Broodstock Nutrition: Enhancement of Egg Quality in Channel Catfish

Herbert E. Quintero* and D. Allen Davis

*Aquaculture/Fisheries Center

University of Arkansas at Pine Bluff

Pine Bluff, AR 71602

Tel: (870) 341-3093

E-mail: quinteroh@uapb.edu

Abstract

To facilitate our understanding of the interaction of nutrition and reproductive performance from female channel catfish, two experiments were performed in earthen ponds. The first experiment evaluated the interaction of feed quality (42 and 32% protein) and feed frequency (feed offered 3 or 6 times per week) in two strains (high and low spawning strains). The second experiment assessed the influence of different lipid sources and n3:n6 ratios using a commercial catfish feed containing 32% protein and 5% lipid as the basal diet. Reproductive performance in terms of spawning and egg production was not influenced by changing protein level of the diet from 32 to 42%. Increasing the feeding frequency from 3 to 6 times per week negatively affected spawning in one of the strains, but did not affect egg production. Age and period of spawning affected reproductive performance. In addition to having bigger eggs than their younger counterpart, older fish performed better than younger fish in terms of spawning success, and egg production. Biochemical composition of the eggs was affected significantly by dietary treatment in terms of lipid, fatty acids and free amino acid content.

Lipid supplementation on a 32% protein, 5% lipid commercial catfish diet using soybean and /or, linseed oil, or menhaden fish oil enriched with docosahexaenoic acid (DHA) and arachidonic acid (ARA) had no significant effects ($p<0.05$) on spawning success neither egg production. The quantity of fry produced per female body weight and fry survival from fish fed top-coated feed with menhaden fish oil enriched ARA and DHA were two to five fold greater than those obtained from fish fed with feed supplemented with vegetable oils.

Keywords: Nutrition, Catfish, *Ictalurus*, Broodstock

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Introduction

The catfish industry is one of the largest and best developed aquaculture segments in the United States. However, catfish production has declined due to decreasing catfish operations and reduction in water surface used for production; thus, during 2005 catfish sales represented 68.7% of total sales from aquaculture production with a total value of 461.9 million dollars, while in 2013 catfish sales added 375.9 million dollars in sales which represented 51.3% of total sales from US aquaculture production in that year (USDA 2014). In order to maintain a competitive level in the market, the US catfish industry started to work on the development of technological solutions to improve production, one of them has been implementing hybridization programs with the objective of produce a fish that combine some of the more desirable characteristics of two parent species (Kerby & Harrell 1990).

The interspecific hybrid obtained from crossing channel catfish, *Ictalurus punctatus*, & x blue catfish, *I. furcatus*, was identified as the most suitable for culture conditions, because attributes related to increased growth rates, improved yield and better disease resistance (Aphis, 2011; Arias *et al*., 2012). Hence, there is a trend to grow hybrids even though there are additional costs associated with their production, in fact it is estimated that during 2010 approximately 3400 ha were dedicated to culturing hybrids, while in 2014 it was estimated at 5,128 hectares (Aphis, 2011; USDA, 2014).

Implementation of the hybridization program had to overcome some reproductive limitations associated with the artificial spawning, because a chronically low hatch out rate, typically half that of pure channel catfish (Dunham & Argue, 2000). One of the first steps to overcome this challenge was to conduct basic research on brood stock nutrition to optimize egg quality, hatch and fry production. This article presents results from broodstock nutrition research conducted to improve egg quality in channel catfish.

