes to increase the size and weight of the gonads without altering plasma vitellogenin content suggesting that vitamin E may be involved in the transport of lipoproteins during this period. Results from liver analysis show that hepatosomatic index values were not significantly different among treatment groups. The significant lower hepatosomatic values were possibly due to the fact that female Nile tilapia (*Oreochromis niloticus*) may had high demand of energy during the vitellogenesis stage when the protein, lipid and glycogen from external source (experiment diet) and internal source (somatic tissues and cells) were synthesized by the liver and absorbed by oocytes for future embryonic development, providing no opportunity for the liver to increase its weight since the energy was transformed for the vitellogenesis process.

Coconut oil slightly improved growth and reproductive performance in female Nile tilapia. In general, reproductive parameters best performance under 6% of coconut oil is recommended to obtain high seed production. Coconut oil can promote great growth rate and efficient feed conversion at 6% of coconut oil in fish feed without reducing survival rate. The results of this study suggest that in under improved diet formula growth and reproduction development can be promoted from introducing coconut oil as an alternative vegetable oil to replace marine lipid source in fish feed in order to reduce production cost. It’s recommended a continuous

research on this topic to evaluate the growth and reproduction performance of combination of several of vegetable oils including coconut oil in fish feed.

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APPENDIX A

Material and methods

Water quality analysis

Water samples after been collected were measured for water temperature and pH using a pHmeter followed by filtration of suspended materials using a filter paper no. 42. After add 20ml of filtered water samples in 150ml test tubes, chemicals such as manganese sulphate solution, sodium hypochlorite solution and phenate reagent were mixed at room temperature before it been measured and recorded its absorbance with spectrophotometer at 630nm. Prior to this, standard solution were prepared to obtain the standard curve equation in order to calculate total ammonia values. Unionised ammonia were calculated using ammonia calculator program after obtain total ammonia values, water temperature and pH readings (see appendix II figure 6b, 6c).

$$\mathbf{Y = 0.253 * X + 0.035} \qquad \text{(A.1)}$$

(standard solution absorbance equation)

Where: Y = total ammonia concentration

X = water samples absorbance reading

Dissolved oxygen

To procede with dissolved oxygen analysis, chemicals such as manganous sulphate and alkaline-iodine-azide and sulphuric acid 97% were added to the water samples to precipitate whole suspended solids in BOD bottle before titrate the water sample solution with sodium tiosuphate (Na_2S_2O3) standard solution in presence of starch indicator (see appendix II figure 6a). Dissolved oxygen parameter calculation as it follows:

$$\mathbf{Dissolved\ oxygen\ (mg/l) = [(VT) \times N \times 8 \times 1000] / V} \qquad \text{(A.2)}$$

Where: VT= total volume of standard Na2S2O3 at end point

N = normality of standard Na2S2O3

V=Sample volume in ml

Total suspended solid

To obtain total suspended solid in water samples, it was filtered in pre-dried filter paper GF/C no. 72 using a sucking machine where all particles were retained. These

filter papers containing all particles were submitted to heat in oven at 105^{o}C overnight (see appendix II figure 6d).

$$\textbf{TSS (mg/L) = (F-T) x (1000/V)} \qquad \text{(A.3)}$$

Where: F = Final weight of filter paper (mg)

T = Initial weight of filter paper (mg)

V = Volume of water samples (mL)

The period of this experiment were of three months to evaluate the effects of coconut oil on growth and reproduction performance when it were submitted to female broodstock Nile tilapia.

Table A.1Water quality parameters raw data samples collected during experiment period

