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DIFFERENTIAL ROLE OF DIETARY FATTY ACIDS ON GONADAL RECRUDESCENCE IN THE AFRICAN CATFISH *CLARIAS GARIEPINUS* (BLOCH.)

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Freshwater African male catfish *Clarias gariepinus* were maintained under oil rich diets differing in fatty acid contents for six weeks during the pre-spawning phase. The diet was supplemented with linseed oil (rich in C18:3 n-3 linolenic acid) or sunflower oil (rich in C18:2 n-6 linoleic acid) or coconut oil (rich in C12:0 dodecanoic acid) (5% w/w each). Gonadosomatic index (GSI) was recorded and, plasma levels of testosterone (T), tri and tetra-iodothyronin (T_3 and T_4), total lipids, triglycerides, phospholipids and total cholesterol were estimated. GSI was significantly high in linseed oil fed group as compared to other groups, whereas it was significantly low in coconut oil fed group. Plasma levels of T as well as T_3 followed the same pattern whereas, change in the T_4 level was significant only in fish fed with coconut oil rich diet. Plasma total lipid content was significantly high in response to fatty acids feeding. Level of phospholipid was elevated in the fish fed with linseed oil supplemented diet whereas declined in group maintained under coconut oil rich feed. Total cholesterol was higher in both n-3 and n-6 fatty acids maintained groups with no change in the saturated fatty acid fed fishes. Results indicated that short chain n-3 fatty acid was gonado-stimulatory, whereas saturated fatty acid was inhibitory. Short chain n-6 fatty acid had no impact on the gonadal activity in this catfish during the prespawing phase of the reproductive cycle.

INTRODUCTION

A perusal of literature suggests an important nutritional role of fatty acids on fish reproduction. Fatty acids are reported to be essential for sperm quality and spawning response (Nandi *et al*., 2007), breeding performance (Nandi *et al*., 2001), steroidogenesis (Mercure and Van Der Kraak, 1995), vitellogenesis and egg development (Navas *et al*., 1998, Jaya-Ram *et al*., 2008), fertilization (Fernandez-Palacios *et al*., 1997) and larval development (Abi-ayad *et al*., 1997) in several fish species. Dietary saturated fatty acids affected the egg quality in fish (Ballestrazzi *et al*., 2006). Dietary lipid sources significantly influenced the pituitary LH and FSH levels and caused an early maturation in male salmon (Shearer and Swanson, 2000). Dietary unsaturated fatty acid like n-3 PUFA

(polyunsaturated fatty acid) influenced the growth rate of Atlantic salmon (Menoyo *et al*., 2003). Further, unsaturated and saturated fatty acids play differential role in modulating the reproductive activity of fish. Spawning performance was significantly influenced by dietary HUFAs (highly unsaturated fatty acids) in Nile tilapia (*Oreochromis niloticus*) depending on the salinity of water in which they were reared (El-Sayed *et al*., 2005). Long chain PUFAs mainly eicosapentaenoic acid (EPA, C20:6 n-3) and arachidonic acid (AA, C20:4 n-6) showed significant influence on the hCG stimulated steroid synthesis in female goldfish, *Carassius auratus* (Mercure and Van Der Kraak., 1995). Higher levels of estradiol-17β (E_2) as well as gonadotropin II (GtH II) hormone were observed on female sea bass, *Dicentrarchus labrax*, with docosahexaenoic acid (DHA, C22:6 n-3) or EPA rich diet (Navas *et al*., 1998). However, levels of testosterone (T) and E_2 decreased in addition to plasma lipid fraction, if *D. labrax* were fed with diet deficient in n-3 fatty acid (Cerda *et al*., 1995).

It is known that PUFAs as well as saturated fatty acids inhibit the thyroid binding in rats (Inoue *et al*., 1989). Oleic acid (C18:1 n-9) was the predominant PUFA and tetradecanoic acid (C14:0) was the most potent saturated fatty acid reported in this investigation. Though the role of thyroid hormone in catfish reproduction is well established (Yadav *et al*., 1986, Sinha and Singh, 1990), the impact of dietary PUFA on thyroid hormone levels in fish is rudimentary.