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Broodstock management

One key factor in broodstock management is the nutritional status of the broodstock fish. Bromage (1995) indicate that both quality and quantity of the diet may influence gamete and fry quality. This is because the contents of an egg must be incorporated into the egg when it is an oocyte within the ovary in a process called vitellogenesis (Sargent, 1995; Wiegand, 1996; Brooks *et al*., 1997). Food restriction itself can affect spawning success, and thus has been reported to cause an inhibition of gonadal maturation in several species such as goldfish *(Carassius auratus* L), European sea bass (*Dicentrarchus labrax* L), and male Atlantic salmon (*Salmo salar* L) (Izquierdo *et al*., 2001). Dietary components as diverse as protein and fatty acids have all been shown to affect egg and embryo survival (Brooks *et al.*, 1997). Proteins act as a source of amino acids and as a reservoir of materials used during the many biosynthetic activities that are essential for the early stages of embryogenesis (Metcoff, 1986). Takeuchi *et al*. (1981) reported higher hatch rates for eggs from trout maintained on a low protein diet (36%), whereas Roley (1983) found that the eggs produced by trout brood fish fed on a 47% protein diet had higher survival than those produced by fish given either 27 or 37% protein diets.

Lipid and fatty acid composition of brood stock diets have been identified as major dietary factors that determine successful reproduction and survival of offspring. Highly unsaturated fatty acids (HUFA) with 20 or more carbon atoms affect directly or through their metabolites, fish maturation and steroidogenesis (Fernandez-Palacios *et al*., 1995; Izquierdo *et al.*, 2001). Altering the lipid composition of brood stock diet effected egg quality in the European sea bass. Eggs considered to be of better quality had a higher content of total n-3 fatty acids, which included enhanced levels of both docosahexaenoic acid and eicosapentaenoic acid (Brooks *et al.,* 1997). In contrast to these studies, an analysis of the lipid and fatty acid composition of Atlantic halibut eggs showed that batches of eggs with widely differing viabilities had very similar lipid compositions (Bruce *et al*., 1993). In the case of Nile tilapia, performance was much higher in fish fed a basal diet

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supplemented with soybean oil (high in n-6 fatty acids) and relatively low in fish fed a diet supplemented with 5% cod liver oil (high in n-3 fatty acids) (Santiago & Reyes, 1993).

Catfish broodstock management in US

Catfish broodstock management in commercial breeding operations in U.S. follows different practices in regards to feed management, and varies according to the season. Feeding in the spring through early summer (pre-spawning and spawning) is mostly either on a daily basis (35.2%) or every other day (35.1%), followed by every third day (18.9%), less often than every third day (5.4%), and no feed (5.4%) (USDA, 2010). The use of feed in breeding operations is identified based on the protein level content, thus 16.7% of the breeding operations use a 28% crude protein level feed, 69.4% of them use a 32% crude protein content, 5.6% of the operations use 35% crude protein level, and 8.3% of them use other protein levels. Finally, about 48.7% of the commercial operations used forage fish as a supplemental food source for broodfish (USDA, 2010)

Interaction nutrition and reproductive performance

To facilitate our understanding of the interaction of nutrition and reproductive performance from female channel catfish, two experiments were performed in earthen ponds. The first experiment evaluated the interaction of feed quality (42 and 32% protein), feed frequency (feed offered 3 or 6 times per week), channel catfish strains (high and low spawning strains) and brood fish age. The second experiment assessed the influence of different lipid sources and n3:n6 ratios using a commercial catfish feed containing 32% protein and 5% lipid as the basal diet.

Results of those experiments showed that reproductive performance in terms of spawning and egg production was not influenced by changing protein level of the diet from 32% to 42%, but affected egg size. Increasing the feeding frequency from three to six times

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per week negatively affected spawning in one of the strains, did not affect egg production and egg fertilization, but had a significant effect on egg size.

Brood fish age affected reproductive performance, with older fish performing better than younger fish in terms of spawning success and egg production. Similar findings were presented by Santiago (1979) who reported a very low spawning success in channel catfish 3-year-old females (12.7%). Davis *et al*. (2005) also suggested that age rather than size is a more important component of maturation. In the present study, there was not significant relation between egg size and either fish weight or fish length, but there was a significant difference in egg diameters due to fish age (older females had bigger eggs). Also, females given higher protein diets, and fed more frequently tended to have larger eggs. According to Shatunovskii (2006) this phenomenon of age affecting egg size, can be attributed to an increased reproductive function in ontogeny, which is realized as a more active synthesis of ovovitellin in the liver and its storage in oocytes as well as to an elongated period of trophoplasmatic growth of oocytes. This situation is also seen in walleye, where female age accounted for a greater amount of variation in egg mass than fork length or size (Johnston, 1997).

