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Inclusion of crude palm oil in the broodstock diets of female Nile tilapia, *Oreochromis niloticus*, resulted in enhanced reproductive performance compared to broodfish fed diets with added fish oil or linseed oil

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ABSTRACT

The intensive farming of tilapia is rapidly expanding and the need to produce sufficient quantities of quality fry is becoming crucial to meet increasing global demands for stocking tilapia farms. The present study was conducted to evaluate the effects of dietary lipid source on the reproductive performance of tilapia broodfish. Four isonitrogenous (35% protein) and isolipidic (10%) casein-based diets were formulated with added fish oil (FO), FO and crude palm oil (FO + CPO; 1:1), CPO or linseed oil (LSO) as the lipid source, respectively. Pre-spawning female Nile tilapia (*Oreochromis niloticus*, GIFT strain) was individually color-tagged, and six females and two males were stocked into a one-tonne breeding tank. Each diet was fed to two tanks of broodfish and the reproductive performance of 12 individual female fish was monitored over 25 weeks. Female broodfish fed the two CPO-based diets showed significantly ($P<0.05$) larger gonad sizes and lower intraperitoneal fat compared to fish fed the FO or LSO diets. First spawning occurred earliest in broodfish fed the CPO diet at 30.8 ± 9.9 days compared to 44.1, 45.5 or 76.3 days for fish fed the FO + CPO, FO or LSO diet, respectively. The highest number of actively spawning tilapia was observed in fish fed the FO + CPO diet, followed by fish fed the CPO, FO or LSO diet, respectively. At the end of 25 weeks, tilapia fed the two CPO-based diets produced the highest total number of eggs per fish due to the shorter inter spawning interval and higher spawning frequency. Mean diameter, volume and weight of eggs did not vary among dietary treatments. Egg hatchability was significantly higher in broodfish fed the CPO-based diets. The fatty acid composition of the muscle, gonad, egg and newly hatched larvae was influenced by dietary lipid source. However, evidence of preferential fatty acid conservation, conversion and utilization was also observed in these tissues. The fatty acid composition of tilapia eggs did not vary over four consecutive spawns. The gonads, eggs and larvae of tilapia fed the CPO diet contained the highest relative concentration of saturates, monoenes, arachidonic acid and n-6/n-3 ratio. The high total n-3 PUFA concentration observed in the gonads of fish fed the LSO diet, and to a lesser degree the FO diet, seemed to be detrimental to the reproductive performance of tilapia. In conclusion, the inclusion of CPO in tilapia broodstock diets can be a cost-effective method to increase tilapia fry production.

1. Introduction

The global production of tilapia is expected to exceed three million tonnes in 2010 and estimated to increase to about 8.9 million tonnes by the year 2020 (Tacon and Metian, 2008). This rapid rise in the global production of tilapia is due in part to the increasing intensification of farming systems and this has led to a critical need for large quantities of fingerlings for stocking grow-out systems. Furthermore, it is increasingly important to produce high quality tilapia fry due to the low fecundity of broodfish. Tilapia of the *Oreochromis* genus, the major farmed species, are female mouth-brooders and exhibit high parental care with relatively low number of eggs produced in each clutch. The problem in the mass production of tilapia seed is further exacerbated due to the low degree of female spawning synchrony and reduction in spawning rigor with time (Mires, 1982). Broodstock nutrition is recognized as a major factor that can influence fish reproduction and subsequent larval quality of many fish species (Izquierdo et al., 2001). The development of cost-effective and nutrient optimized broodstock feeds for tilapia is both pertinent and crucial. There is currently a paucity of information concerning the nutrient requirements of tilapia broodfish especially as it relates to lipid nutrition but some information is available on protein and energy requirements (Gunasekera et al., 1996; Siddiqui et al., 1998; El-Sayed and Kawanna, 2008; Lupatsch et al., 2010).