As reported by Acharia *et al*. (2000), the influence of dietary PUFAs (C18:3 n-3 or C18:2 n-6) on the gonadal or somatic growth was temperature dependent in the Indian female catfish, *Clarias batrachus*. Dietary C18:3 n-3 was gonado-stimulatory whereas C18:2 n-6 was favourable for somatic growth when fish were maintained at high temperature. In addition, plasma and gonadal lipid contents were also altered in response to these diets. However, in a related study conducted during the post-spawning phase, the gonadosomatic index, plasma level of T and E_2 were significantly higher, when the fish were injected wit*h Mystus* gonadotropin compared to the n-3 PUFA diet alone (Acharia *et al*., 2001). Since the gonadal activity of fish varies from season to season, it is relevant to study the effect of these diets during different phases of the reproductive cycle, especially to know whether the effect of these diets on the gonadal activity vary with the reproductive status or not.

Therefore, in the present study, the African male catfish *Clarias gariepinus* were maintained under dietary regimes of C18 n-3 PUFA, C18 n-6 PUFA and saturated fatty acid (mainly dodecanoic acid C12:0) for six weeks in the month of April. In addition to body weight and GSI, total lipids, triglycerides, phospholipids, total cholesterol and testosterone (T), tri-iodo (T_3) and tetra-iodo thyronin (T_4) in plasma were estimated.

MATERIALS AND METHODS

Adult male specimens of freshwater catfish *C. gariepinus* (body weight 250-300 g) were collected in first week of April from local fish market in Delhi and maintained under normal photoperiod and temperature (12L:12D; 28±1 ^{0}C) in aquaria during their pre-spawning phase. After a fortnight of acclimation, fishes were divided into four groups (12 fish per group) and fed with different test diets for six weeks. Feed (2% of body weight) was provided to fish once a day. Initially they did not accept the feed but after 4 to 5 days of practice, they started feeding. The leftover pellets were removed. The four test groups were as follows:

1. Basal diet i.e. diet without any oil (fatty acid) supplementation (BD)
2. Basal diet supplemented with linseed oil (LSO)
3. Basal diet supplemented with sunflower oil (SO)
4. Basal diet supplemented with coconut oil (CO)

Preparation of diet

Basal diet was prepared by mixing maize powder (75% wt/wt), linseed oil cake powder (12.5% wt/wt) and dry fish powder (12.5% wt/wt). Agrimin (1% wt/wt) (Glaxo India) was added as a source of vitamins and minerals (Acharia *et al*., 2000). Test diets were prepared by incorporating linseed oil (LSO), sunflower oil (SO) and coconut oil (CO) to the basal diet at 5% (W/W). Ingredients were mixed homogenously in grinder (Yorko high speed homogenizer, India). Water was added to the mixture to make into dough. It was then passed through a sieve of 2 mm diameter. After partial drying at 40 ^{o}C, semi moist pellets were provided to the fish throughout the duration of experiment. Fatty acid content of different oils is listed in table 1.

Table 1. Percent by weight of total fatty acids of linseed oil, sunflower oil and coconut oil

Fatty acids*	Linseed oil	Sunflower oil	Coconut oil
10:0	-	-	6.00
12:0	-	-	47.00
14:0	-	-	18.00
16:0	3.00	7.00	9.00
18:0	7.00	5.00	3.00
18:1	21.00	19.00	6.00
18:2 (n-6)	16.00	68.00	2.00
18:0 n-3)	53.00	1.00	-
Total			
Saturated	10.00	12.00	83.00
Monounsaturated	21.00	19.00	6.00
n-3 PUFA	53.00	1.00	-
n- PUFA	16.00	68.00	2.00

