Iranian Journal of Fisheries Sciences 15(3) 1187- 1201 2016

An experimental analysis of the effects of dietary lipid sources and feeding ration on the reproductive performance, egg and larval quality of Nile tilapia, *Oreochromis niloticus* (L.)

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Received: April 2013 Accepted: August 2015

Abstract

This study investigates the effects of dietary lipid sources on the growth and reproductive performance of Nile tilapia over three consecutive spawnings. Fish were reared using three experimental diets, with the goal of replacing dietary fish oil with palm oil. Three experimental diets and a commercial trout diet was used as the control. The effect of dietary lipid on the growth performance, spawning interval, fecundity, relative fecundity (number of eggs per unit weight), egg size, egg fertilization and hatching rate in addition to an assessment of larval quality was investigated. Growth was significantly (p<0.05) influenced by the source of dietary lipid used. The source of the dietary lipid, however, had no significant effect on the diameter of the eggs, as well as their volume or dry weight. Despite this, relative fecundity was found to be significantly different between fish fed experimental diets and those fed the control diet; there was no difference between those fed the PO and mixed PO:CO diets (p>0.05). Similar results were observed for the egg to body weight ratio (EW: BW) and the inter-spawning interval (ISI) for the fish fed control diet (diet 4). The total fecundity (number of eggs produced per fish) obtained from the fish fed the mixed oil diet (PO:CO) was significantly higher (p<0.05) than from those fed the palm oil and control diets. This study suggests that palm oil can replace fish oil in diets fed to *O. niloticus* with no subsequent negative effects on the eggs and larval quality.

Keywords: Nile tilapia, *Oreochromis niloticus*, Diet, Growth, Lipid, Reproduction, Egg, Larval quality

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Introduction

Nile tilapia, *Oreochromis niloticus* (L.) is a widely cultured fish species. It grows and reproduces under a wide range of environmental conditions, tolerates handling stress and performs well in different culture systems. In 2013, the global production of Nile tilapia reached 4,677,613 metric tons (mt), representing approximately 84% of the total farmed tilapia species worldwide (FAO, 2014). After carps, tilapia species are now the second most commonly farmed fish in about 100 countries throughout tropical and sub-tropical regions (Fitzsimmons, 2006). One of the most important goals in fish feed industry is production of good quality food which enhances the quality of the fish eggs, fertilization, survival and growth rate of the larvae produced Broodstock nutrition affects reproduction as well as egg and larval quality in fish (Izquierdo *et al*., 2001). Some feed components are known to greatly influence the spawning quality in several species (Watanabe *et al*., 1985; Verakunpiriya *et al*., 1996; Ng, 2012).

Broodstock productivity remains one of the most significant constraints to commercial production costs and thus knowledge of the factors affecting broodstock productivity is of immense importance to the further development of tilapia culture (Cerdà *et al*., 1994a, b, 1995; Bell *et al*., 1997; Bruce *et al*., 1999; Ng 2012). The nutritional composition of diets, notably the lipids and essential fatty acids (EFA) as nutritional factors, are of key importance and can have a marked effect on egg and larval quality (Watanabe *et al*., 1984, 1985; Harel *et al*., 1994; Fernandez-Palacios *et al*., 1995; Navas *et al*., 1997; Furuita *et al*., 2000). Nevertheless, marine fish oils are traditionally used as the main dietary source of lipid in many commercial fish feeds. The aquaculture sector is currently the world's largest consumer of fish oil; it is estimated that aquafeed consume about 90% of global supply of fish oil and the demand for fish oil in the expanding aquaculture industry will outstrip supply (Tacon and Metian 2008; Turchini *et al*., 2009). Given the fact that supply from wild feed grade fisheries will remain static in the next decade the availability, growth and profitability of aquaculture could be negatively impacted (Sargent and Tacon, 1999; Tidwell and Allan, 2002; Pike and Barlow, 2003; Tacon and Metian, 2008; Pike, 2005). In order to sustain rapid aquaculture development, the industry cannot continue to rely on finite stocks of marine pelagic fish for oil supply. However, one potentially under-utilised replacement for fish oil in broodstock feed is palm oil. Palm oil is similar to other vegetable oils that have been reported in numerous scientific papers to be able to replace a significant part of fish oil in fish diets without negatively affecting fish growth, feed utilisation and survival (Legendre *et al*., 1995; Al-Owafeir and Belal, 1996; Ng *et al*., 2000; Tortensen *et al*., 2000; Bell *et al*., 2002; Ng *et al*.,

2003; Ng and Low, 2005; Ng *et al*., 2006). Nevertheless, in addition to its relatively low cost and high availability, palm oil also has many additional advantages over other vegetable oils when used in aqua-feed formulation (Ng *et al*., 2004).

