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Effect of lipid supplementation on reproductive performance of female channel catfish, *Ictalurus punctatus*, induced and strip-spawned for hybridization

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Abstract

The influence of different lipid sources and n3:n6 ratios on reproductive performance of female channel catfish, *Ictalurus punctatus* was evaluated. A commercial catfish feed was top coated with 2% oil and offered to brood stock females fish during 70–85 days before spawning season. Four dietary treatments were formulated using the following top coating ratios: diet 1, soybean oil 9.5 g kg^{-1} and linseed oil 10.5 g kg^{-1}; diet 2, soybean oil 17.5 g kg^{-1} and linseed oil 2.5 g kg^{-1}; diet 3, 20.0 g kg^{-1} linseed oil, and diet 4, 10.0 g kg^{-1} menhaden fish oil, supplemented with 5.0 g kg^{-1} arachidonic acid (ARA), and 5.0 g kg^{-1} docosahexaenoic acid (DHA). Fatty acid composition of the eggs reflected the effect of dietary treatment offered during spring season. Supplementation of ARA, EPA and DHA in commercial catfish feed in the form of menhaden fish oil with purified liquid algae extracts of ARA and DHA produced from two to five times the number of fry per female body weight when compared to the effect of fed top coated with vegetable oils. Although, this effect was not statistically significant it may represent an economical improvement for the industry.

KEY WORDS: channel catfish, egg production, lipid supplementation

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Introduction

The interspecific hybrid between channel catfish, *Ictalurus punctatus* (Rafinesque) females and blue catfish, *I. furcatus* (Lesueur) males has been described as the most suitable for culture conditions compared to channel catfish due to better growth, increased resistance to low oxygen levels and diseases, ease of harvest, and higher carcass yield (Giudice 1966; Dunham *et al*. 1983, 1990; Smitherman *et al*. 1983). Inconsistent spawning and fry rearing are currently bottlenecks to the continued commercialization of hatchery technologies (Tave & Smitherman 1982; Dunham & Smitherman 1987). Hence, improvement and/or development of new techniques dealing with brood stock management, spawning, gamete quality, fertilization, and hatchery protocols are required to provide strategies that will advance this technology.

Genetic, physiological, environmental, and nutritional background from brood stock can influence gamete production in terms of quality and quantity (Brooks *et al*. 1997; Schreck *et al*. 2001). In particular, the nutritional status of the brood stock determines the quality of the egg, since all the content of an egg must be incorporated when the egg is an oocyte within the ovary during vitellogenesis (Sargent 1995; Wiegand 1996; Brooks *et al*. 1997). Lipids can be incorporated into egg yolk from dietary sources, or from reserves that are stored before vitellogenesis and subsequently mobilized, or possibly synthesized *de novo* (Wiegand 1996). A considerable amount of research has addressed the effect of dietary essential fatty acid content on reproductive performance with varying results. In some cases, as in gilthead seabream (*Sparus aurata* L.), fecundity increased significantly with an increase in dietary n-3 highly unsaturated fatty acids (HUFAs), but an excess also caused detrimental effects (Fernández-Palacios *et al*. 1995; Izquierdo *et al*. 2001). There are studies where no correlation between egg quality and levels of fatty acids was demonstrated, such as in goldfish (Wiegand *et al*. 1991), and Atlantic halibut (Bruce *et al*. 1993; Wiegand 1996). In Nile tilapia has been determined a requirement of dietary n-3 HUFAs for optimum spawning performance of broodfish reared in brackish water (El-Sayed *et al*. 2005). Also, a more recent study with the white seabream (*Diplodus sargus*) evidenced some gender related differences in terms of lipid class accumulation and mobilization (Perez *et al*. 2007).

Regarding the channel catfish, there are a few studies that evaluate the effect of dietary lipids on fatty acid composition of fry (Yingst & Stickney 1979), and also in young fish (Gatlin & Stickney 1982; Hedrick *et al*. 2005). Effects of dietary lipid source using poultry fat and menhaden fish oil with different dietary concentration have been conducted on channel catfish brood stock to evaluate their reproductive performance and egg composition, production, and quality (Sink & Lochmann 2008).

The aim of the present study was to evaluate the effect of linolenic acid (18:2n6), linolenic acid (18:3n3), and the highly unsaturated fatty acids (HUFAs): arachidonic acid (20:4n6, ARA), eicosapentaenoic acid (20:5n3, EPA), and docosahexaenoic acid (22:6n3, DHA) on female channel catfish brood stock.

Materials and methods

Dietary treatments

Four experimental diets were formulated using a commercial formulation feed consisting of 320 g kg^{-1} protein, and 50 g kg^{-1} lipids (manufactured by ARKAT Feeds Inc., Dumas, AR, USA). The feed was then top-coated with 20 g kg^{-1} lipids from different sources. Diet 1–3 used soybean and linseed oil as primary lipid sources. Diet 1 was top coated with 9.5 g kg^{-1} of soybean oil and 10.5 g kg^{-1} of linseed oil (SBO–LSO); diet 2 was top coated with 17.5 g kg^{-1} of soybean oil and 2.5 g kg^{-1} linseed oil (SBO); diet 3 was top coated with 20.0 g kg^{-1} linseed oil (LSO). Diet 4 was top coated with 10.0 g kg^{-1} of menhaden fish oil, 5.0 g kg^{-1} of DHA rich oil, and 5.0 g kg^{-1} of ARA rich oil. The HUFA oils used in diet 4 were liquid algae extracts with about 40% purity of the DHA or ARA (manufactured by Advanced Bionutrition ABN, Columbia, MD, USA) (MFO) (Table 1). Lipid supplementation in those proportions targeted the following n3:n6 ratios 1:1, 1:4, 4:1, and 3:2 for diets 1 through 4, respectively. Lipid supplementation was added by spraying the feed as it was tumbled in a rotary mixer. The feed was stored at –20 °C until needed.

Experimental fish

A total of 187 female 4-year-old channel catfish (Kansas strain) were maintained at the E. W. Shell Fisheries Research Center, Auburn University, Auburn AL, USA. The fish were randomly stocked in January 2005, in eight ponds (0.04 ha) at a density of ~600 kg ha^{-1}, using two ponds per treatment. Females were weighed and measured at the beginning and at the end of the experiment. The acclimation period was approximately 2 months, and the trial period was 70–85 days depending on when fish were removed for spawning. Floating feed was offered every other day during the warmest part of the day between 15:00 and 17:00 h, at an estimated rate of 2.75% of total biomass of brood fish stocked per pond. Water quality parameters were taken twice daily for dissolved oxygen and temperature and twice weekly for pH, ammonia-N, and nitrite-N.