Lipids and fatty acids have been reported to play a major role in broodstock nutrition and greatly influence the quality of developing

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eggs and larvae (Izquierdo et al., 2001; Tocher, 2010). Omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), as well as n-6 LC-PUFA such as arachidonic acid (ARA, 20:4n-6), are known to significantly influence reproductive performance in many farmed fish. It has been reported that dietary DHA/EPA and EPA/ARA ratios may influence egg and larval quality (Bell and Sargent, 2003; Furuita et al., 2007; Wilson, 2009). These and other studies have shown a direct impact of fatty acid composition in broodstock diets on the fatty acid composition of egg lipids (Tocher, 2010). Furuita et al. (2000) reported that a minimum of 1.5–2.0% of n-3 LC-PUFA in the broodstock diets of the Japanese flounder, *Paralichthys olivaceus*, was necessary to ensure high quality eggs and larvae. A dietary level of 1.8% ARA as a percentage of total fatty acids was reported to have beneficial effects on the spawning performance and egg and larval quality of Atlantic halibut, *Hippoglossus hippoglossus* (Mazorra et al., 2003). Marine fish oils are rich in LC-PUFA and are traditionally used as the major lipid source in aquafeeds, including broodstock feeds. However, it is estimated that aquafeeds currently consume about 90% of the global supply of fish oil (FO) and the demand for FO from the expanding aquaculture industry will imminently out strip supply (Tacon and Metian, 2008; Turchini et al., 2009). Considering the high demand, impending short supply and rising costs of FO, much research is currently being conducted on finding suitable alternative lipid sources for use in aquafeeds (Turchini et al., 2009).

Vegetable oils are viable alternatives as they are readily available, renewal and more cost-effective compared to FO. Many studies have reported that vegetable oils can partially or fully replace FO in fish diets without compromising growth performance as long as the essential fatty acid requirements of the fish are met (Turchini et al., 2009). However, all the major vegetable oils produced do not contain LC-PUFA in their fatty acid profile. Considering the reported importance of this group of fatty acids in broodstock nutrition, finding suitable lipid alternatives will be more challenging and requires concerted research effort. There is currently very limited information on the effect of dietary vegetable oils and FO on the reproductive performance of tilapia.

In the present study, linseed oil (LSO) which has very high concentrations of linolenic acid (18:3n-3) was selected as a potential replacement for dietary FO in the broodstock diets of Nile tilapia. Previous research has indicated that Nile tilapia is capable of elongating and desaturating 18:3n-3 to LC-PUFA (Olsen et al., 1990) and that this bioconversion is greater in fish fed vegetable oil (Tocher et al., 2002). We have also chosen to evaluate crude palm oil (CPO) as a potential lipid source. In a previous study where red hybrid tilapia, *Oreochromis* sp., was fed the CPO-based diets from stocking to marketable size, we noticed that the gonado-somatic index of both the female and male fish was much higher compared to fish fed the FO-based diet (Bahurmiz and Ng, 2007). It would be interesting to further verify this observation and to determine whether the larger gonads may eventually lead to enhanced reproductive performance and improved egg and larval quality. Research into the use of palm oil products and by-products has generally indicated that substantial amounts of dietary FO can be replaced by palm oil in the grow-out feeds of various commercially important farmed species (Ng et al., 2007; Ng and Gibon, 2010). However, to date, not much is known on the impact of palm oil on fish reproduction. Shiranee and Natarajan (1996) reported that the supplementation of CPO in the diets of pearlspot (*Etroplus suratensis*) imparted a beneficial effect on ovarian development and maturation. A recent study by Hajizadeh et al. (2008) reported preliminary results which suggested that palm oil can be successfully used in tilapia broodstock diets. Unfortunately, they were not able to compare the reproductive performance of palm oil-fed fish to that of fish fed a FO diet due to high mortality of fish fed the FO diet. The present study was conducted to comprehensively evaluate the effects of dietary lipid source (FO, LSO and CPO) on the spawning performance, and egg and larval quality, and the fatty acid composition of various reproductive products of Nile tilapia.