Studies on the effect of dietary lipid source on spawning performance of tilapias are limited and in the available studies, the brood-fish dietary history is commonly unknown. Only Santiago and Reyes (1993) studied the effects of dietary lipid source on reproductive performance and tissue lipids of Nile tilapia broodstock. They found that cod liver oil (rich in *n*−3 HUFA) resulted in poor reproductive performance, while highest fry production was obtained from fish fed a diet supplement with soybean oil (rich in n-6 fatty acids). El-Sayed *et al*. (2005) studied the effect of dietary lipid source on spawning performance on Nile tilapia at different salinities and found that tilapia needed fish oil for better reproductive performance in brackish water while plant oil (soybean oil) is required for freshwater rearing. However, dietary lipid sources have not been examined under one culture system, including serial spawning and over the entire life-cycle of the fish. This study investigated the effect of different dietary lipid sources and feeding ration on the reproductive performance and egg and larval quality over three consecutive spawnings in Nile tilapia *O. niloticus* which were reared for their entire life-cycle in a recirculating system.

Materials and methods

Diet preparation

Three experimental diets in this study were made at the Institute of Aquaculture, University of Stirling. The dry ingredients were first weighed and mixed for approximately 30 minutes using a Hobart mixer to ensure that the mixture was thoroughly homogenised. Then equal to 10% of the weight of the diet oil was added; this was either cod liver oil (CO), palm oil (PO) or a mixture of PO and CO (9:1 ratio), respectively and mixed for a further 15 minutes. Then enough water was added to the mixture and pellets of appropriate size was made by a California pellet mill (model CL2, San Francisco, California). The proximate composition of experimental diets were analysed and are shown in Table 1.

Feeding procedure

In the present study four diets and two rations, including the control diet, were examined. Diet 1(D1) containing cod liver oil, diet 2 (D2) containing palm oil, diet 3 (D3) containing palm and cod liver oil (9:1 ratio) and diet 4 (D4) a commercial trout feed containing fish meal and fish oil as control.

Culture system and experimental design

The experiment was designed in two phases:

- Growth performance stage.

In the first phase, the first feeding larvae were supplied from a tropical aquarium at Institute of aquaculture, Stirling University and stoked in 20 litre tanks at a density of 210 larvae per tank. The experiment was set up using 4 diets and two rations and duplicated for each diets and ration, respectively for a period of 120 days. Fry were fed 5 times to twice a day with their respective diets of appropriate sizes. The diet sizes were varied according to fry size (see Table 2). Growth data of rationed fish used in each treatment are provided along with the relevant feeding regime. Growth responses of *O. niloticus* fry were recorded as initial and final mean weights, weight gain and specific growth rate, data are shown in Table 3. Fish were then transferred to larger tanks (60 litres) and the same feeding regime was maintained until spawning. All fish were kept in gravity-fed recirculation tanks linked to several settling tanks, faecal traps and filtration units incorporating filter brushes and bio-rings (Dryden aquaculture, UK) for particulate filtration and maximizing bio-filtration. Water was pumped from the system collector tank to a sand filter tank and then sent to a header tank (227-l capacity) via a water pump (Beresford Pumps, UK).

Water temperature was maintained at 27±1°C (using a 3-kW thermostatically controlled water heater). Water was oxygenated via an aeration system and water quality was monitored weekly, including dissolved oxygen (O_2) and water temperature. The levels of pH, nitrite and ammonia were monitored with aquarium water quality kits (C-Test kits, New Aquarium Systems, UK). To maintain good water quality, a partial change of water (10% of total volume) was carried out once a week; the system was refilled with fresh, aerated and preheated water.

