EFFECTS OF DIETARY LIPID SOURCE ON EGG AND LARVAL QUALITY OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L.)

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Abstract

This study investigated the effects of dietary lipid sources on reproductive performance of Nile tilapia for three consecutive spawnings with the goal of replacing dietary fish oil with palm oil. In this study tilapia were fed solely with the selected experimental diet during their entire life, from onset of exogenous feeding until termination of spawning. Three isonitrogenous (40% crude protein), isoenergitic (20 KJg^{-1}) experimental diets were made containing either 10% cod liver oil (CO), palm oil (PO) or mixed palm and cod liver oil (9:1 ratio; PO&CO) using soybean protein concentrate as the protein source. In addition a commercial trout diet was used as a control. The influence of dietary lipid on spawning intervals, fecundity, relative fecundity (egg number per unit weight), egg size, fertilisation and hatching rate and larval quality was investigated. Dietary lipid sources had no significant effect on egg diameter, egg volume and egg dry weight. However, relative fecundity was significantly ($P<0.05$) different in fish fed control diet while those fish fed PO and mixed PO&CO were not different ($P>0.05$). Similar results were observed for egg weight to body weight ration (EW: BW) and inter spawning interval (ISI) for fish fed diet 4. Moreover, total fecundity (number of eggs produced per fish) obtained from fish fed the mixed oil diet (PO & CO) was significantly ($P<0.05$) higher than for those fed the palm oil and control diets. This study suggests that palm oil can replace fish oil with no negative effect on egg and larval quality in *O. niloticus*.

Keywords: Nile tilapia, *Oreochromis niloticus*, Diet, Lipid, Reproduction, Egg and larval quality

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus* is a widely cultured species because it grows and reproduces under a wide range of environmental conditions and tolerates handling stress. Tilapias perform well in extensive, semi-intensive and intensive culture systems. Farmed Nile tilapia production reached 1,703,125 mt, about 84% of total farmed tilapia production, in 2006 (FAO, 2006). Tilapias are now the second most popularly farmed fish after carps, and currently tilapia are cultured in about 100 countries in tropical and subtropical regions. One of the most important aspects in fish seed production is production of fertilized eggs that result in larvae with high survival and growth. Broodstock nutrition affects reproduction and egg and larval quality in fish (Izquierdo *et al.*, 2001). Some feed components are known to greatly

influence spawning quality in several species (Verakunpiriya *et al.,* 1996; Watanabe *et al.,* 1985).

Broodstock productivity remains one of the most significant constraints to commercial production costs and thus knowledge of factors affecting broodstock productivity is of immense importance to further development of tilapia culture. In particular, lipids and essential fatty acids (EFA) are nutritional factors that greatly affect egg and larval quality (Fernandez-Palacios *et al.,* 1995; Furuita *et al.,* 2000; Harel *et al.,* 1994; Navas *et al.,* 1997; Watanabe *et al.,* 1984; Watanabe *et al.,* 1985). Nevertheless, marine fish oils are traditionally used as the main dietary lipid source in many commercial fish feeds. Aquafeeds currently use about 70% of the global supply of fish oil and by the year 2010, fish oil use in aquaculture is estimated to reach about 97% of the world supply (Tacon, 2003).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 potential replacement for fish oil in aquafeeds is palm oil. In this respect, 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 utilization and survival (Al-Owafeir and Belal, 1996; Bell *et al.,* 2002; Legendre *et al.,* 1995; Ng *et al.,* 2000; Ng *et al.,* 2006; Ng *et al.,* 2003; Ng and Low, 2005; Tortensen *et al.,* 2000). Nevertheless, in addition to its 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).