Parameter	Treatment 1 (3%)	Treatment 2 (6%)	Treatment 3 (9%)	Treatment 4 (12%)
Unionised ammonia (month 1)	0.00153 ± 0.000ab	0.00184 ± 7.28E-05a	0.0014 ± 0.00025ab	0.00125 ± 1.15E-05ab
Unionised ammonia (month 2)	0.00064±0.0003	0.00043 ± 4.8E-05	0.00060 ±0.00012	0.00049 ±7.34E-05
Unionised ammonia (month 3)	0.00059±8.22E-05	0.00048±9.34E-05	0.00063±3.47E-05	0.00065 ±4.59E-05
Total ammonia (month 1)	0.0713 ±0.0040ab	0.0830 ±0.0045a	0.0696 ±0.0045b	0.0613 ±0.0031b
Total ammonia (month 2)	0.080 ±0.0049a	0.0783 ±0.0012ab	0.0673 ±0.0037b	0.0706 ±0.0062ab
Total ammonia (month 3)	0.0816 ±0.0059	0.083 ±0.0078	0.0816 ±0.0081	0.0856 ±0.0034
DO (month 1)	7.29 ± 0.465	7.25 ± 0.112	7.40 ± 0.294	7.11 ± 0.313
DO (month 2)	6.82 ± 0.496	6.17 ± 0.306	6.77 ± 0.187	6.64 ± 0.269
DO (month 3)	7.27 ± 0.492	6.78 ± 0.080	7.21 ± 0.236	7.42 ± 0.438
TSS (month 1)	0.0596 ±0.036	0.146 ± 0.0892	0.0313 ± 0.0180	0.055 ± 0.0357
TSS (month 2)	0.0140 ± 0.0045	0.0136 ± 0.0032	0.0123 ± 0.0038	0.0116 ± 0.0032
TSS (month 3)	0.0143 ± 0.0076	0.0143 ± 0.0020	0.0243 ± 0.0221	0.0150 ± 0.0036
TPT (month 1)	27.20 ± 0.4	27.15 ± 0.13	27.25 ± 0.28	27.31 ± 0.20
TPT (month 2)	25.13 ± 0.38	25.15 ± 0.17	25.41 ± 0.14	25.26 ± 0.20
TPT (month 3)	23.48 ± 0.08	23.53 ± 0.08	23.51 ± 0.13	23.50 ± 0.09
pH (month 1)	7.31 ± 0.09	7.36 ± 0.04	7.28 ± 0.08	7.29 ± 0.03
pH(month 2)	7.02 ± 0.23	6.85 ± 0.11	7.11 ± 0.14	6.98 ± 0.12
pH(month 3)	7.10 ± 0.07	7.06 ± 0.03	7.13 ± 0.02	7.12 ± 0.04

Abbreviations:DO: Dissolved oxygen; TSS: Total suspended solid; TPT: Temperature

Table A.2Mean values of water quality parameters

Parameter	Treatments (mean±sd)			
	Treatment 1 (3%)	**Treatment 2 (6%)**	**Treatment 3 (9%)**	**Treatment 4 (12%)**
Unionised ammonia (mg/L)	0.00092± $1.31*10^{-04}$	0.00094± $2.94*10^{-05}$	0.00091± $9.81*10^{-05}$	0.00080± $3.58*10^{-05}$
Total ammonia (mg/L)	0.077 ±0.0017^{ab}	0.081±0.0022^{a}	0.073±0.0044^{ab}	0.072±0.0040^{b}
DO (mg/L)	7.27± 0.46	6.89± 0.11	7.28± 0.17	7.23± 0.32
TSS (mg/L)	0.029± 0.0091^{b}	0.058± 0.0121^{a}	0.023± 0.0095^{b}	0.027± 0.0046^{b}
pH	7.20± 0.099	7.15 ± 0.023	7.20 ± 0.021	7.16 ± 0.032
TPT (^{o}C)	25.88±0.15	25.88±0.06	25.99±0.13	25.98±0.12

Abbreviations:DO: Dissolved oxygen; TSS: Total suspended solid; TPT: Temperature; pH: pH

Fish feed preparations

All the ingredients were mixed using rotating machine (see appendix II figure 2a)as distilled water were added gradually until it was obtained a soft dough consistency followed by its submission to heat and pressure in extruder machine to make pellets dough. The dough were pelleted using a 5mm mincer and air-dried under shade and overnight (see appendix II figure 2c). The pellets dough were grinded to 5mm diameter using grinding machine according to the size of Nile tilapia broodfish to be fed (Gunasekera et al. 1995) (see appendix II figure 2b). After air dry in indoors facility to avoid the lipid and vitamin content in the experiment diets to be degraded or peroxidised that consequently would be harmful to the fish samples, experimental fish feed were stored in fridge at 4^{o}C (Bhujel, 2000; El-Sayed et al. 2005; Gammapila et al. 2007). The test of ability of Nile tilapia fingerlings to eat fish feed pellets were conducted and it sunk but fish ate it.