Biochemical composition of the eggs was affected significantly by dietary treatment in terms of fatty acids and free amino acid content, but not in the protein and lipid content (Table 1). The most abundant fatty acids were 16:0, 18:0, 18:1n9, 18:2n6, 20:3n3, and 22:6n3. There was a significant effect of protein level on the fatty acid composition of the eggs, except for 14:0,16:0, 18:2n6 and 20:1n9. Proportions and absolute values of linolenic acid and highly unsaturated fatty acids (ARA, EPA, and DHA), as well as the n3:n6 ratios were significantly higher in eggs from fish fed 42% protein diet (Table 1 and 2). The ratios DHA:EPA, ARA:EPA, and ARA:DHA were significant higher in eggs from fish fed 32% protein diet (Table 2). Egg viability at 48 hours of fertilization was not significantly different among treatments (feed protein x feed frequency) for each strain.

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The second experiment had as a goal to evaluate the effect of polyunsaturated fatty acids PUFAs (linolenic acid, 18:2n6 and linolenic acid, 18:3n3) in different ratios, as well as the effect of highly unsaturated fatty acids HUFAs (arachidonic acid - ARA, 20:4n6; eicosapentaenoic acid - EPA, 20:5n3; and docosahexaenoic acid - DHA, 22:6n3) on channel catfish, *Ictalurus punctatus* brood stock females to produce hybrid catfish fry. A commercial catfish feed (32% protein, 5% lipid) was top-coated with 2% lipids from different sources, using soybean and /or, linseed oil, or menhaden fish oil enriched with docosahexaenoic acid (DHA) and arachidonic acid (ARA) (Quintero *et al*., 2011). Results of this study showed that reproductive parameters, such as spawning success, number of eggs either as per gram of egg mass or per female body weight did not exhibit significant effect from dietary treatments, but fatty acid composition of the eggs was affected by broodstock dietary treatment provide prior to the spawning season (Table 3 and 4). 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. Freshwater fish are able to convert C18 PUFA to the biologically active C20 and C22 HUFA. Many freshwater fish posses both the fatty acid Δ6- and Δ5- desaturases required for the production of 20:5n3 and 22:6n-3 from 18:3n3, and of 20:4n-6 from 18:2n6 (Sargent 1995; Sargent *et al.*, 2002). 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 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). Linoleic acid (C18:2n6) and linolenic acid (C18:3n3) are considered primary precursors of highly unsaturated fatty acids, especially in freshwater fishes (Sargent *et al.*, 2002) and were found in lower proportions than those found in the feeds. These differences are more likely to be related to

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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 indicate either a selective mobilization of this fatty acid from other tissues or elongation and desaturation 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 n3 configuration in order to prevent essential fatty acid deficiencies (Farkas *et al*., 1977). There was not a defined trend in proportions of n3 HUFAs in the eggs, specifically EPA and DHA, 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. Egg quality of channel catfish, it appears, is affected by more than the relative abundance of n3 HUFAs. The ratio between ARA (20:4n6) and EPA (20:5n3), or DHA (22:6n3) could be more determinant in that outcome, as was suggested by Bell & Sargent (2003). They consider that both 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 make the generalization that a high ARA:EPA in fish eggs may be mandatory for survival. Moreover, embryogenesis could be influenced by essential fatty acids C20:3n6, C20:4n6, C20:5n3, and C20:6n3, since they are precursors for eicosanoid production, which in turn result in metabolites that include prostaglandins, leukotrienes and lipoxins (Leray *et al*., 1985; Mokoginta *et al*., 1998; Bell & Sargent, 2003).