Two spawning trials were evaluated using 95 and 92 fish in each period. Upon initiation of the spawning cycle, one pond from each treatment (randomly selected) was drained, and all the females were harvested and selected based on external characteristics (abdominal fullness, softness and palpability of the ovaries, redness or swollen appearance of the genitals). At harvest the total length, body weight, and girth were recorded and the selected fish placed in labelled soft mesh bags. Selected females were transferred to holding tanks (3.0 × 0.47 × 0.61 m with a water volume from 670 to 837 L) supplied with continuous flow-through water. GMP grade luteinizing hormone-releasing hormone analogues (LHRHa) from American Peptide (Vista, CA, USA) was injected into females to stimulate egg release. Interperitoneal hormone injections were administered in two doses, a priming injection of 30 μg kg^{-1} LHRHa, followed 12 h later by a resolving

Table 1 Dietary treatments based on lipid supplementation (20 g kg^{-1} top coated oil) from channel catfish commercial diet (320 g kg^{-1} protein level, 50 g kg^{-1} lipid), and final observed n3:n6 ratios in supplemented oil and experimental feed

Diet	Soybean oil (high n6) (g kg^{-1})	Linseed oil (high n3) (g kg^{-1})	MFO + ARA + DHA (g kg^{-1})	n3:n6 (Oil)	n3:n6 (Feed)
1. SBO–LSO	9.5 g kg^{-1}	10.5	—	1.0:1.02	1.0:2.4
2. SBO	17.5 g kg^{-1}	2.5	—	1.0:3.1	1.0:4.6
3. LSO	—	20.0	—	2.4:1.0	1.0:1.5
4. MFO	—	—	20.0	3.2:2.0	1.0:2.5

MFO, Menhaden fish oil; ARA, Arachidonic acid (algal extract: 40% ARA); DHA, Docosahexaenoic acid (algal extract: 40% DHA); SBO, Soybean oil; LSO, Linseed oil.

dose of 150 μg kg^{-1}. Data pertaining to the number of fish stocked and those selected for strip spawning are presented in Table 2.

Blue catfish (*Ictalurus furcatus*) male brood stock varied in age from 6 to 9 years. Fish were communally stocked in pods and belong to one of the following strains: Rio Grande (RG), D&B, Forks D&B, and Auburn X Rio Grande. They were harvested prior to ovulation and were chosen based on secondary sexual characteristics. These characteristics were large, muscular pads on the dorsal surface of the head, darkened coloration, well-developed genital papilla, and indications of possible aggressive tendencies toward other males as evidenced by the presence of wounds in and around the head region (Kristanto 2004).

Collection and fertilization of gametes

To obtain the sperm, the males were sacrificed, their testes removed, cleaned, and then macerated in a saline solution. This sperm-fluid homogenate was then filtered for solid tissue masses and blood contamination was kept to a minimum. The sperm was evaluated in terms of quantity and quality to minimize adverse effects on hybridization. Then, it was diluted and refrigerated prior to stripping of the eggs and fertilization. Sperm concentration of 6.5×10^7 per 100 g of eggs was utilized for fertilization.

Females were monitored for ovulation 24 h after the second injection. Females that released eggs when pressure was applied to the abdomen were removed from the holding tank and anesthetized in 250 mg L^{-1} tricaine methane sulfonate (MS-222) (Argent Chemical Laboratories, Redmond, WA, USA) buffered with sodium bicarbonate. Females were then stripped and eggs were collected in metal pans lubricated with vegetable shortening. Those females that did not express eggs were rechecked later. Stripping of gametes ceased when all females had been stripped or attempts to strip them had been made. Eggs were weighed and total number of eggs per spawn, and total number of eggs per female body weight were determined, based on counting of eggs sub-samples. After weighing, eggs and sperm were gently swirled together and allowed to sit for 2–10 min until they formed a mass, which was transferred to a water hardening trough for 15 min. Finally, the egg masses were transferred to an egg basket in a hatching trough. The troughs had an air supply and a paddle wheel which was turned on when the youngest egg mass in the trough was at least 3-h old. Eggs were treated with formalin (100 ppm) and copper sulphate (32 ppm) to prevent fungus growth every 8 h.

The egg masses were evaluated 24 h before hatching to determine the proportion of eggs that were alive. Each mass was weighed and inspected visually to estimate the number of

Table 2 Relative weight condition (W_r) before and after dietary treatments

Diet	1. SBO–LSO	2. SBO	3. LSO	4. MFO	*P*-values
Relative weight (W_r)					
Initial W_r	76.8 ± 8.5^{b}	83.3 ± 7.8^{a}	74.9 ± 11.7^{b}	77.6 ± 10.9^{b}	8×10^{-4}
Final W_r	125.7 ± 13.2	126.3 ± 11.9	121.8 ± 13.6	125.5 ± 12.4	0.3572
Difference	48.8 ± 15.4	42.5 ± 14.1	46.9 ± 19.8	47.6 ± 16.2	0.3766
Fish spawned					
First period	12 (24)	10 (24)	8 (23)	13 (24)	
Second period	12 (23)	12 (21)	16 (24)	6 (15)[1]	
Reproductive performance parameters: First period of spawning					
Eggs/g	55 ± 6	63 ± 10	57 ± 13	53 ± 9	0.1405
Eggs/kg[2]	8592 ± 1381	9410 ± 1514	7748 ± 2025	8805 ± 1352	0.1687
Egg mass/kg[3]	158.3 ± 34.7	154.7 ± 34.8	136.0 ± 26.9	170.9 ± 38.1	0.1817
Egg diameter, mm	3.69 ± 0.18^{a}	3.45 ± 0.30^{b}	3.66 ± 0.26^{a}	3.71 ± 0.23^{a}	<0.0001
Fry kg^{-1} female	630 ± 341	796 ± 397	293 ± 412	1602 ± 341	0.08
Hatch (%)	7.16 ± 5.34	8.05 ± 5.02	3.78 ± 4.52	20.85 ± 8.23	0.069

Number of channel catfish females, *Ictalurus punctatus*, Kansas strain, 4-year-old, spawned from total number of fish per dietary treatment (in parentheses); number of eggs and egg diameter from channel catfish females per dietary treatment; fry per kilogram of female, and hatching percentage of eggs after being fertilized with sperm from blue catfish males, *Ictalurus furcatus*, related to female brood stock dietary treatments (*P*-values from Tukey–Kramer test).

[1] Females were reduced from 24 to 15 due to mortality at harvest.

Means followed by the same letter are not different ($P > 0.005$, Tukey–Krammer test).

Eggs/g: number of eggs per gram of egg mass.

Eggs/kg: number of eggs per kilogram of spawning female body weight.

Egg mass/kg: total weight of egg mass (g) per kilogram of female body weight.

eggs that were developing. This allowed an estimation of number of fry per egg mass, which later was related to the total number of fry obtained in each trough to determine individual female performance.

Egg measurements

Two samples of unfertilized eggs were taken from each female during stripping. One sample was placed in the freezer (–70 °C) for later biochemical analysis, and the other sample was preserved using a 5% solution of formalin. The latter was used to determine the egg weight, as well as the egg diameter. Egg weight was determined from total number of eggs in the sample related to the total weight of the sample. A sub-sample randomly chosen, consisting of 15 eggs was measured. The software Image Pro-Express v. 4.5.1.3 (Media Cybernetics, Bethesda, MD, USA) was used to determine egg diameter measurements.