Table 1: The proximate composition of the three experimental diets and a commercial trout diet fed to *Oreochromis niloticus* for a period of 120 days. The composition of each component is presented as a percentage of the diet ± 1 standard deviation.

Proximate analysis	Diet 1	Diet 2	Diet 3	Diet 4
Moisture	15.1 ± 0.2	14.3 ± 1.1	14.2 ± 1.1	8.0 ± 1.1
Crude protein	40.5 ± 0.3	41.0 ± 0.1	40.8 ± 0.2	40.6 ± 0.1
Crude lipid	10.0 ± 0.2	9.8 ± 1.0	9.7 ± 1.3	7.02 ± 1.1
Carbohydrate	24.1 ± 0.1	22.2 ± 0.1	23.1 ± 0.1	24.3 ± 0.1
Ash	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.2	10.0 ± 0.2
Crude fibre	7.7 ± 0.7	7.3 ± 0.8	7.3 ± 0.8	7.8 ± 0.9
Gross energy (KJg^{-1})	20.4 ± 0.1	20.4 ± 0.1	20.3 ± 0.2	23.9 ± 0.2

Table 2: Ration of the diet expressed as a percentage of the body weight given to the *Oreochromis niloticus* at key time points throughout the 120-day experimental period.

Age (days)	Full ration (%)	Half ration (%)
0-20	30	15
20-40	20	10
40-80	10	5
80-100	5	2.5
100-120 and thereafter	3	1.5
120 and thereafter	3	1.5

Table 3: The respective growth of the *Oreochromis niloticus* fry and their utilization of the four experimental diets over the 120 experimental period.

Parameters	CO based diet (Diet 1)		Palm oil based diet (Diet 2)		9:1 PO:CO diet (Diet 3)		Commercial trout diet (control, Diet 4)	
	Full ration	Half ration	Full ration	Half ration	Full ration	Half ration	Full ration	Half ration
I_W (g)	0.015 ± 0.02	0.015± 0.02	0.015 ± 0.02	0.015 ± 0.02	0.015 ± 0.02	0.015 ± 0.02	0.015 ± 0.02	0.015 ± 0.02
F_W (g)	10.6 ± 1.79^{a}	9.0 ± 2.57^{ab}	19.5 ± 1.5^{c}	13.1 ± 1.2^{d}	18.5 ± 1.36^{c}	13.0 ± 1.76^{d}	19.8 ± 1.45^{c}	16.2 ± 1.37^{cd}
W_G (g)	716.2± 9.12	539.4±54.67	2072.2± 34.67	1294.4±45.65	1942.9±9.65	1356.9±45.17	2169.6 ± 39.18	1532.5± 51.23
SGR (%/day)	3.2 ± 0.23^{a}	2.2 ± 0.26^{b}	3.0 ± 0.09^{c}	2.5 ± 0.13^{c}	2.9 ± 0.17^{c}	2.6 ± 0.13^{c}	3.0 ± 0.16^{c}	2.8 ± 0.14^{c}
FCR	3.4 ± 0.25^{a}	3.9 ± 0.33^{ab}	2.3 ± 0.17^{c}	2.2 ± 0.23^{c}	2.4 ± 0.14^{c}	2.3 ± 0.16^{c}	2.2 ± 0.13^{c}	2.2 ± 0.19^{c}
SR (%)	45.0 ± 5.77^{a}	40.0 ± 10.12^{a}	70.1 ± 7.63^{b}	71.2 ± 8.11^{b}	69.9 ± 7.67^{b}	69.3 ± 8.26^{b}	73.2 ± 6.56^{b}	71.9 ± 7.45^{b}

I_W=initial weight, F_W=final weight, W_G=weight gain (as total mean weight), SGR=specific growth rate, SR=survival rate and FCR= food conversion ratio. Values are the mean ± 1 S.D. of two replicate, and values within the same row with different letters are significantly different (p<0.05).