*Number of carbon atoms: number of double bonds

Collection and analyses of samples

At the end of the experiment, each fish was weighed, blood collected by heparinised syringes fitted with 26-gauge needle from the caudal artery and centrifuged at 1500 xg at 4 ^{o}C to collect plasma. Samples were stored at -80 ^{o}C until analysis. Fish were sacrificed by decapitation; testes were extirpated and weighed to calculate GSI. Plasma samples were processed for estimation of T, T_3 and T_4 by competitive ELISA using commercial kits (DRG EIA-1599 for T; CALBIOTECH T3043T for T_3 and CALBIOTECH T4044T for T_4). Total lipids, total cholesterol, phospholipids and triglycerides were estimated by colorimetric method using commercial kits (CALBIOTECH, RANDOX cat. No/KA.NR TL 100 and Bayer diagnostic kit).

Statistical analysis

T_4 to T_3 ratio and cholesterol to phospholipid ratio were calculated and data was expressed as mean±SE. One-way ANOVA ($P<0.05$) was applied to know the overall effect of diet factor on different parameters (Brunning and Kintz, 1977). Tukey's test was applied as a supplementary test to know the significant difference between means of basal and different oil fed groups.

RESULTS

LSO rich diet elevated the level of GSI significantly ($p<0.05$), but SO supplemented feed showed no effect on GSI values; whereas a statistically significant decline ($p<0.05$) was noticed in CO rich feed as compared to BD (Fig. 1). Plasma levels of T were minimum (16.76±0.41) in fish fed with CO rich feed and maximum (34.33±1.07) in LSO supplemented diet (Fig. 2). Similar trend was observed with plasma T_3 concentration in different fatty acid fed groups (Fig. 3). However, experimental PUFAs had no impact on plasma T_4 but CO rich diet reduced its level remarkably when compared with BD (Fig. 3). T_4 to T_3 ratio was higher in BD as well as SO (1.22 and 1.25 respectively) fed groups whereas lower in LSO and CO fed fishes (Table 3).

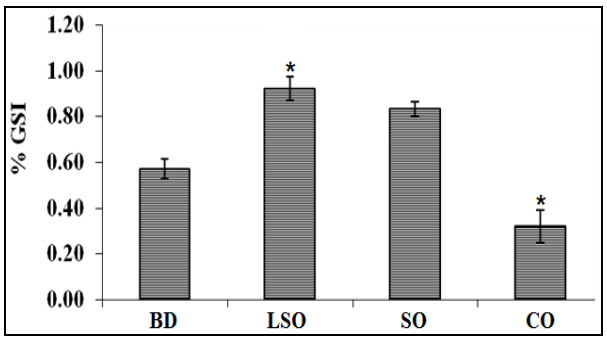

Fig. 1. Changes in the levels of gonadosomatic index (% GSI) in response to feeding dietary fatty acids in the male African catfish *Clarias gariepinus*. *significant from BD

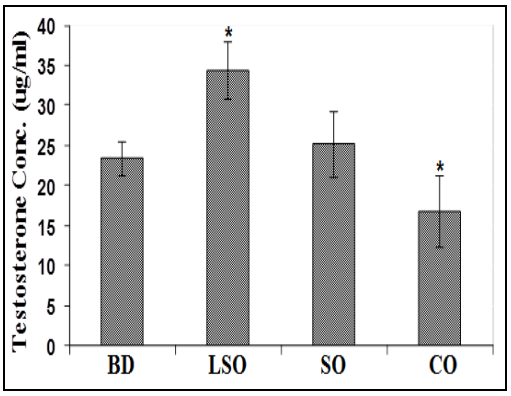

Fig 2. Changes in the plasma levels of testosterone in response to feeding different fatty acid rich diet in the male African catfish *Clarias gariepinus.* *significant from BD