Analytical procedures

Analysis of diets was conducted to determine crude protein, crude lipids, energy content, and moisture. Crude protein and crude lipid content (g kg^{-1}) of the diets was analyzed using the micro Kjeldahl method and the ether extraction method (Soxtec Avanti 2055 Manual Extraction Unit, Foss Tecator, Höganäs, Sweden), respectively. A 15-mg sample was used to determine energy content using a 1425 micro bomb calorimeter (Parr Instrument Company, Moline, IL, USA).

Protein, lipid, free amino acids, fatty acid profiles of eggs, and egg diameter were determined from samples of unfertilized eggs taken from females spawned during the first spawning period. A total of 43 individual samples were analyzed in triplicate for the parameters mentioned previously, except for free amino acids which was analyzed by duplicate. The crude lipid of the egg samples were extracted by using the method of Folch *et al.* (1957). Approximately 400 mg of eggs was placed in 20 × 125-mm borosilicate glass screw-cap tubes. Samples were homogenized in a chloroform/methanol solution, followed by addition of water. Two separate phases were obtained, the upper phase was discarded, and the lower phase was placed in a new tube. The sample was dried by flushing with nitrogen. The lipid fraction was determined as a percentage on a wet weight basis. Following lipid extraction, methanolic potassium hydroxide (1 mL, 0.5 N KOH) was added to each tube, capped tightly, vortexed, and heated in a water bath at 70 °C for 20 min. After cooling, an esterification agent (1 mL 14% boron trifluoride–methanol, Sigma-Aldrich, Dallas, TX, USA) was added to each tube, flushed with nitrogen, capped tightly, reheated in a water bath at 70 °C for 45 min and then cooled. Extraction of fatty acid methyl esters (FAMEs) was facilitated by the addition and vortexing of exactly 2 mL of hexanes (Fisher Scientific, Fair Lawn, NJ, USA) and 1 mL saturated NaCl solution. The upper (hexane) phase containing FAMEs was transferred via Pasteur pipette to 13 × 100 mm borosilicate glass vial. These samples were flushed with nitrogen and stored in the freezer for chromatographic analysis. FAMEs were analyzed using a hydrogen flame ionization gas chromatograph (GC-17A Ver. 3, Columbia, MD, USA) equipped with capillary column (Omegawax 530, 30 m × 0.53 mm × 0.5 μm film thickness, Supelco 2-4019, Sigma-Aldrich, Oslo, Norway) using helium as the carrier gas. Injector port and detector temperatures were maintained at 260 and 270 °C, respectively. Samples containing FAMEs were injected into the column in 1 μL of dichloromethane (DCM-methylene chloride HPLC-GC/MS, Fisher Scientific) using an auto-sampler (AOC-20i, Shimadzu, Columbia, MD, USA). The column oven temperature was initially 140 °C, and then was increased to 260 °C at a rate of 3.0 °C min^{-1}. Total run time was 42.0 min per sample. Sample FAMEs were identified and quantified by comparing peak retention times and area counts to those of serially diluted mixtures of the following reference standards: PUFA-3, Supelco 37 Component FAME Mix, and GLC 90 (Supelco, Bellefonte, PA, USA). Non-adecanoic acid methyl ester (C19:0) (Sigma–Aldrich Inc., St Louis, MO, USA) served as the internal standard. The results of the individual fatty acids were expressed as relative percentage of total identified FAMEs and as μg per g of egg.

Free amino acids were determined as total ninhydrin positive substances (TNPS) using a colorimetric determination (Lee & Takahashi 1966). Approximately 380 mg of egg sample was macerated and placed in 5 mL of 80% ethanol at 4 °C for 48 h to extract intracellular NPS. Then a volume of 0.1 mL was added to 1.9 mL of a ninhydrin–citrate–glycerol mixture (0.5 mL of 1% ninhydrin solution in 0.5 M citrate buffer (pH 5.5), 1.2 mL of glycerol, and 0.2 mL of 0.5M citrate buffer (pH 5.5). This solution was heated in a boiling water bath for 12 min, and then cooled in a tap-water bath at room temperature. The tube was shaken and read at 570 μm within 1 h from the procedure. A reagent blank and a standard amino acid – norleucine – solution were run at the same time to verify and standardize the determinations. Free amino acids were reported as μmol g^{-1} of eggs and μmol per 100 eggs.

Statistical analysis

The experiment was conducted using a randomized complete block design. The ponds were grouped into two blocks according to period of spawning. Relative weight (W_r) was used as a condition index to determine whether or not feeding had an effect on the fish during the spring season. This condition index is determined by the equation:

$$W_r = (W/W_s) \times 100$$ (Anderson & Neumann 1996)

where W is the weight, in grams, and W_s is a length-specific standard weight obtained from a weight-length regression for channel catfish. The equation for W_s in catfish is:

$$\log_{10}(W_s) = -5.800 + 3.294 (\log_{10} \mathrm{TL})$$ (Brown *et al.* 1995)

where TL is the total length of the fish.

Analysis of spawning success was performed using logit models, considering spawning as a binary response (Quintero *et al.* 2007a). The model included the effect of treatments (lipid supplementation) on spawning using period of spawning as a covariate. The number of eggs per gram of egg mass and the number of eggs per gram of female body weight were determined using the zero-inflated negative binomial regression (ZINB) (Quintero *et al.* 2007b). This model used treatment (lipid supplementation) and period of spawning as the explanatory variables. Analysis of variance (ANOVA) was performed for egg diameter measurements to detect treatment differences due to lipid treatments.

Protein and lipid egg content (g kg^{-1}) were analysed using beta regression modelling (Ferrari & Cribari-Neto 2004). Fatty acid composition was analyzed using the ANOVA procedure following an arcsin transformation. Tukey's test was applied for multiple comparison of mean values. If the responses were not normally distributed, the Kruskal–Wallis non-parametric test was applied to the non-transformed data. Regression analyses were performed on ratios of essential fatty acids (DHA:EPA, ARA:DHA, ARA:EPA) and fry hatch. Multivariate statistics was used to evaluate the differences and similarities in the results. The relative amounts of linoleic acid, linolenic acid, ARA, EPA, and DHA in the samples were transformed using arcsin square root of the percentage values, and then they were subjected to principal component analysis. Two new coordinates, the principal components (PCs), were generated in the direction of the largest and second largest variation of the samples. The relation among the samples was then displayed by projecting them on the plane. This allowed a display of the major trends within the data set without significant loss of total original variation. The GLM, Logistic, Genmod, NLMixed, and Princomp Procedures from SAS® version 9.1 (SAS Institute Inc., Cary, NC, USA) were used.