- Broodstcok stage

After growth performance investigation, each group of fishes were transferred to larger individual tanks (60 litres) and the same feeding regime continued, and the fishes were frequently checked for sexual maturation. Then the sexually matured females and some males were collected randomly from their respective populations as our broodstock. They were measured (weight and total length) and tagged with Passive Integrated Transponder-PIT tags (Trovan, UK) under anaesthesia by immersion in 1:10 000 ethyl 4-aminobenzoate (Sigma Aldrich, UK). The tagged fishes were allowed to recover completely in clean aerated water prior to being placed in their respective tanks with the same feeding regime until they were ready to spawn.

Spawning investigation

The female broodstock were then transferred into glass tanks. Each tank incorporated two, three or four (depending on the fish size) vertical dividers constructed from translucent Perspex, thus respectively creating three or four separately partitioned 'holding spaces' within each tank where female broodstock could be introduced and maintained individually (Coward and Bromage, 1999). The system condition was the same as the culture condition described above.

Collection of eggs and the eggs total lipid and fatty acid composition

Fish were checked at two hourly intervals during the day for evidence of spawning. In females undergoing ovulation and oviposition, the genital papilla were considerably swollen and extended. The fish were manually stripped under anaesthesia and eggs were fertilised in a petri-dish by adding the sperm taken from the males maintained under similar conditions as females. Fishes were measured and weighed prior to returning into experimental tank after recovering in clean aerated water and all data recorded.

Petri-dishes containing fertilised eggs were scanned and the image was analysed using MR Grab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001) to determine their total fecundity (Rana, 1988). Fertilised eggs were then placed in round-bottomed plastic containers (Rana, 1986) supplied with clean, U.V. sterilized water and left until hatching. A sub-sample of 50 eggs per spawning was taken, prior to incubation and each egg individually measured to the nearest 0.1mm with a dissecting microscope (Olympus Optical Ltd., UK) connected to a video camera by specific calibration utilising Image Pro software (Macromedia V. 4). Since tilapia eggs are ellipsoid, it was important to measure both axes (long and short axis) in order to calculate egg diameter and volume in accordance with the referenced method (Coward and Bromage, 1999). The fertilization (%) and hatching rate (%) and inter-spawning-interval (ISI is the time elapsed between two consecutive spawnings in a female) were also determined.

After measuring the egg sizes, they were then weighed and subsequently oven dried at 70ºC for 24 hours (h). The mean average dried egg weight was determined to the nearest 0.1mg. The egg weight as percentage of body weight (EW: BW) was calculated according to Coward and Bromage (1999) using the equation given below:

$$EW: BW = (EDW \times TF/W) \times 100$$

where: EDW = egg dry weight (mg) , TF = total fecundity and W = fish body weight (g)

A batch of unfertilised eggs (1g approximately) was taken in order to determine total lipid and fatty acid composition of each diet and ration regime prior fertilisation. Samples were placed into a freezer at -70 ^{o}C until used.

Larval quality

Larvae from each individual fish at 10 days post-fertilisation were sacrificed by overdose of anaesthetic and weighed to the nearest 0.1mg. The length was also measured to the nearest 0.1mm using MRGrab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001).

Statistical analysis

Statistical analyses were performed using SPSS for Windows (ver. 15) and Minitab (ver. 15). Statistical significance between treatments was evaluated at the 5% probability level. A general linear model (GLM) ANOVA was used for further analysis of the data. Values are expressed as the mean ± S.E.M. (standard error mean).

Results

Growth performance

The growth rate of fish fed diet1 (cod liver oil) was significantly (p<0.05) lower than others at either full or half ration. Growth responses were significantly influenced by the dietary lipid source. In general, during a period of 120 days, the growth rate increased with the ration size increase. Diet 4, a commercial trout diet (Skretting, UK), as control had the highest weight gain (19.76 and 16.21 g), diet 2 (19.53 and 13.12 g) followed by diet 3 (18.3 and 12.96 g) and the least (10.61 and 6.99 g) was diet 1 for full and half ration, respectively. However, in the case of specific growth rate, the control was significantly higher (2.6 %.day^{-1}) than diet 1 (2.2 %.day^{-1}). Weight gain of fish fed diets 2 and 3 which had palm and mixed palm and cod liver oil as the lipid source were not significantly different from the control at either full or half rations. With respect to SGR (specific growth rate), diets 2 and 3 resulted in the same values as the control. This meant that these diets were not significantly different from the control. Food conversion ratio (FCR) followed the same trend as SGR with the exception of diet 1 which was significantly higher at both full and half ration than other diets.