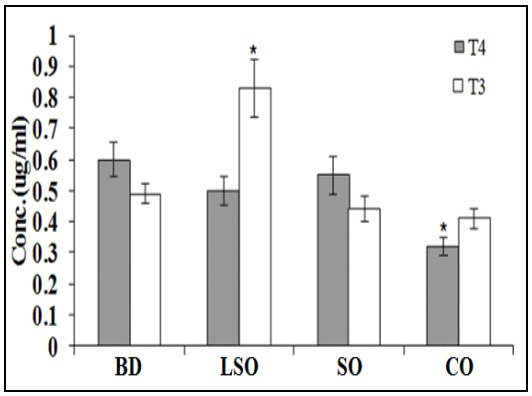

Fig 3. Change in the plasma levels of T4 and T3 in response to feeding different fatty acid rich diets in the male African catfish *Clarias gariepinus*. * significant from BD

Plasma total lipids (g/ml) exhibited an overall enhancement in its concentration in all the three groups maintained under different oil supplemented feeds. However, no significant differences in lipid contents were observed among these groups (Table 2).

Table 2. Plasma levels of various classes of lipids in the African male catfish, *Clarias gariepinus* in response to different diets. Values were expressed as mean± SE (n=8). Data were analysed by one-way ANOVA ($p<0.05$)

Lipid fractions	Feeding regimes			
	BD	LSO	SO	CO
Total lipid (g/ml)	0.74 ± 0.09	0.80 ± 0.082*	0.91 ± 0.07*	0.89±0.02*
Triglyceride (mg/ml)	1.29 ± 0.093	1.33 ± 0.088	1.35 ± 0.076	1.32 ± .066
Phospholipid (mg/ml)	0.67 ± 0.054	1.85 ± 0.12*	0.89 ± 0.081	0.38 ± 0.025*
Total Cholesterol (mg/ml)	2.02 ± 0.19	3.9 ± 0.09*	3.5 ± 0.28*	2.7 ± 0.17

Plasma triglyceride level (mg/ml) remained uninfluenced by any of the three experimental diets whereas augmentation of phospholipids (mg/ml) was highly significant on feeding short chain n-3 fatty acid (18:3 n-3). SO showed no effect on it. A noticeable reduction in the phospholipids (0.38±0.025) was recorded in the saturated fatty acid fed fish. Interestingly, no correlation was found between the dietary saturated fatty acids and plasma total cholesterol concentration. However, unsaturated fatty acid feeding (LSO and SO) increased its level (Table 2). Cholesterol to phospholipid ratio was

maximum (7.11) in CO fed group and minimum (2.1) in LSO fed group. In fishes fed with BD and SO fortified diet, the ratio was 3 and 3.93 respectively (Table 3).

Table 3. Ratio of T_4 to T_3 and total cholesterol (Chol) to phospholipids (PL) in plasma of African male catfish *Clarias gariepinus* fed on different dietary regimes

Ratio	Feeding regimes			
	BD	LSO	SO	CO
T_4/T_3	1.22	0.6	1.25	0.78
Chol/PL	3.0	2.1	3.93	7.11

DISCUSSION

The present study indicated that dietary fatty acids play a determinant role in modulating the reproductive activity in the male African catfish, *C. gariepinus* during the pre-spawning phase. A significant increase in GSI as well as T and T_3 in the C18 n-3 PUFA fed fishes showed that LSO plays a stimulatory role in gonadal recrudescence which is consistent with our earlier report (Acharia *et al*., 2000) in another catfish, *C. batrachus*. Since elevation in GSI by SO oil compared to basal diet was not statistically significant it was considered that this particular fatty acid had no effect on gonadal recrudescence at this phase. Interestingly, testicular size was reduced in fish fed with CO (rich in dodecanoic acid C12:0) supplemented diet. Since body weight remained unaltered (data not shown) in response to any of the experimental fatty acid supplemented diets, it is assumed that C18:3 n-3 or C18:2 n-6 or C12:0 had no impact on the somatic growth in this fish during the pre-spawning phase particularly. Similar observation was reported in the earlier studies performed in the Indian female catfish *C. batrachus* (Acharia *et al*., 2000, 2001). However, in this species the differential role of n-3 or n-6 PUFA for gonadal activity was temperature dependent. Though linoleic acid (C18:2 n-6) was stimulatory for increasing the body weight in *C. batrachus* at high temperature, with no effect on ovarian activity, we could not find similar results in case of *C. gariepinus*, may be due to difference in species or sex studied or the difference in the phase in which experiment was conducted. A significant reduction in the GSI in the CO fed groups indicates its inhibitory role in the gonadal recrudescence, which was further evident by the regressed testes noticed in these groups compared to BD. The evidence of direct utilization of dietary fatty acids in reproduction has been observed in turkey. Incorporation of dietary 22:5 n-3 and 22:6 n-3 fatty acids into the spermatozoa leading to better reproductive activity was found in aged turkeys (Blesbois *et al*., 2004). In female carp *Catla catla*, a mixture of dietary n-3 and n-6 PUFAs was even more effective on maturation and fecundity than the individual fatty acid supplementation (Nandi *et al*., 2001). Interestingly, males of the same species responded better in terms of sperm quality and spawning response when they were fed with PUFA enriched diet compared to control (Nandi *et al*., 2007).