Channel catfish females fed with feed top-coated with soybean oil (high n-6) produced eggs that were found to be significant smaller than the eggs produced by females under other treatments. Fatty acid composition of the eggs reflected the dietary lipid supplementation, making clear the incorporation of essential fatty acids during the pre-spawning season. Multivariate statistics using Principal Components Analysis (PCA) for the five fatty acids (linoleic acid, linolenic acid, ARA, EPA, and DHA) contained in the egg samples, lead us to differentiate groups of eggs based on scores assigned for each principal component which were reflection of the dietary treatments. Linseed oil (rich in linolenic acid C18:3n3) displayed the lowest hatch, which could be related to higher levels of ARA (C20:4n6),

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causing alteration in the immune cell composition. Similarly, higher proportions of C20:3n6 observed in diets 1 (SBO-LSO) and 2 (SBO) could be affecting embryo development due to immune response alterations. Supplying ARA, EPA, and DHA directly to brood stock females in diet 4 (MFO) increased fry production from two to five times when compared to females fed with diets top coated with soybean and/or linseed oil. Hence, commercial producers may consider using highly unsaturated fatty acids as lipid supplement on their brood stock diets to improve their fry production.

The quantity of fry produced per female body weight and fry survival from fish fed top-coated feed with menhaden fish oil enriched ARA and DHA were two to five fold greater than those obtained from fish fed with feed supplemented with vegetable oils. This difference was not significant ($p = 0.08$) and their impact on a commercial basis could be very important. Based on the results of these studies, it is recommended that the minimum dietary requirements for ARA, eicosapentaenoic acid (EPA) and DHA be evaluated for enhancement of egg quality in the channel catfish. Finally, further investigation is required to elucidate the mechanism that regulates fatty acid composition in eggs, in particular that related to proportions of linolenic acid and arachidonic acid, and the physiological implications of such relation reproductive performance parameters evaluated on channel catfish females, either spawning success, number of eggs per gram of egg mass, number of eggs per body weight, egg mass per body weight, fry production, or fry hatch.

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Table 1 - 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 42% and 32% protein level offered three times per week.

Parameter	42% -3 times/week	32% -3 times/week	p-values
Feed			
Moisture (%)	9.41 ± 0.09	7.12 ± 0.19	0.0048
Protein (%)	40.74 ± 0.83	33.99 ± 1.03	0.0187
Lipids (%)	6.22 ± 0.05	5.48 ± 0.11	0.0137
Energy (cal)	4,437 ± 6	4,231 ± 17	0.0036
Eggs			
Protein (%)	17.80 ± 1.93	17.21 ± 1.71	0.1590*
Protein (mg/individual egg)	3.20 ± 0.98	2.95 ± 0.94	0.1531
Lipids (%)	6.82 ± 1.05	6.49 ± 1.05	0.6159*
Lipids (mg/individual egg)	1.12 ± 0.42	1.02 ± 0.39	0.1415
Free amino acids as total ninhydrin positive substances (TNPS)			
TNPS (µmol/gr egg mass)	3.84 ± 1.89	2.43 ± 2.05	<0.0001
TNPS (µmol/individual egg)	6.91 ± 3.60	3.74 ± 3.09	<0.0001
Ratios			
DHA[1]:EPA[2]	6.46 ± 1.98	13.27 ± 3.09	0.0001
ARA[3]:DHA[1]	0.012 ± 0.005	0.015 ± 0.003	0.0004
ARA[3]:EPA[2]	0.08 ± 0.07	0.19 ± 0.07	<0.0001

*The smallest p-value ≥0.05 from Beta regression coefficients

1 – DHA: Docosahexaenoic acid

2 – EPA: Eicosapentaenoic acid

3 – ARA: Arachidonic acid

Table 2 - Fatty acid analysis from eggs of channel catfish females, *Ictalurus punctatus*, by dietary treatments, commercial catfish diet 42% and 32% protein level offered three times per week