Statistical analysis

Data on growth performance, survival rate, reproduction performance, gonadosomatic and hepatosomatic indexes, environment parameters and proximate composition of whole fish body were subjected to one-way ANOVA (R-package). Differences were considered significant at 0.05 probability level for all data. Values were expressed in mean ± standard deviation (SD).

APPENDIX B

Photos

Figure 1a Fish husbandry

Figure 1b Water management

Figure 1c Fish husbandry

Figure 1d Experiment design

Figure 2aRotating machine and extruder

Figure 2b Grinder to make small pellets

Figure 2cPellets prepared and dried in animal farm

Figure 3a Proximate analysis. Crude moisture

Figure 3b Proximate analysis Total ash

Figure 3c Proximate analysis Crude fiber

Figure 3d Proximate analysis Crude lipid

Figure 3e Proximate analysis Crude protein

Figure 4a Growth parameters (Standard length)

Figure 4b Growth parameters (Weight)

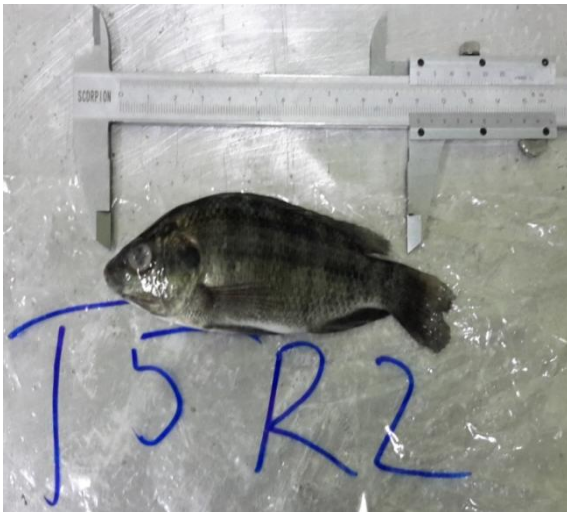


Figure 5a Reproduction parameters (standard length)

Figure 5b Reproduction parameters (Weight)

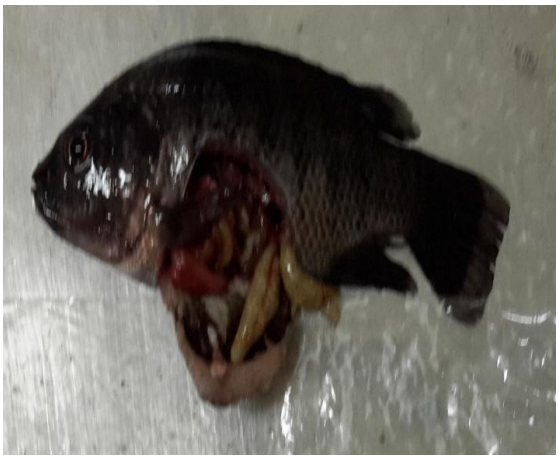
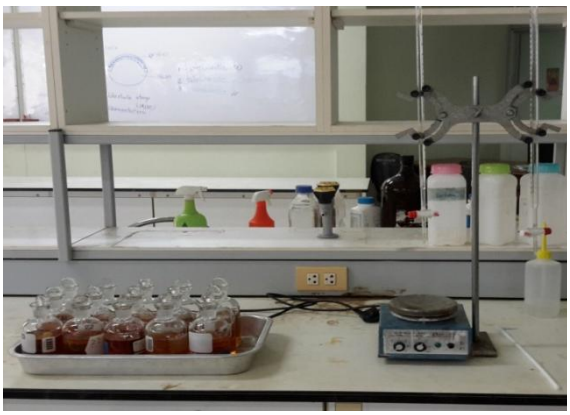


Figure 5c Reproductive analysis (desiccation)

Figure 5d Reproductive analysis (gonad weight)



6a Water quality analysis (Dissolved oxygen analysis)

6b Water quality analysis (Total ammonia analysis)

6c Water quality (pH and temperature analysis)

6d Water quality (total suspended solid)

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