Fecundity and egg size

A total of 125 spawns were recorded over three consecutive spawnings for all diet treatments. In diet one, containing cod liver oil (COD 1) only one fish spawned three times, however, due to the high mortality of fish from the previous phase of the experiment and poor egg quality data obtained from the group of fish fed diet 1 was discarded from the analyses. Egg size and fecundity were analysed among the dietary treatment and spawning numbers using two-way ANOVA. As a result of no significant (p>0.05) interaction being observed between diet and spawning numbers, spawning data were pooled and analysed using GLM one-way ANOVA, comparing differences between diet treatments. There were no significant (p>0.05) differences between egg diameter, egg volume, egg wet and dry weight and total egg volume from fish fed diet 2, 3 and 4, respectively (Table 4).

Relative fecundity ranged from 5.5 ± 1.84, 5.5 ± 2.17 and 3.6 ± 1.68 for fish fed diet 2, 3 and 4, respectively. However, a significant (p<0.05) difference occurred in relative fecundity for fish fed diet 4 (control) but for fish fed diets 2 and 3 were not significant (p>0.05) (Table 4). Similar results were observed when comparing the EW: BW which ranged from 1.4 ± 0.06, 1.3 ± 0.08 and 0.9 ± 0.08 (Table 4). Mean total fecundity in the present study ranged from 629 to 823, the effect of dietary lipid source on total fecundity of fish fed diet 3 was significantly (p<0.05) higher than fish fed diet 2 and 4, respectively, but for fish fed diet 2 and 4 was not significant (Table 4).

Inter spawning intervals (ISI)

The average spawning intervals in the present study ranged from 14-24 days. Significant (p<0.05) differences were detected when comparing ISI between the diet groups. The longest ISI was found in fish fed diet 4 (control) and the shortest was found for fish fed diet 2 (PO), however, ISI in fish fed diets 2 and 3 was not significantly (p>0.05) different (Fig.1).

Larval quality

Larval batches of each fish were recorded and maintained individually for three consecutive spawnings and grouped as fish fed diet 2, 3 or 4, respectively. Mean values of larval length and weight were analysed using GLM two-way ANOVA. The effects of dietary lipid sources on larvae length and weight over three serial spawnings were significant. However, these levels were not constant and due to no significant difference being observed in egg dry weights between treatments, it was concluded that these slight differences could not be due to diets; therefore the larvae length and weight data were pooled together to determine mean differences between the diets. Table 5 shows that both larval length and weight from fish fed diet 2 were significantly (p<0.05) lower than for larvae obtained from fish fed diet 3 and 4 but between diet 3 and 4 the difference was not significant (p>0.05).

Table 4: Spawning performance of each group of *Oreochromis niloticus* fed an experimental diet for their entire life-span up to the point at which they spawned.

Parameters	Treatments		
	PO diet (Diet 2)	**PO:CO oil diet (9:1) (Diet 3)**	**Control (Diet 4)**
Total fecundity (total no. of eggs produced)	752.6 ± 32.01[b]	823.3 ± 46.59[a]	662.9 ± 36.10[b]
Relative fecundity (no /g)	5.5 ± 0.23[a]	5.5 ± 0.38[a]	3.6 ± 0.31[b]
Egg diameter (mm)	2.2 ± 0.03[a]	2.2 ± 0.03[a]	2.2 ± 0.03[a]
Egg volume (mm^3)	5.2 ± 0.22[a]	5.4 ± 0.22[a]	5.6 ± 0.24[a]
Total egg volume (mm^3)	3902.7 ± 236.45[a]	4385.7 ± 267.11[a]	3654.6 ± 237.07[a]
Egg dry weight (mg)	2.6 ± 0.05 [a]	2.5 ± 0.09[a]	2.7 ± 0.09[a]
Egg wet weight (mg)	6.1 ± 0.1[a]	6.1 ± 0.16 [a]	6.6 ± 0.21 [a]
EW: BW (%)	1.4 ± 0.06[a]	1.3 ± 0.08[a]	0.9 ± 0.08[b]
Fertilisation rate (%)	76.3 ± 1.40 [a]	78.5 ± 1.82 [a]	75.9 ± 2.2 [a]
Hatchability (%)	59.5 ± 1.04 [a]	60.1 ± 1.75 [a]	61.4 ± 1.35 [a]
ISI (day)	14.0 ± 0.71 [a]	19.0 ± 1.52 [b]	24.0 ± 2.74 [c]