The effect of dietary lipid source on spawning performance of tilapias has not been sufficiently studied. Only Santiago and Reyes (1993) studied the effects of dietary lipid source on reproductive performance and tissue lipids of Nile tilapia. 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) and El-Sayed *et al.* (2005) studied the effect of dietary lipid source on spawning performance at different salinities and found that tilapia need 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 fish. This study investigated the effect of different dietary lipid sources on egg and larval quality over three consecutive spawnings in Nile tilapia *O. niloticus* which had bean reared for their entire life cycle on their respective diet regime 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 and the proximate composition for these are presented in (Table 1) and (Table 2), respectively. The dry ingredients were first mixed for approximately 30 minutes using a Hobart mixer to ensure that the mixture was well homogenised and then blended by adding 10% oil from cod liver oil (CO), palm oil (PO) or a mixture of PO and CO (9:1 ratio), respectively for further 15 minutes. Water was added at 20-30% V/W to give a pelletable mixture. Diets were made as pellets of appropriate size using a California pellet mill (model CL2, San Francisco, California).

Table 1. Feed ingredients and formulation of experimental diets (g/100g total diet)

Ingredients	Diet 1	Diet 2	Diet 3
Soybean concentrate	55	55	55
Casein	0.5	0.5	0.5
Corn starch	17.5	17.5	17.5
Cod liver oil	10	---	---
Palm oil	---	10	---
PO& CO (9:1)*	---	---	10
DCP**	2	2	2
Fish hydrolysate	5	5	5
DL-methionine	0.5	0.5	0.5
Vitamin premix	2	2	2
Minerals premix	2	2	2
Carboxy methyl cellulose	3.5	3.5	3.5
α-cellulose	2	2	2
TOTAL	100	100	100

PO &CO = combination of cod liver and palm oil**DCP= dicalcium phosphate.

Table 2. Proximate composition of experimental diets (composition of diet expressed as %)

Proximate analysis	Diet 1	Diet 2	Diet 3
Dry mater	15.1±0.18	14.3±1.05	14.2±1.1
Crude protein	40.5±.0.28	41.01±0.07	40.8±0.19
Crude lipid	10±0.15	9.8±1.05	9.7±1.25
Carbohydrate	24.11	22.2	23.1
Ash	5.3 ±0.006	5.3 ±0.045	5.1±0.25
Crude fibre	7.7±01	7.3±.79	7.3±.84
Gross energy (KJg^{-1})	20.4±0.121	20.4±0.112	20.3±0.23

Culture system and experimental design

Female broodstock were maintained in glass tanks, each tank incorporated two, three or four (depending on fish size) vertical dividers constructed from translucent Perspex, thus respectively creating three or four separately partitioned 'holding spaces' within each tank into which female broodstock could be introduced and maintained individually (Coward and Bromage, 1999). All fish were maintained in gravity-fed recirculation aquaria incorporating various sizes of covered fish holding 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) (Figure 1).

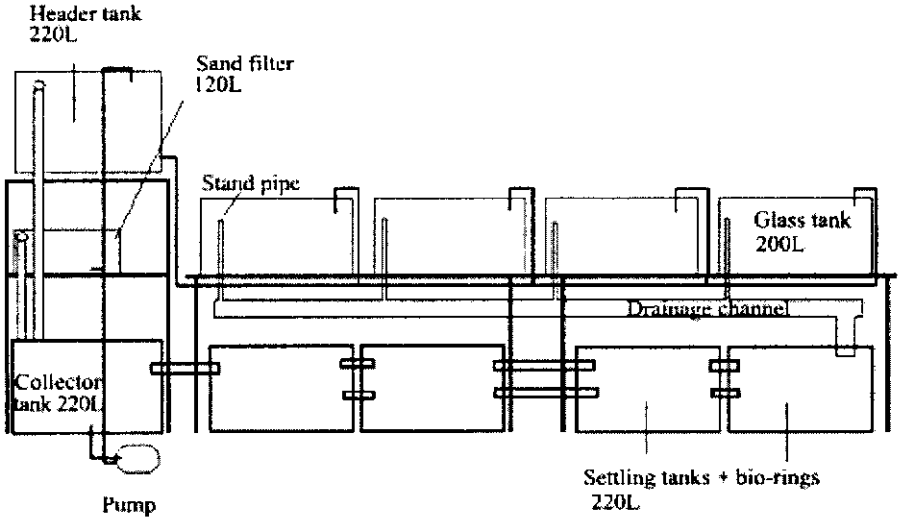


Figure 1. Lateral view of the closed recirculating system used to hold experimental fish