Results

Lipid supplementation to the experimental diets had slightly different n3:n6 ratios to those initially targeted, especially for diet 2 which reached 1.0:3.1 instead of 1.0:4.0, and diet 3 which was 2.4:1.0 instead of 4.0:1.0 (Table 1). Those differences are most likely due to oil composition and experimental error. The commercial diet used for this experiment was rich in linoleic acid (C18:2n6) which affected the n3:n6 ratios from the experimental diets, with the following final ratios: 1.0:2.4, 1.0:4.6, 1.0:1.5, and 1.0:2.5 for diets 1–4, respectively (Table 1).

Final relative weights of channel catfish females brood stock were increased over 60% during the spring season with final values over 120 across all the treatments (Table 2), which evidenced acceptance of the experimental diets. Dietary treatments and period of spawning did not improve egg-stripping success (Table 3). Fertilization problems across all the treatments during the second period of spawning were probably linked to sperm quality, and thus data for reproductive performance were restricted to the first period of spawning.

Reproductive performance parameters during the first spawning period were not affected significantly by dietary treatments. Thus, differences observed in number of eggs per gram of egg mass between treatments were statistically not significant and they were in the range of minimum 53 eggs g^{-1} (diet 4, MFO) to a maximum of 63 eggs g^{-1} (diet 2, SBO) (Table 2). Number of eggs per body weight (kg) were also not significantly different among treatments, reaching 9410 eggs kg^{-1} in fish fed diet 2 (SBO), 8805 eggs kg^{-1} in fish

Table 3 Results of logistic regression analysis evaluating differences in spawning success from channel catfish females, *Ictalurus punctatus*, Kansas strain, 4-year-old after being maintained in dietary treatments using period of spawning as covariate

Variable	df	Parameter estimate	$P > \chi^2$
Diet 1. SBO–LSO	1	−0.012	0.9635
Diet 2. SBO	1	0.0615	0.827
Diet 3. LSO	1	0.025	0.9244
Period 1	1	−0.1482	0.3446

Diet 1, diet 2, and diet 3 correspond to the logistic regression coefficients where the levels of diet are coded as diet 1 (1, 0, 0); diet 2 (0, 1, 0); diet 3 (0, 0, 1); diet 4 (-1, -1, -1); and Period 1 is the logistic regression coefficient where period is coded as period 1 (1), period 2 (-1).

SBO, Soybean oil; LSO, Linseed oil.

fed diet 4 (MFO), 8592 eggs kg^{-1} in fish fed diet 1 (SBO–LSO), and 7748 eggs kg^{-1} in fish fed diet 3 (LSO). Egg masses per female body weight were not significantly affected by lipid supplementation. Diet 4 (MFO) produced the largest masses with a mean value of 170.9 g kg^{-1}, and diet 3 (LSO) produced the smallest masses with 136.0 g kg^{-1}. Egg diameters from females fed diet 2 (SBO) were significantly smaller than those from females fed diets 1, 3, and 4 (Table 2). Although fry produced per female body weight was not significantly affected by treatments ($P = 0.08$), there was a large difference in the number of fry obtained for fish fed diet 4 (MFO). Females given diet 4 (MFO) produced five times more fry than females given diet 3 (LSO) and over two times those produced given diet 1 (SBO–LSO) and diet 2 (SBO). Fry hatch displayed a similar pattern to that of fry per female body weight, with only a weak relationship to dietary treatment (P-value = 0.0686) (Table 2).

Biochemical composition of the test diets did not differ significantly for moisture, protein, lipids, and energy (Table 4). Protein content of the eggs was significantly affected by the diets. Eggs from fish fed diet 4 (MFO) had significantly lower protein content (mg kg^{-1}) than eggs from other treatments. In terms of the amount of protein per unit egg, eggs from fish fed diet 2 (SBO) had significantly lower amounts of protein than eggs from other treatments (2.73 ± 0.39 mg per egg). This is related to the fact that those eggs were significantly smaller (Tables 2 & 4). There was not significant effect from dietary treatments on lipid content, but in terms of amount of lipids per unit egg, there was an effect related to the size of the egg, with eggs from diet 2 (SBO) having significantly less amount of lipid in eggs that those from fish fed diet 4 (MFO), but not different form the amount of lipid in eggs of fish fed diet 1 (SBO–LSO) or diet 3 (LSO) (Table 4). The content of free amino acids differ significantly among treatments, with the highest amount both as μmol g^{-1} egg and μmol per 100 eggs in eggs from diet 4 (MFO), followed by diet 2 (SBO), diets 1 (SBO–LSO) and 3 (LSO), respectively (Table 4).

Fatty acid composition from experimental diets exhibited marked differences reflecting the composition of oils used to top coat the feed (Table 5, Fig. 1a–e). In general terms C16:0, C18:1n9, C18:2n6 and C18:3n3 accounted for around 88% of total fatty acids in diets 1–3, while these same fatty acids represented only 70% from diet 4 (MFO). Similarly, C20:4n6 (ARA), C20:5n3 (EPA), and C22:6n3 (DHA) represented less than 1% in diets through 3, but almost 11% in diet 4 (MFO). Regarding linolenic (18:3n3) and linoleic (18:2n6) acids, their ratios were 1.0:2.6 in diet

Table 4 Proximate analysis from commercial channel catfish feed, and biochemical composition from eggs of channel catfish females, *Ictalurus punctatus*, including proteins, lipids, free amino acids as total ninhydrin positive substances (TNPS), and ratios between essential fatty acids from dietary treatments

Diet	SBO–LSO	SBO	LSO	MFO	*P*-values
Feed					
Moisture (g kg^{-1})	73.6 ± 2.5	71.5 ± 2.0	75.5 ± 1.0	71.8 ± 1.2	0.5512
Protein (g kg^{-1})	334.3 ± 4.0	335.8 ± 1.7	335.3 ± 3.1	333.9 ± 3.6	0.9726
Lipids (g kg^{-1})	71.2 ± 0.4	69.4 ± 2.0	71.2 ± 4.8	72.1 ± 2.6	0.7402
Energy (cal)	4128 ± 15	4207 ± 47	4230 ± 66	4220 ± 72	0.5312
Eggs					
Proteins					
g kg^{-1}	168.1 ± 1.8^{a}	166.5 ± 1.2^{a}	167.4 ± 2.4^{a}	163.3 ± 1.3^{b}	0.0360*
mg per egg	3.07 ± 0.30^{a}	2.73 ± 0.39^{b}	3.02 ± 0.46^{a}	3.16 ± 0.42^{a}	0
Lipids					
g kg^{-1}	72.7 ± 1.3	71.9 ± 0.9	74.0 ± 1.7	75.4 ± 0.9	0.2681**
mg/ egg	1.12 ± 0.19^{ab}	0.97 ± 0.12^{b}	1.14 ± 0.28^{ab}	1.27 ± 0.40^{a}	0
Free amino acids as total ninhydrin positive substances (TNPS)					
μmol g^{-1}	3.29 ± 2.26^{c}	5.85 ± 1.83^{b}	3.72 ± 2.02^{c}	7.37 ± 1.63^{a}	<0.0001
μmol/eggs[1]	5.92 ± 4.15^{c}	9.62 ± 3.45^{b}	7.06 ± 4.74^{bc}	14.48 ± 4.45^{a}	<0.0001
Ratios					
DHA:EPA	7.54 ± 2.09^{b}	10.93 ± 3.62^{a}	8.14 ± 1.96^{b}	8.60 ± 1.97^{b}	<0.0001
ARA:DHA	0.021 ± 0.004^{b}	0.016 ± 0.003^{c}	0.026 ± 0.005^{a}	0.013 ± 0.002^{d}	<0.0001
ARA:EPA	0.149 ± 0.02^{c}	0.173 ± 0.054^{b}	0.204 ± 0.034^{a}	0.108 ± 0.028^{d}	<0.0001

SBO, Soybean oil; LSO, Linseed oil; MFO, Menhaden oil, supplemented with arachidonic acid (ARA~40%) and docosahexaenoic acid (DHA~40%); DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; ARA, Arachidonic acid.