Fatty acid	42% - 3 times/week	32% - 3 times/week	p-value
14:0	0.99 ± 0.01	0.90 ± 0.04	0.0961
16:0	18.78 ± 0.02	18.37 ± 0.03	0.0702
16:1n7	3.08 ± 0.02	2.37 ± 0.04	<0.0001
18:0	12.17 ± 0.02	13.10 ± 0.02	<0.0001
18:1n9	28.67 ± 0.11	30.70 ± 0.05	0.0001
18:2n6	7.25 ± 0.07	6.97 ± 0.04	0.2471
19:0	5.92 ± 0.04	5.89 ± 0.04	0.8472
18:3n3	0.41 ± 0.01	0.26 ± 0.002	<0.0001
20:1n9	1.08 ± 0.01	1.13 ± 0.01	0.1607
20:2n6	1.16 ± 0.004	1.42 ± 0.01	<0.0001
20:3n6	2.65 ± 0.02	3.25 ± 0.01	<0.0001
20:3n3	3.88 ± 0.04	5.72 ± 0.01	<0.0001
20:4n6	0.08 ± 0.002	0.06 ± 0.001	<0.0001
20:5n3	1.20 ± 0.03	0.35 ± 0.01	<0.0001
22:4n6	0.24 ± 0.003	0.37 ± 0.002	<0.0001
22:5n6	1.11 ± 0.02	2.56 ± 0.02	<0.0001
22:5n3	0.74 ± 0.004	0.55 ± 0.01	<0.0001
22:6n3	7.34 ± 0.03	4.38 ± 0.08	<0.0001
Σ n-6	11.37 ± 0.08	13.10 ± 0.05	<0.0001
Σ n-3	13.66 ± 0.02	11.31 ± 0.07	<0.0001
n-3/n-6	1.22 ± 0.18	0.84 ± 0.12	<0.0001

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Table 3 - 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	1-SBO-LSO	SBO	LSO	MFO	p-values
Feed					
Moisture (%)	7.36 ± 0.25	7.15 ± 0.20	7.55 ± 0.10	7.18 ± 0.12	0.5512
Protein (%)	33.43 ± 0.40	33.58 ± 0.17	33.53 ± 0.31	33.39 ± 0.36	0.9726
Lipids (%)	7.12 ± 0.04	6.94 ± 0.20	7.12 ± 0.48	7.21 ± 0.26	0.7402
Energy (cal)	4,128 ± 15	4,207 ± 47	4,230 ± 66	4,220 ± 72	0.5312
Eggs					
Protein (%)	16.81 ± 0.18[a]	16.65 ± 0.12[a]	16.74 ± 0.24[a]	16.33 ± 0.13[b]	0.0360*
Protein (mg/ egg)	3.07 ± 0.30[a]	2.73 ± 0.39[b]	3.02 ± 0.46[a]	3.16 ± 0.42[a]	0.0001
Lipids					
Percentage (%)	7.27 ± 0.13	7.19 ± 0.09	7.40 ± 0.17	7.54 ± 0.09	0.2681**
mg/individual egg	1.12 ± 0.19[ab]	0.97 ± 0.12[b]	1.14 ± 0.28[ab]	1.27 ± 0.40[a]	0.0003
Free amino acids as total ninhydrin positive substances (TNPS)					
µmol/gr egg mass	3.29 ± 2.26[c]	5.85 ± 1.83[b]	3.72 ± 2.02[c]	7.37 ± 1.63[a]	<0.0001
µmol/egg	5.92 ± 4.15[c]	9.62 ± 3.45[b]	7.06 ± 4.74[bc]	14.48 ± 4.45[a]	<0.0001
Ratios					
DHA[1]:EPA[2]	7.54 ± 2.09[b]	10.93 ± 3.62[a]	8.14 ± 1.96[b]	8.60 ± 1.97[b]	<0.0001
ARA[3]:DHA[1]	0.021 ± 0.004[b]	0.016 ± 0.003[c]	0.026 ± 0.005[a]	0.013 ± 0.002[d]	<0.0001
ARA[3]:EPA[2]	0.15 ± 0.02[c]	0.17 ± 0.05[b]	0.20 ± 0.03[a]	0.11 ± 0.03[d]	<0.0001