Water temperature was maintained at 27±1 °C (using a 3-kW thermostatically controlled water heater). Water was oxygenated via airstones in the header tank and each aquarium by a low-pressure blower. The water inflow was constant at 252 l h^{-1} $tank^{-1}$. Water quality was monitored twice a month, including dissolved oxygen (O_2) and water temperature. The levels of pH, nitrate, nitrite and ammonia were evaluated 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.

Before starting the experiment, the female broodstock, *O. niloticus* were previously reared under experimental conditions for their entire life from onset of exogenous feeding until spawning; female broodsocks were then collected randomly from their respective populations and 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, UK). The fish were allowed to recover completely in clean aerated water prior to being placed to their respective glass tank.

Fish were fed three times daily (9:13:17) at 3% of body weight with the experimental diets and a commercial pelleted trout feed (Skretting, UK) as a control.

Spawning investigation

Fish were checked at two hourly intervals during the day for the evidence of spawning. In females undergoing ovulation and oviposition the genital papilla were considerably swollen and extended. Fish were manually stripped under anaesthesia and eggs were fertilised on a Petri-dish by adding the sperm from males maintained under the same diet regime as well as the same method of females. Fish 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 using a scanner and the scanned picture analysed using MRGrab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001) to determine total fecundity (Rana ,1988) where total fecundity is the number of eggs in a freshly spawned batch of eggs. Fertilised eggs were then placed in round-bottomed plastic containers (Rana, 1986) supplied with clean, U.V. sterilised 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., U.K.) 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 according to method of (Coward and Bromage, 1999). The fertilisation (%) and hatching rate (%) and inter-spawning-interval (ISI time elapsed between one spawn and the next) were also determined.

After measuring egg size, eggs were then weighed and subsequently oven dried at 70°C for 24h. Mean egg dried weight was determined to the nearest 0.1mg. The EW: BW ratio was determined (Coward and Bromage, 1999).

(EW: BW= (EDW*TF/W*100)

Where: EW: BW= egg weight to body weight ratio (%), EDW=egg dry weight (mg), TF= total fecundity and W= fish weight (g).

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 utilising MRGrab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001).

Feeding procedure

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

Table 3. Experimental design

Lipid source of diets	Diet	Protein source	Feeding rate (%BW $day^{-1)}$	No. replicate	Spawning no. per fish
Cod liver oil	D1	Soybean Concentrate	3	2	3
Palm oil	D2	Soybean Concentrate	3	2	3
Mixed PO*&CO** (9:1)	D3	Soybean Concentrate	3	2	3
Control	D4	Fish meal	3	2	3

*PO=palm oil and **CO=cod liver oil

Statistical analysis

Statistical analyses were performed using SPSS for windows (version 15) and Minitab (version 15). Statistical significance between treatments was evaluated at the 5% probability level. General linear model (GLM) ANOVA was used further analysis of data. Values are expressed as means ± S.E.M.

RESULTS

Fecundity and egg size

A total of 125 spawns were recorded over three consecutive spawnings for all diet treatments. In diet CO (diet 1) only one fish spawned three times, however, due to 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 this 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 for 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).

Table 4. Spawning performance of *O. niloticus* fed different dietary lipid sources

Parameters	Treatments		
	Palm oil diet (Diet 2)	P&CL oil diet (9:1) (Diet 3)	Control (diet 4)
Total Fecundity	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 weigh (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±0.71 ^{a}	19±1.52 ^{b}	24±2.74 ^{c}

In each raw means with different superscripts are significantly different (ANOVA, Tukey's test, $P < 0.05$).

Data are means ± SEM of two replicates.