[1] Total amount of free amino acids (μmol) per 100 eggs.

* The largest *P*-value <0.05 from Beta regression coefficients.

** The smallest *P*-value ≥0.05 from Beta regression coefficients.

1 (SBO–LSO), 1:0:5.3 in diet 2 (SBO), and 1.0:1.5 in diet 3 (LSO), which accounted for most of the final n3:n6 ratios observed in the experimental diets (Tables 1 & 5). Diet 4 (MFO) had a linolenic (18:3n3):linoleic (18:2n6) ratio equals to 1.0:8.5, but its final n3:n6 ratio was much lower: 1.0:2.5, due to additional sources of EPA and DHA, which were coming in the fish meal oil, and the liquid algae extracts (Tables 1 & 5).

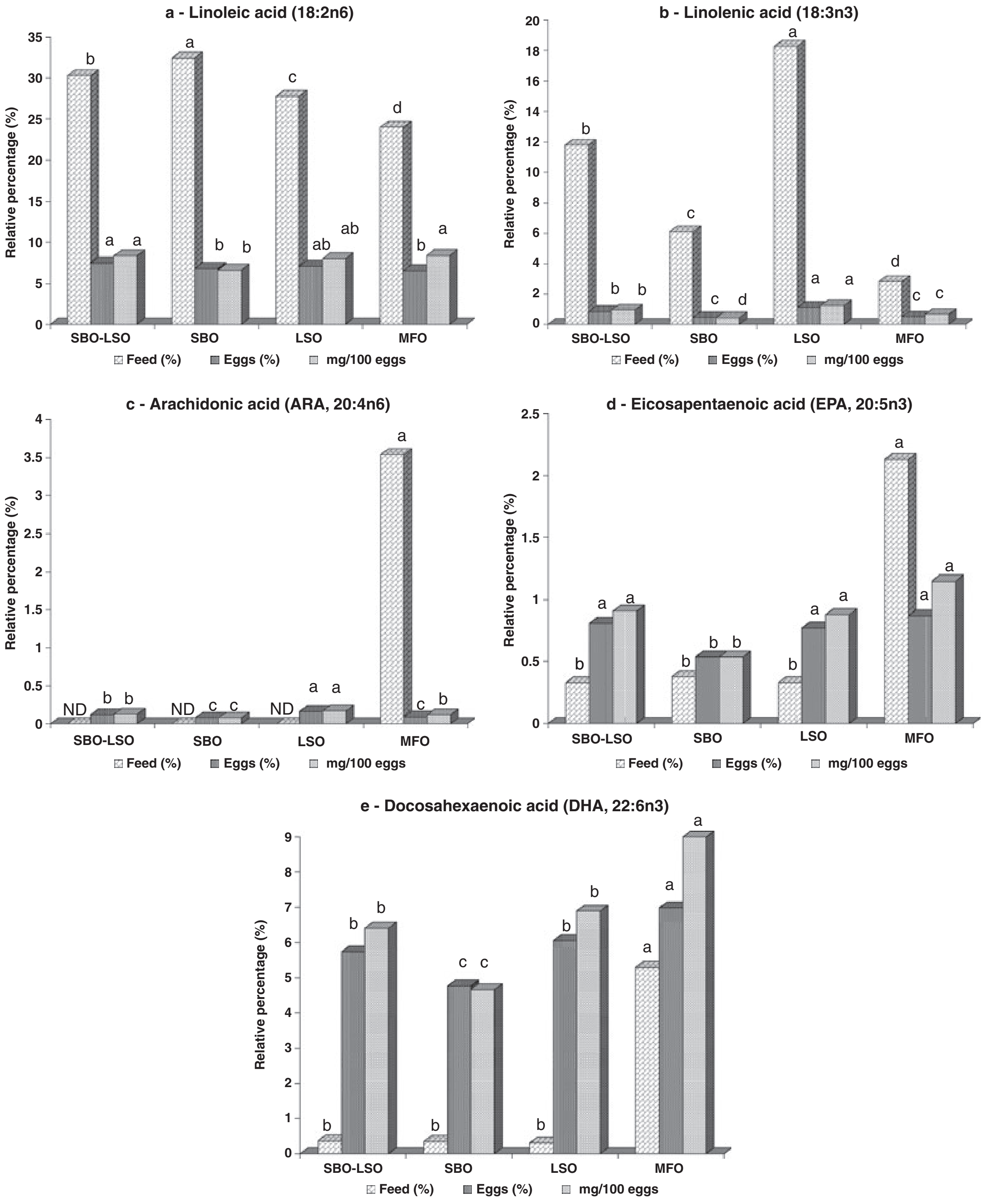
Relative proportions of fatty acid composition from unfertilized egg samples for each treatment are displayed in Table 6, Fig. 1a–e. The most abundant fatty acids were 16:0, 18:0, 18:1n9, 18:2n6, 20:3n3, and 22:6n3. Relative proportions of linoleic acid (18:2n6) were not different in eggs of fish fed SBO (diet 2), LSO (diet 3) and MFO (diet 4), but were significantly higher in eggs from females given diets 1 (SBO–LSO) and 3 (LSO) (Fig. 1a). Linolenic acid (18:3n3) and arachidonic acid (ARA – 20:4n6) were significantly higher in eggs from females given diet 3 (LSO), and significantly lower in diets 2 (SBO) and 4 (MFO) (Fig. 1b,c). Eicosapentaenoic acid (EPA – 20:5n3) was significantly lower in eggs from diet 2 (SBO) (Fig. 1d). Docosahexaenoic acid (DHA – 22:6n3) was significantly higher in eggs from diet 4 (MFO), while eggs from diet 2 (SBO) were significantly lower in terms of relative proportion and absolute values (mg per 100 eggs) (Fig. 1e). The n3:n6 ratio from unfertilized eggs was significantly different among treatments, diet 4 (MFO) was significantly higher with 1.26:1.0, while diet 2 (SBO) was significantly lower with 1.0:1.04. Diets 1 (SBO, LSO) and 3 (LSO) were not significantly different among them with n3:n6 ratios equal to 1.09:1.0 and 1.14:1.0, respectively (Table 6).