2. Materials and methods

2.1. Experimental broodstock diets

Four isonitrogenous (35% crude protein) and isolipidic (10%) broodstock diets were formulated (Table 1). The ingredient composition of all diets was similar, with the exception of the added lipid sources. Casein and gelatin were used as the major protein sources, together providing 27% of dietary protein. To improve the palatability of the diets, fish meal and soybean meal, which collectively provided the remaining 8% of protein, were added. The dietary residual oil originating from fish meal and soybean meal were minimal (about 0.84%). Fish oil (FO) or linseed oil (LSO) was added to the experimental broodstock diets, respectively, as the sole lipid source. For the remaining two diets, dietary FO was replaced with crude palm oil (CPO) at 50% (CPO + FO) or 100% (CPO) substitution level, respectively. All oils were purchased from various local suppliers or grocery stores. The experimental diets were prepared by mixing the dry ingredients with oil and water in a Hobart mixer. The moist dough was screw-pressed through a 3-mm die and the feed pellets formed were broken down into 2 to 5 mm long pellets, fan-dried and stored

Table 1
Ingredient and proximate composition (g/100 g dry diet) of the tilapia broodstock diets.

	Diets[a]			
	FO	FO + CPO	CPO	LSO
Ingredient[b]				
Casein	22.61	22.61	22.61	22.61
Gelatin	6.00	6.00	6.00	6.00
Soybean meal[c]	10.23	10.23	10.23	10.23
Danish fish meal[d]	3.86	3.86	3.86	3.86
Fish oil	9.16	4.52	–	–
Crude palm oil	–	4.63	9.16	–
Linseed oil	–	–	–	9.16
Corn starch	24.76	24.76	24.76	24.76
Vitamin mix[e]	3.00	3.00	3.00	3.00
Vitamin C[f]	0.50	0.50	0.50	0.50
Mineral mix[g]	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
CMC[h]	2.00	2.00	2.00	2.00
α-cellulose	14.88	14.88	14.88	14.88
Proximate composition				
Moisture	12.5	12.9	12.1	13.5
Protein	36.3	36.8	36.0	35.9
Lipid	10.2	10.6	10.1	10.3
Ash	4.0	4.0	3.9	3.8
Fiber	6.4	5.5	5.6	6.1
NFE[i]	43.2	43.8	43.7	43.7

[a] FO, fish oil; FO + CPO, fish oil + crude palm oil (1:1); CPO, crude palm oil; LSO, linseed oil.

[b] All purified feed ingredients were purchased from Sigma-Aldrich (MO, USA) except for α-cellulose and cornstarch which were purchased from Liang Traco (Penang, Malaysia).

[c] Contains protein 48.8 g, lipid 3.6 g, fiber 4.8 g, and ash 6.8 g per 100 g dry matter.

[d] Contains protein 77.8 g, lipid 12.3 g, and ash 13.1 g per 100 g dry matter.

[e] Formulation (g/kg): α-tocopheryl acetate, 2; inositol, 5; choline bitartrate, 136.06; niacin, 4.5; riboflavin, 1; pyridoxine·HCl, 1; thiamin·HCl 0.92; D-calcium panthothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione 1.67; D-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; and cellulose, 834.167.

[f] Added as 20 mg/kg L-ascorbic acid in cellulose.

[g] Formulation (g/kg): calcium phosphate monobasic, 135.5; calcium L-lactate hydrate, 327.0; ferric citrate, 29.7; magnesium sulfate·$7H_2O$, 132.0; potassium phosphate dibasic, 239.8; sodium phosphate monobasic·H_2O, 87.2; sodium chloride, 43.5; potassium iodide, 0.15; cuprous chloride, 0.2; manganous sulfate·H_2O, 0.8; cobalt chloride·$6H_2O$, 1.0; zinc sulfate·$7H_2O$, 3.0; and sodium selenite, 0.011.

[h] Carboxyl methyl cellulose.

[i] Nitrogen-free extract = 100 − (protein + lipid + ash + fiber).