* The largest p-value <0.05 from Beta regression coefficients

** The smallest p-value ≥0.05 from Beta regression coefficients

1 – DHA: Docosahexaenoic acid

2 – EPA: Eicosapentaenoic acid

3 – ARA: Arachidonic acid

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Table 4 - Fatty acid analysis from eggs of channel catfish females, *Ictalurus punctatus*, held under dietary treatments, commercial catfish diet 32% protein, 5% lipids, top-coated with 2% oil, and offered three times per week

Fatty acid	SBO-LSO	SBO	LSO	MFO	p-values
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}	0.0040
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
Σ n-6	13.14 ± 1.07^{ab}	13.33 ± 0.75^{a}	12.56 ± 1.35^{b}	11.56 ± 0.75^{c}	<0.0001
Σ 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

Values followed by the same letter are not different (p>0.005, Tukey-Krammer test)

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ISBN 978-607-27-0593-7, pp. 259-273.

References

Anderson A. J., Arthington A. H., Anderson S. (1990) Lipid classes and fatty acid composition of the eggs of some Australian fish. Comparative Biochemistry and Physiology, Part B: Biochemistry and molecular biology, 96B, 267-270

Animal and Plant Health Inspection Service, APHIS (2011) Use of channel x blue hybrid in U.S. catfish operations. Veterinary services, Info sheet.

Arias, C. R., Cai, W., Peatman, E., Bullard, S. A. (2012) Catfish hybrid *Ictalurus punctatus* x *I. furcatus* exhibits higher resistance to columnaris disease than the parental species. Diseases of Aquatic Organims, 100, 77-81. doi: 10.3354/dao02492.

Bell J. G., Sargent J. R. (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218, 491-499

Bromage N. (1995) Brood stock management and seed quality - general considerations. In: Bromage, N. R., Roberts, R. J. (Eds.), Brood stock management and egg and larval quality. Blackwell Science Inc., Cambridge, Great Britain, 424 pp

Brooks S., Tyler C. R. ,Sumpter J. P. (1997) Egg quality in fish: what makes a good egg?. Reviews in Fish Biology and Fisheries 7, 387-416

Bruce M. P., Shields R. J., Bell M. V., Bromage N. R. (1993) Lipid class and fatty acid composition of eggs of Atlantic halibut *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive brood stock. Aquaculture Fisheries Management 24, 417-422

Davis K. B., Simco B. A., Silverstein J. T. (2005) Relationship of gonadal development to body size and plasma sex hormone concentrations in female channel catfish. North American Journal of Aquaculture 67, 259-264

Dunham, R. A., Argue, B. J. (2000). Reproduction among channel catfish, blue catfish and their F1 and F2 hybrids. Transactions of the American Fisheries Society 129, 222-231

Durland, E. R., Quintero, H. E., Davis, D. A., Dunham, R. A. (2010). Influence of forage fish and dietary lipid supplements on egg quality and fry production in channel catfish (*Ictalurus punctatus*) x blue catfish (*Ictalurus furcatus*) hybridization. Aquaculture nutrition 16, 153-162. doi: 10.1111/j.1365-2095.2009.00647.x

Farkas T., Csengeri I., Majoros F., Oláh J. (1977) Metabolism of fatty acids in fish. I. Development of essential fatty acid deficiency in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture 11, 147-157

Fernández-Palacios H., Izquierdo M. S., Robaina L., Valencia A., Salhi M., Vergara J. M. (1995) Effect of n-3 HUFA level in brood stock diets on egg quality of gilthead sea bream (*Sparus aurata* L). Aquaculture 132, 325-337

Quintero, H. and D. Allen Davis. 2015. Broodstock nutrition: enhancement of egg quality in channel catfish. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México,
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Harel M., Tandler A., Kissil G. W., Applebaum S. W. (1994) The kinetics of nutrient incorporation into body tissues of gilthead sea bream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. British Journal of Nutrition 72, 45-58

Henderson R. J., Tocher D. R. (1987) The lipid composition and biochemistry of freshwater fish. Progress in Lipid Research 26, 281-347