Principal component analysis (PCA) allows to identify patterns in the data, expressing it to highlight their similarities and differences. The primary axis is the single vector describing the direction of largest variation in the data, and is termed the principal component one (PC1). This analysis described 83% of the variance in the data set and is therefore a good representation of the relationship among the diets and the fatty acids present in egg samples. Figure 2 shows the coefficients plotted for the first two principal components. The first PC accounted for 63% of the variance, with all five fatty acids loaded positively onto the axis. The PC2 axis represented 20% of the variance. EPA and DHA were positively loaded on this axis, whereas linoleic, linolenic and arachidonic acid were negatively loaded. The projections of each individual data onto the PCs are defined as scores, which reveal relationships between them. Scores for diet 4 (MFO) grouped together on the positive side of PC2, and both sides of PC1. Scores for diet 2 (SBO) grouped mostly in the negative side of PC1 and PC2, whereas scores for diets 1 (SBO–LSO) and 3 (LSO) gathered in the positive side of PC1 and negative from PC2.

Table 5 Fatty acid analysis from dietary treatments (commercial catfish diets 320 g kg^{-1} protein, 50 g kg^{-1} lipids, top-coated with 20 g kg^{-1} oil)

Fatty acid	Diet 1. SBO–LSO	Diet 2. SBO	Diet 3. LSO	Diet 4. MFO
14:0	0.83 ± 0.03	0.89 ± 0.07	0.78 ± 0.03	2.99 ± 0.13
16:0	16.19 ± 0.32	16.69 ± 0.26	14.27 ± 0.14	16.73 ± 0.21
16:1n7	1.75 ± 0.04	1.78 ± 0.08	1.71 ± 0.07	3.37 ± 0.14
18:0	4.65 ± 0.11	4.64 ± 0.01	3.91 ± 0.07	4.82 ± 0.10
18:1n9	30.15 ± 0.42	31.67 ± 0.23	28.94 ± 0.06	26.90 ± 0.27
18:2n6	30.31 ± 0.64	32.41 ± 0.57	27.77 ± 0.36	24.01 ± 0.68
18:3n6	ND*	ND*	ND*	0.42 ± 0.03
19:0	1.33 ± 0.19	2.45 ± 0.11	1.45 ± 0.16	2.25 ± 0.07
18:3n3	11.76 ± 0.46	6.11 ± 0.34	18.28 ± 0.21	2.83 ± 0.12
20:1n9	0.78 ± 0.04	0.89 ± 0.05	0.69 ± 0.15	1.03 ± 0.03
20:2n6	0.30 ± 0.01	0.33 ± 0.03	0.32 ± 0.04	0.35 ± 0.02
20:3n6	0.18 ± 0.02	0.20 ± 0.01	0.21 ± 0.01	0.50 ± 0.03
20:3n3	0.21 ± 0.004	0.23 ± 0.002	0.21 ± 0.01	ND*
20:4n6	ND*	ND*	ND*	3.54 ± 0.18
20:5n3	0.33 ± 0.02	0.38 ± 0.02	0.33 ± 0.02	2.13 ± 0.10
22:4n6	0.02 ± 0.02	0.04 ± 0.002	0.04 ± 0.002	0.07 ± 0.01
22:5n6	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.14 ± 0.01
22:5n3	0.09 ± 0.001	0.11 ± 0.01	0.10 ± 0.004	0.41 ± 0.02
22:6n3	0.37 ± 0.04	0.35 ± 0.01	0.31 ± 0.01	5.30 ± 0.24
$\sum$ n-6	30.60 ± 0.63	32.74 ± 0.57	28.12 ± 0.34	28.69 ± 0.45
$\sum$ n-3	12.76 ± 0.50	7.17 ± 0.38	19.24 ± 0.20	11.53 ± 0.41
n-3/n-6	0.42 ± 0.03	0.22 ± 0.01	0.68 ± 0.01	0.40 ± 0.02

ND, not detected; SBO, Soybean oil; LSO, Linseed oil; MFO, Menhaden oil, supplemented with arachidonic acid (ARA~40%) and docosahexaenoic acid (DHA~40%).



a - Linoleic acid (18:2n6)

b - Linolenic acid (18:3n3)

c - Arachidonic acid (ARA, 20:4n6)

d - Eicosapentaenoic acid (EPA, 20:5n3)

e - Docosahexaenoic acid (DHA, 22:6n3)

Figure 1 Relative proportions of linoleic, linolenic, ARA, EPA and DHA acids from catfish feed and eggs, and calculated values in µg of fatty acid per 100 eggs. a - Linoleic acid (18:2n6), b - Linolenic acid (18:3n3), c - Arachidonic acid (ARA, 20:4n6), d - Eicosapentaenoic acid (EPA, 20:5n3), e - Docosahexaenoic acid (DHA, 22:6n3).

Table 6 Fatty acid analysis from unfertilized eggs of channel catfish females, *Ictalurus punctatus*, held under dietary treatments, commercial catfish diet 320 g kg^{-1} protein, 50 g kg^{-1} lipids, top-coated with 20 g kg^{-1} oil, and offered three times per week