Plasma level of T was increased by LSO enriched feed which again indicates a stimulatory role of α-linolenic acid (C18:3 n-3) for gonadal recrudescence in *C. gariepinus*. This finding is in agreement with our previous study in female *C. batrachus* (Acharia *et al*., 2000). Modulation of steroidogenesis by PUFAs has been reported by several workers (Meikle *et al*., 1989; Speizer *et al*., 1991; Elliott and Goodfriend, 1993). Arachidonic acid (C20:4 n-6) via its conversion to eicosanoids, stimulates T production in goldfish ovaries (Van Der Kraak and Chang, 1990) and testes (Wade and Van Der Kraak, 1993). Mercure and Van Der Kraak (1995) noticed an inhibitory action of long chain linolenic (C18:3 n-3) and linoleic acid (C18:2 n-6) in gonadotropin stimulated T production by full grown pre-maturational ovarian follicles *in vitro* in goldfish, *C. auratus*. Differential role of long chain n-3 or n-6 PUFA on the *in vitro* testicular steroidogenesis in goldfish is apparent when n-6 fatty acid (arachidonic acid, C20:4 n-6) stimulates testosterone synthesis, whereas eicopentaenoic acid (C20:5 n-3) inhibits it (Wade *et al*., 1994). In the present study, the selective action of short chain PUFA in LSO fed group in increasing the T production is apparent whereas, in CO fed group the T level was declined. This data is contrary to our previous work (Acharia *et al*., 2000) where sunflower oil raised the plasma T level in female *C. batrachus* during the post-spawning phase of its reproductive cycle. Since during this phase, fish undergoes physiological activities more for somatic growth, it was assumed that T was utilized for some metabolic activity other than reproduction. Present study was conducted in pre-spawning phase (active phase for gonadal growth). Data show that, like in female *C. batrachus* (Acharia *et al*., 2000; 2001), linoleic acid (C18:2 n-6) may not play any significant role on reproduction in this species. On the other hand, saturated fatty acid (CO) reduces the T level significantly. Although no report is available regarding the effect of dietary saturated fatty acids on hormonal fluctuation in fish, in mammals octadecanoic acid (C18:0) and hexadecanoic acid (C16:0) (Glass *et al*., 1981, Vermeulen, 1996) reduced plasma T levels. However, mechanism of this phenomenon is still unresolved excepting a study, which exhibited the apoptotic role of hexadecanoic acid (C16:0) and octadecanoic acid (C18:0) on rat Leydig cells *in vitro* (Lu *et al*., 2003). In the present study, a significant reduction in the plasma phospholipid concentration in CO fed group further confirms the inhibitory effect of saturated fatty acid on testicular activity in this species.