For each factor, letters with a different superscript are significantly different (ANOVA, Tukey's test, $p<0.05$) form one another. Data are the mean ± 1. S.E.M of two replicates. CO: Cod Liver Oil. PO: Palm Oil.

Table 5: The larval performance of the *Oreochromis niloticus* whose parents had been reared on one of the experimental diets for a period of 120 days up until they spawned.

Parameters	Treatments		
	Diet 2	**Diet 3**	**Diet 4**
Larvae length (mm)	9.3 ± 0.64[a]	9.6 ± 0.67[bc]	9.5 ± 0.66[c]
Larvae weight (mg)	9.8 ± 1.43[a]	10.2 ± 1.57[bc]	10.3 ± 1.61[c]

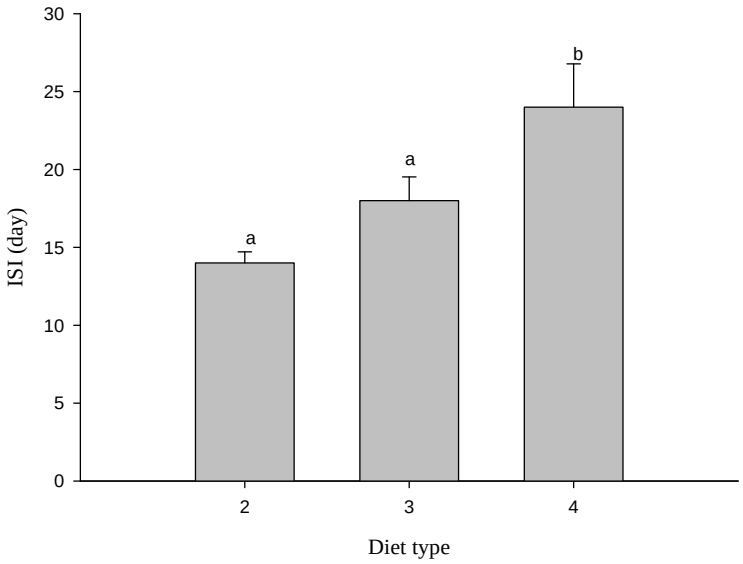

Figure 1: The mean ± 1 S.E.M inter-spawning interval (ISI day^{-1}) for each group of *Oreochromis niloticus* fed one of the experimental diets. Columns with a different superscript are statistically different (ANOVA, Tukey's test, $p<0.05$) from one another.

Discussion

One of the principal objectives of the present study was to investigate fish oil based diets, commonly used by aquafeed industry, with alternative oil sources. Fish oil is produced from small marine pelagic fish and represents a finite resource (Ng *et al.*, 2003). Because of several factors, including over fishing, resulting in dwindling catch and environmental changes which necessitate tight regulations, future demand for wild-caught fish will exceed supply (Sargent *et al.*, 1999). Hence the need to evaluate potential substitutes for fish oil, an important ingredient in the formulation of aquafeeds. Palm oil, currently the second most abundant vegetable oil in the world, presents a viable alternative to fish oil in aquafeeds (Ng, 2002).

A fishmeal based diet contains approximately 6-7 wt% fish oil. Therefore to avoid any effect of fish oil in the experimental diet, the protein sources of diets were changed to soybean concentrate containing 65 wt% protein and a trace amount of lipid.