Fatty acid	1. SBO–LSO	2. SBO	3. LSO	4. MFO	*P*-value
14:0	0.63 ± 0.12^{b}	0.67 ± 0.14^{b}	0.71 ± 0.11^{ab}	0.75 ± 0.10^{a}	0.0003
16:0	17.49 ± 0.75	17.25 ± 0.70	17.27 ± 0.26	17.62 ± 0.57	0.0485
16:1n7	2.06 ± 0.45^{b}	1.91 ± 0.29^{b}	2.35 ± 0.25^{a}	2.35 ± 0.38^{a}	<0.0001
18:0	13.09 ± 1.44^{b}	14.51 ± 1.34^{a}	13.04 ± 1.31^{b}	13.47 ± 1.39^{b}	0.0001
18:1n9	29.54 ± 1.15	29.48 ± 1.27	29.95 ± 1.22	30.21 ± 1.38	0.0537
18:2n6	7.46 ± 0.81^{a}	6.79 ± 0.90^{b}	7.08 ± 1.11^{ab}	6.53 ± 0.79^{b}	0.0001
18:3n6	0.45 ± 0.39^{b}	0.59 ± 0.26^{a}	0.58 ± 0.23^{a}	0.40 ± 0.11^{ab}	0.0075
19:0	6.61 ± 1.07^{ab}	7.09 ± 1.32^{a}	6.99 ± 0.78^{a}	6.25 ± 0.93^{b}	0.004
18:3n3	0.85 ± 0.18^{b}	0.45 ± 0.20^{c}	1.11 ± 0.22^{a}	0.50 ± 0.11^{c}	<0.0001
20:1n9	0.97 ± 0.17^{b}	0.98 ± 0.15^{b}	0.92 ± 0.15^{b}	1.14 ± 0.19^{a}	<0.0001
20:2n6	1.18 ± 0.14^{ab}	1.25 ± 0.13^{a}	1.14 ± 0.13^{b}	1.24 ± 0.20^{ab}	0.0348
20:3n6	3.04 ± 0.26^{a}	3.05 ± 0.25^{a}	2.87 ± 0.31^{a}	2.63 ± 0.25^{b}	<0.0001
20:3n3	6.00 ± 0.42^{b}	6.38 ± 0.79^{a}	5.42 ± 0.49^{c}	5.38 ± 0.33^{c}	<0.0001
20:4n6	0.12 ± 0.03^{b}	0.08 ± 0.03^{c}	0.16 ± 0.03^{a}	0.09 ± 0.02^{c}	<0.0001
20:5n3	0.81 ± 0.23^{a}	0.54 ± 0.37^{b}	0.77 ± 0.14^{a}	0.87 ± 0.27^{a}	<0.0001
22:4n6	0.40 ± 0.04^{a}	0.41 ± 0.04^{a}	0.34 ± 0.05^{b}	0.36 ± 0.03^{b}	<0.0001
22:5n6	1.68 ± 0.35^{b}	2.42 ± 0.60^{a}	1.53 ± 0.22^{b}	1.56 ± 0.37^{b}	<0.0001
22:5n3	0.94 ± 0.16^{a}	0.72 ± 0.22^{b}	0.89 ± 0.17^{a}	0.76 ± 0.13^{b}	<0.0001
22:6n3	5.73 ± 0.69^{b}	4.77 ± 0.94^{c}	6.05 ± 0.58^{b}	6.98 ± 0.60^{a}	<0.0001
$\sum$ n-6	13.14 ± 1.07^{ab}	13.33 ± 0.75^{a}	12.56 ± 1.35^{b}	11.56 ± 0.75^{c}	<0.0001
$\sum$ n-3	14.33 ± 1.20^{a}	12.86 ± 1.70^{b}	14.25 ± 0.79^{a}	14.49 ± 1.08^{a}	<0.0001
n-3/n-6	1.09 ± 0.07^{b}	0.96 ± 0.10^{c}	1.14 ± 0.09^{b}	1.26 ± 0.10^{a}	<0.0001

Means followed by the same letter are not different ($P > 0.005$, Tukey–Krammer test).

SBO, Soybean oil; LSO, Linseed oil; MFO, Menhaden oil, supplemented with arachidonic acid (ARA~40%) and docosahexaenoic acid (DHA~40%).

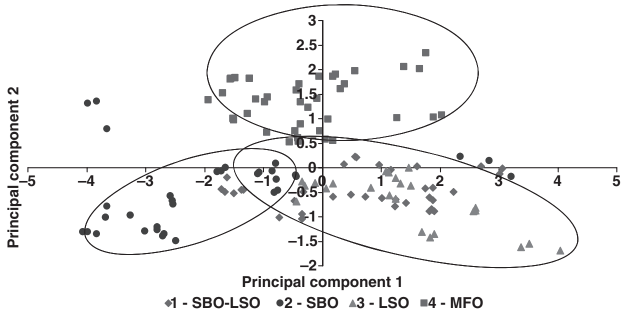


Figure 2 Plot of factor scores from five fatty acids (linoleic, linolenic, ARA, EPA, and DHA) present in unfertilized eggs from females channel catfish, *Ictalurus punctatus*, after principal components analysis with respect to first (PC 1) and second (PC 2) principal components analysis.

Discussion

The results of this study showed that fatty acid composition of the eggs was affected by brood stock dietary treatment provided during 70–85 days before the spawning season. This has been observed in species that eat during sexual maturation and throughout the spawning season (Harel *et al.* 1994). Results suggest that dietary essential fatty acids are readily incorporated into the eggs, and also mechanisms to elongate and desaturate fatty acids are very active in channel catfish. Preferential accumulation of certain fatty acids was also observed especially with regards to saturated fatty acids such as C16:0 and C18:0, and monoenes as C: 16:1 and C18:1n9, which represented from 62.2 to 63.7% of total fatty acids in eggs. This characteristic has been noted in other freshwater and marine fish and could be related to the fact that these fatty acids are heavily catabolized to generate metabolic energy in fish (Kaitaranta & Linko 1984; Tocher & Sargent 1984; Henderson & Tocher 1987; Anderson *et al.* 1990; Wiegand 1996; Sargent *et al.* 2002; Perez *et al.* 2007).

Accumulation of linoleic acid (C18:2n6) and linolenic acid (C18:3n3) in eggs was low, which could be related to the fact that those are considered primary precursors of highly unsaturated fatty acids, especially in freshwater fishes (Sargent *et al.* 2002). For instance, proportions of linoleic acid were around 21–27% of those found in all diets while linolenic acid were 6.1–7.4% in diets 1–3, and 17.7% in diet 4. These differences are more likely related to active processes in generation of HUFAs, such as ARA, EPA, and DHA, which tend to be deposited selectively into fish eggs (Henderson & Tocher 1987; Wiegand 1996; Sargent *et al.* 2002). Thus, a relatively high presence of ARA (C20:4n6), EPA (C20:5n3), and DHA (C22:6n3) in eggs from diets 1 to 3 indicate either a selective mobilization and re-allocation of this fatty acid from endogenous reserves in other tissues or an elongation and desaturation mechanism of C18:2n6 and C18:3n3. The elongation and desaturation of linolenic acid (C18:3n3) occurs in the absence of long-chain fatty acids of the n-3 configuration to prevent essential fatty acid deficiencies (Farkas *et al.* 1977). Also, linoleic acid C18:2n6 can be further desaturated and elongated to C22:5n6 (Sargent *et al.* 2002). This mechanism of synthesis from 18-carbon precursors has also been suggested as a key source of HUFAs in catfish eggs (Sink & Lochmann 2008). It is remarkable that differences observed in the proportions of linolenic and linoleic acid and their correspondent DHA (22:6n3) and C22:5n6 from unfertilized eggs produced by fish fed diets 1–3 followed the proportions observed in the correspondent experimental diets. For instance, dietary linolenic acid (18:3n3) had a higher proportion in diet 3, followed by diet 1, and the minimum proportion was observed in diet 2, accordingly, unfertilized eggs followed the same trend in term of the presence of docosahexaenoic acid (22:6n3). A similar pattern was detected for dietary linoleic acid (18:2n6) and the presence 22:5n6 in the eggs, where higher proportions were detected in eggs produced by fish fed diet 2, and the lower proportions corresponded to eggs from fish fed diet 3. Correspondence with this observation was also found in rainbow trout fed dietary C18:2n6 which resulted in a marked increase of C20:4n6 and C22:5n6, and also dietary C18:3n3 increased the proportion of C22:6n3 in the liver, kidney and heart (Castell *et al.* 1972).