Izquierdo M. S., Fernández-Palacios H., Tacon A. G. J. (2001) Effect of brood stock nutrition on reproductive performance of fish. Aquaculture 197, 25-42

Johnston T. A. (1997) Within-population variability in egg characteristics of walleye, *Stizostedion vitreum*, and white sucker, *Catostomus commersoni*. Canadian Journal of Fisheries and Aquatic Sciences 54, 1006-1014

Kaitaranta J. K., Linko R. R. (1984) Fatty acids in the roe lipids of common food fishes. Comparative Biochemistry and Physiology, Part B: Biochemistry and molecular biology, 79B, 331-334

Kerby J. H., Harrell R. M. (1990) Hybridization, genetic manipulation, and gene pool conservation of striped bass. Chapter 11. In: Harrel R. M., Kerby J. H & Minton R. V. (Eds.). Culture and propagation of Striped Bass and its hybrids. Striped Bass Committee, Southern Division. American Fisheries Society. Bethesda, MD, USA

Leray C., Nonnotte G., Roubaud P., Leger C. (1985) Incidence of –3 essential fatty acid efficiency on trout reproductive processes. Reproduction nutrition development 25, 567-581.

Metcoff J. (1986) Intracellular amino acid levels as predictors of protein synthesis. Journal of the American College of Nutrition 5, 107-120

Mokoginta I., Takeuchi T., Moeljohardjo D. S., Sumawidjaja K., Fardiaz D. (1998) The effect of different ratios of n-6/–3 fatty acids in brood stock diets on egg quality of catfish, *Clarias batrachus*. Asian Fisheries Science 11, 157-168

Quintero, H. E., Durland, E., Davis, D. A., Dunham, R. (2011). Effect of lipid supplementation on reproductive performance of female channel catfish, *Ictalurus punctatus*, induced and strip-spawned for hybridization. Aquaculture Nutrition, 17, 117-129. doi: 10.1111/j.1365-2095.2009.00709.x

Roley D. D. (1983) The effect of diet protein level, feeding level and rearing water temperature on the growth and reproductive performance of rainbow trout broodstock. PhD. Dissertation. University of Washington. 271 pp

Santiago A. C. (1979) Effects of feeding regime on reproductive performance of female channel catfish in ponds. PhD. dissertation. Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA

Santiago C. B., Reyes O. S. (1993) Effect of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) brood stock. Journal of Applied Ichthyology 9, 33-40

Sargent J. R. (1995) Origins and functions of egg lipids: nutritional implications. In: Bromage, N. R., Roberts, R. J. (Eds.), Brood stock management and egg and larval quality. Blackwell Science Inc., Cambridge, Great Britain, 424 pp

Sargent J. R., Tocher D. R., Bell J. G. (2002) The lipids. In: Halver, J. E., Hardy, R. W. (Eds.), Fish nutrition. Third Edition. Academic Press, San Diego, CA, USA, 824 pp

Shatunovskii M. I. (2006) Some patterns of age and geographical variation in fish fecundity. Biology Bulletin 33, 2: 195-198

Takeuchi T., Watanabe T., Ogino C., Saito M., Nishimura K., Nose T. (1981) Effects of low protein-high calorie diets and deletion of trace elements from a fish meal diet on reproduction of rainbow trout. Bulletin of the Japanese Society of Scientific 47, 645-654

Tocher D. R., Sargent J. R. (1984) Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids 19, 492-499

United States Department of Agriculture, USDA (2010) Catfish 2010 - Part I: Reference of Catfish health and production practices in the United States, 2009. Animal and Plant Health Inspection Service, Veterinary Services.

United States Department of Agriculture, USDA (2014) 2012 Census of Agriculture, Census of Aquaculture (2013) Vol 3, special studies, part 2. National Agricultural Statistics Service

Wiegand M. D. (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Reviews in fish biology and fisheries 6, 259-286

Quintero, H. and D. Allen Davis. 2015. Broodstock nutrition: enhancement of egg quality in channel catfish. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México,
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