Larval quality

Larval batches of each fish were recorded 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 significance levels were not constant and due to no significant difference in egg dry weights between treatments 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 5. Larval performance of Nile tilapia (*O. niloticus*) fed different dietary lipid sources over three consecutive spawning.

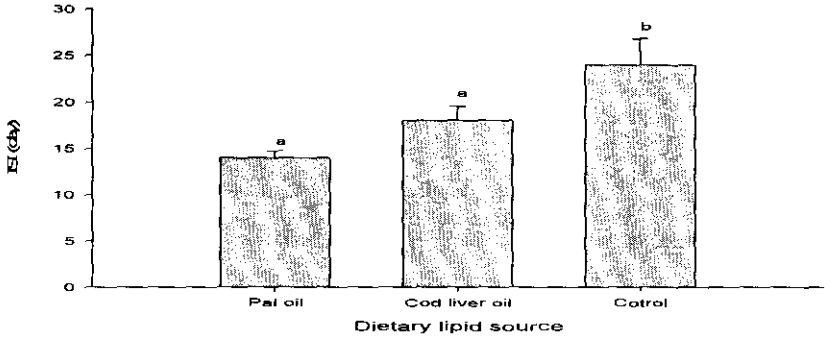
Parameters	Treatments		
	Diet 2	Diet 3	Diet 3
Larvae length (mm)	9.3 ± 0.64^{a}	9.6 ± 0.67^{bc}	9.5 ± 0.66^{c}
Larvae weight (mg)	9.8 ± 1.43^{a}	10.2 ± 1.57^{bc}	10.3 ± 1.61^{c}

Values are means ± S.D In each row means with different superscripts are statistically different (ANOVA, Tukey's test, $P<0.05$).

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.

Figure 2. Inter -Spawning- -Intervals (ISI day-1) of *O. niloticus* fed on different dietary lipid source



Values are mean ± S.E.M. In each column means with different superscripts are statistically different (ANOVA, Tukey's test, $P<0.05$).

DISCUSSION

One of the principal objectives of the present study was to investigate fish oil based diets, commonly used by industry, with alternative oil sources. Fish oil is produced from small marine pelagic fish and represents a finite fishery 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% 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% 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 (Al-Owafeir and Belal, 1996; Bell *et al.,* 2002; Legendre *et al.,* 1995; Ng *et al.,* 2000; Ng *et al.,* 2006; Ng *et al.,* 2003; Ng *et al.,* 2004; Ng and Low, 2005; Tortensen *et al.,* 2000). 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 of the reproductive performance of tilapia fed solely their respective experimental diets for their entire life cycle. The present 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&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 obtained the fish fed diet 2, 3 and 4 respectively. The fish group fed diet 1 (cod liver oil) had 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 the present study are in agreement with the previous studies (Kanazawa *et al.,* 1980; Ng, 2004; Ng *et al.,* 2004; Takeuchi *et al.,* 1983) that reported depressed growth of tilapia with oils having high levels of n-3 PUFA and (Santiago and Reyes, 1993; Watanabe, 1982) who found that fish fed a cod liver oil diet had poor egg quality but this result contradicted the results of growth gain of tilapia that reported by Santiago and Reyes (1993). On the other hand the reason for lower growth gain could be due to the palatability of the diet which consisted of soybean meal and cod liver oil. However, further investigations are required to support this assumption.

Usually fertilised eggs of *O. niloticus* take about 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 3-4 days were required for hatching and a further 6 days to absorb the yolk-sac . Usually, yolk-sac is absorbed gradually over 6 days after hatching at 28°C when eggs are orally incubated (Coward and Bromage, 1999; Macintosh and Little ,1995). 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 need tiny amounts 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% 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 diet because no significant difference occurred in 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 longest ISI and conversely small females the shortest ISI. This might simply imply that ISI was longer, and fish need 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.

CONCLUSION

Dietary lipid source (palm oil) had no significant effect on egg and larval quality. 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 brood stock diets. In addition, comparable performance with commercial control diets and halving of feed requirement should increase profitability of seed production.

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