Linolenic acid (C18:3n3) and linoleic acid (C18:2n6) are dietary essential fatty acids for most of the freshwater fish, but there is also a lot of competitive interaction among them for the conversion of these C18 PUFA to their end-product C20 and C22 PUFA (Sargent *et al.* 1999a). This characteristic may explain the fact that a significantly lower amount of EPA (C20:5n3) was observed in unfertilized eggs from fish fed diet 2. Thus, diet 2 had the highest amount of dietary linoleic acid (C18:2n6) decreases conversion of linolenic acid (C18:3n3) to EPA. The opposite effect where linolenic acid (C18:3n3) decreases amount of arachidonic acid (C20:4n6) by competition with linoleic acid (C18:2n6) was not observed due to unknown reasons.

There was not a defined trend in proportions of n-3 HUFAs specifically EPA and DHA in the eggs, when they were related to spawning success, egg production, and/or fry survival. In fact none of those parameters were affected significantly by dietary treatments. Thus, we cannot infer that low or high proportions of n-3 HUFAs by themselves have a negative consequence on fry survival (Ashton *et al.* 1993; Harel *et al.* 1994; Fernández-Palacios *et al.* 1995; Navas *et al.* 1997; Almansa *et al.* 1999; Bruce *et al.* 1999; Furuita *et al.* 2002). Similarly, Sink & Lochmann (2008) did not find any correlation among the ratio of DHA:EPA and hatching success or fry survival from channel catfish fertilized eggs. Egg quality of channel catfish, it appears, is affected by more than the relative abundance of n-3 HUFAs.

Bell & Sargent (2003) state that the concentrations and ratios of the essential HUFAs (DHA, EPA and ARA) are likely to have important influences on both fertilization rates and survival of fish eggs, and that a high ARA:EPA in fish eggs may be mandatory for survival. Moreover, embryogenesis could be influenced by the essential fatty acids C20:3n6, C20:4n6, C20:5n3, and C 20:6n3, since they are precursors for eicosanoid production, which in turn results in metabolites that include prostaglandins, leukotrienes and lipoxins (Leray *et al.* 1985; Mokoginta *et al.* 1998; Bell & Sargent 2003; Perez *et al.* 2007). Prostaglandins, especially ARA-derived PGE2 and leukotriene B4 (LTB4) are associated with modulation of immune cell function. Additionally, low concentrations of these substances are required for normal immune function, while high concentrations are immune suppressive (Kinsella & Lokesh 1990; Bell & Sargent 2003). This effect could explain the fact that high concentration of ARA evidenced in diet 3 (LSO) would be affecting normal development of hybrid catfish embryos, possibly resulting in lower hatching rate (3.78%).

Regarding the effect of linseed oil in this matter, it has been observed that Atlantic salmon fed diets containing this oil showed significant reductions in non-specific immune parameters (Bell *et al.* 1996; Bell & Sargent 2003). Although the mechanism involved in this apparent immunosuppresion is not known, it has been hypothesized that increased levels of membrane C20:3n6 in fish fed vegetable oils may increase competition between ARA (C20:4n6) and C20:3n6 for prostaglandin production, which in turns alter immune cell composition and function (Kinsella & Lokesh 1990; Kelley & Daudu 1993; Bell & Sargent 2003). It is remarkable that the proportion of C20:3n6 was significantly lower in diet 4 (MFO), while diets 1–3 which were top coated with vegetable soybean and linseed oil, had significantly higher proportions of this fatty acid. It is plausible that this higher proportion eventually could be negatively affecting normal development of hybrid catfish embryos.

Concentration and ratio of DHA:EPA:ARA have been suggested as important in larval marine fish nutrition (Sargent *et al.* 1999b). From this study, the ratio that produced the highest fry production per body weight and fry survival was determined to be 77.5:9.7:1.0 from eggs produced under diet 4 (MFO), while the lowest fry survival from diet 3 (LSO) displayed a ratio 37.8:4.8:1.0, which again, highlighted the higher proportion of ARA.

Principal component analysis (PCA) performed on the fatty acids targeted in this research resulted in differentiation of diet 4 (MFO) from diets 1 to 3 (vegetable oil based). Also, scores from diets 1 (SBO–LSO) and 3 (LSO) tended to be mixed in the same region, whereas diet 2 (SBO) appeared to be isolated from the others (Table 6).

Another important component of the egg is the free amino acid pool. Free amino acids (FAA) are used in protein synthesis, as well as fuel in the energy metabolism of developing marine fish eggs and larvae, and to some degree in osmoregulatory functions (Rønnestad & Fyhn 1993). However, in freshwater fish a higher FAA pool is not established during ovulation, possibly because the eggs will not be spawned in a hyper-osmotic environment and they may not be utilized as an energy source during early ontogeny (Gunasekera *et al.* 1999). Free amino acids for channel catfish eggs have not been reported previously, but Wilson & Poe (1985) characterized amino acid composition from channel catfish eggs (4-to 5-days old). They found higher levels of isoleucine, leucine, methionine, threonine, valine, phenylalanine and tyrosine, and lower levels of arginine and lysine when compared to amino acid composition of the whole body tissue. In our case we found significant differences among treatments but there was not clear relation with fry hatch or fry produced per female body weight (Table 4).

Increasing dietary n-3 and n-6 HUFAs in freshwater brood stock fish diets may have a positive impact in reproductive performance. Thus, Nile tilapia brood fish reared in brackish water increased the absolute fecundity and number of eggs per spawn when fish oil was added to the diet when compared to soybean oil and soybean oil/fish oil as lipid dietary sources (El-Sayed *et al.* 2005). Also, Sink & Lochmann (2008) found that increasing dietary fish oil from 4 to 10% improved \significantly spawning performance of channel catfish. In the present study, production parameters fry produced per female body weight and fry hatch did not exhibit significant effect from treatments at the 0.05 level of significance ($P = 0.08$ and 0.0686, respectively). However, production of two to five times the number fry per female body weight from females fed commercial catfish feed 32% top coated with Menhaden fish oil may have an economic impact at commercial level.

Conclusions

Fatty acid composition of the eggs reflected the dietary lipid supplementation, making clear the incorporation of essential fatty acids during the pre-spawning season. This effect was also determined using principal components analysis (PCA) for the five fatty acids (linoleic acid, linolenic acid, ARA, EPA, and DHA) contained in the egg samples, which lead to the differentiation of groups of eggs based on scores assigned for each principal component, which were reflections of the dietary treatments. Reproductive parameters, such as spawning success, the number of eggs per gram of egg mass or per female body weight did not exhibit significant effect from dietary treatments. However, catfish brood stock females fed commercial catfish feed top coated with Menhaden fish oil (MFO) and supplemented with DHA and ARA increased fry production from two to five times when compared to females fed with diets top coated with soybean and/or linseed oil. Therefore, commercial producers may consider using highly unsaturated fatty acids as lipid supplement on their brood stock diets to improve their fry production. Further investigation is required to elucidate the mechanism that regulates fatty acid composition in eggs and the physiological implications of their relations.

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