In the present investigation T_3 level was enhanced by n-3 PUFA but remained unaltered by n-6 PUFA or saturated fatty acid. Nevertheless, T_4 levels remained unaltered in any of the PUFA fed groups. On the other hand, CO was found to be inhibitory for T_4. With the present set of data, it is difficult to explain the role of dietary fatty acids on the thyroid physiology. Since, there is no such evidence on the dietary effect of PUFAs on the thyroid activity in fish; it can be assumed that the increase in T_4 or T_3 under linolenic acid (C18:3 n-3) fortified diet may be due to elevated level of T in these groups, which was further evidenced with no increase in T_3 in the SO maintained fish. This contention can be explained from the findings of Yadav *et al*. (1986) where injection of testosterone

propionate raised the plasma T_3 level almost double and T_4 several times in the freshwater catfish *C. batrachus*. Singh and Raizada (1979) also observed the maximum activation of thyroid gland in response to estrogen in female *Heteropneustes fossilis*. In this study, no fluctuation in the level of T_4 in response to LSO supplemented or SO rich feed was recorded may be because of the fact that rate of T_4 synthesis and conversion into T_3 is same. Maximum T_4 to T_3 conversion was reported in the pre-spawning phase in the *C. batrachus* (Sinha and Singh, 1982). Higgs *et al*. (1982) opined that fish thyroid mainly produces T_4 which behaves as a prohormone, and about 70% of it may be rapidly deiodinated extrathyroidally into T_3 as major if not sole end product. However, unesterified long chain fatty acids especially oleic acid inhibited the thyroid hormone (T_3) binding to its nuclear receptor in rat testes (Inoue *et al*., 1989). Medium chain fatty acid also interfered in the thyroid hormone receptor binding (Thurmond *et al*., 1998).

The experimental diets rich in fatty acids appear to be lipogenic as evidenced by the appreciable enhancement of the plasma total lipid irrespective of the type of fatty acids used in the diets. However, the degree of increase was almost equal in all the three groups may be because the total lipid content of all the three experimental fatty acids (LSO, SO and CO) was almost equal. Dietary linseed oil rich in (C18:3 n-3) again showed its gonadostimulatory effect by increasing the plasma phospholipids and cholesterol. In this species, phospholipids may be one of the important class of lipids required for gonadal activity. Requirement for phospholipids for signal transduction is well documented. In *C. batrachus* (Lal and Singh, 1987), a strong elevation in ovarian phospholipid was observed during the reproductively active phase. In the same species, Acharia *et al*. (2000) reported higher ovarian phospholipid content than plasma phospholipid when the fish were maintained with LSO supplemented diet, but plasma phospholipid level was comparable to that of basal diet fed fish because phospholipid was mobilized to the gonads. Wiegand and Peter (1980) noticed a rise in the plasma triglyceride level with increasing GSI in the maturing *C. auratus*. However, in this species, it was the proportion of free and esterified cholesterol, which varied in response to dietary fatty acids. In our study, we found an increase in plasma cholesterol in the SO fed groups too. Since there was no significant change in the level of T or GSI, the elevated level of cholesterol may be utilized for some other activity in this fish. In immature and mature *Anguilla anguilla*, a strong correlation between the plasma non esterified fatty acids and E_2 was observed by Ccottrill *et al*. (2001). Similarly a change in the total lipid, neutral lipid and polar lipid was observed in response to n-3 PUFA rich or deficient diet in female gilthead seabream (Almansa *et al*., 2001). Mukherjee and Bhattarcharya (1982) observed very low level of free cholesterol in the ovary of *Channa punctatus* with the ovarian development. Cholesterol to phospholipid ratio in the plasma was found high in all the experimental groups including basal diet. A decreasing tendency in the ratio was noticed in LSO maintained fishes whereas it was highest in CO fed fishes when compared with BD.

In conclusion, feeding diets containing varying amounts of fatty acids to African male catfish for six weeks affected the gonadal recrudescence in terms of GSI, hormonal and lipid profile during pre-spawning phase. This study has shown that diet supplemented with linseed oil (rich in C18:3 n-3) enhanced testicular size and elevated T and T_3 levels whereas diet enriched with coconut oil (C12:0) reduced testis size and depressed hormone levels.

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