Previous studies revealed that palm oil could be used as a dietary lipid source with no negative effect on fish growth (Legendre *et al*., 1995; Al-Owafeir and Belal, 1996; Ng *et al*., 2000; Tortensen *et al*., 2000; Bell *et al*., 2002; Ng *et al*., 2003, 2004; Ng and Low, 2005; Ng *et al*., 2006). However, limited information is available on the effect of lipid sources on tilapia reproductive performance. The present study is the first attempt to investigate the effect of dietary lipid source on the reproductive performance of tilapia fed solely their respective experimental diets for their entire life-cycle. This study shows that tilapia broodstock can be maintained and spawned successfully on different dietary lipid sources. The spawning performance of the Nile tilapia fed the two formulated dietary lipid sources (palm and mixed PO and CO) was comparable to those fed a control diet. No significant differences were found in egg wet and dry weights, egg diameter and volume, fertilisation and hatching rate of the fish fed diet 2, 3 and 4, respectively. The fish group fed diet 1 (cod liver oil) had a high mortality in the on-growing stage and only one fish spawned during the experiment which had poor egg quality; the growth gain was lower than other diets. This might be due to the high concentration of (n-3) HUFA in cod liver oil. The results of this work are in agreement with the previous studies (Kanazawa *et al*., 1980; Takeuchi *et al.*, 1983; Ng, 2004; Ng *et al*., 2004) that reported depressed growth of tilapia with oils having high levels of n-3 PUFA and (Watanabe, 1982; Santiago and Reyes, 1993) found that fish fed a cod liver oil diet had poor egg quality. However this result contradicted the results of growth gain of tilapia that were reported by Santiago and Reyes (1993). Alternatively the lower growth gain may have been a result of the palatability of the diet which consisted of soybean meal and pure cod liver oil. It is important to note that further

independent investigations are required to support this hypothesis.

Fertilized eggs of *O. niloticus* commonly take approximately 4 days to hatch at 28°C and development time takes about 6 days (Macintosh and Little, 1995). In the present study, eggs from all treatments were kept at 28 ± 1°C and for 3-4 days for hatching and a further 6 days to absorb the yolk-sac. Yolk-sac is absorbed gradually over 6 days after hatching at 28°C when eggs are orally incubated (Macintosh and Little, 1995; Coward and Bromage, 1999). The results showed that total fecundity of the group of fish fed the mixed oil diet was significantly higher than those fed palm oil or the control diet, this could be due to the ratio of n-6 and n-3. The results indicated that tilapia requires miniscule quantities of n-3 for growth and enhanced reproductive performance; similar results were found by Watanabe (1982) that Nile tilapia fed a basal diet supplemented with soybean oil (high in n-6 fatty acids) had higher fecundity, spawning frequency and fry production and that these were relatively lower in fish fed a 5 wt% cod liver oil supplemented diet (high n-3 fatty acids). In support, Hung *et al* (1998) suggested that *n*−3 HUFA, such as linolenic, EPA and DHA are important for these fish. Similarly, Kanazawa *et al.* (1980) and El-sayed and Garling (1988) found that *T. Zillii* reared in freshwater required n−6 fatty acids for optimum growth.

Larval quality

Larval length and weight were not significantly affected by parents' dietary lipid sources. Nevertheless, both weight and length of larvae from fish fed palm oil were slightly lower than in larvae from fish fed mixed oil or control diets. The lower weights and lengths from fish fed the palm oil diet could not be affected by their diet because no significant difference occurred in the egg dry weight. However, this significance could be due to genetic differences within the broodstock or other parameters.

Inter spawning interval (ISI)

Shortest ISI was observed in the group of fish fed the palm oil diet and the longest in fish fed control diet. In the present study there was no relationship between egg size and ISI, but it was apparent that large females had the longest ISI and conversely small females the shortest ISI. This might simply imply that ISI was longer, and fish required more energy for maintenance and growth than producing eggs. This result agrees with Rana (1988) who reported that within a group of females of the same age class, there is no significant relationship between body size and egg size.

Dietary lipid had no significant effect on reproductive performance. In conclusion, the results of this study suggest that under controlled conditions, lipids of non-marine origin, such as palm oil, can be used

successfully for broodstock diets at 1.5% of body weight. In addition, comparable performance with commercial control diets and halving of feed requirement should increase profitability of feed production.

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