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Influence of supplementary diet on the gonadal development of common carp (*Cyprinus carpio*) male.

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Abstract: The main aim of this present work is to study the influence of supplementary diet on the gonadal development of male common carp (*C. carpio*). Three types of supplementary diet with varying level of fish oil (0%, 2.5% and 5%) was prepared and given to three groups of experimental fishes. Fatty acid analysis showed saturated fatty acids found in three types of supplementary diets are significantly ($P<0.05$) different. The monounsaturated fatty acids in three types of experimental feeds are significantly ($P<0.05$) different. The total polyunsaturated fatty acids was high in feed E2 (27.26%) compared to control feed C (20.17%) and feed E1 (25.44%). The gonad weight observed during the period of this study from January to December, 2013 was statistically ($P<0.05$) significant in all experimental group of fishes. The GSI showed its peak value in the month of August, 2013 in three groups of experimental fishes. The GSI was maximum in Group III fishes compared to Group I and Group II fishes.

Key Words: *C. carpio*, Supplementary diet, Fish oil, Male gonad, GSI.

1. INTRODUCTION:

The development of aquaculture is highly dependent on the reproductive success of fishes. To enhance the fertilization success in fish industry modern techniques were practiced including induced spawning and multiple breeding, ovulation and spermiation and artificial fertilization. The significance of artificial propagation methods is to supply quality fish seed at the required times of the year (Lin and Peter, 1996; Taranger *et al*., 2010; Amano, 2010). The success of artificial fertilization depends on the adequate and quality of fish feed. Rearing of broodstock in insufficient food condition does not show full gamete maturity (Watanabe and Vassallo-Agius, 2003). Nowadays feed manufacturers use fish meal as a source of animal protein but it is very limited due to its high cost and also increases the ammonia level in the rearing medium (Viola and Lahav, 1991; Chakraborty and Chakraborty, 1998; Stibranyiova and Paraova, 2000).

In recent years fish nutrition has gained special importance in aquaculture particularly brood stock nutrition. Gonadal maturity and quality of egg improved by micronutrients such as polyunsaturated fatty acids, vitamins C and E, carotenoids and trace elements. They play a key role in the development of egg yolk (Matty, 1985; Singh *et al*., 2000a, 2002; 2009; Pandey *et al*., 2003). Spawning behaviour in fish is greatly influenced by the total lipids and essential fatty acids present in the diet (Mourente *et al*., 1989; Dhert *et al*., 1991; Navas *et al*., 1997; Mazorra *et al*., 2003; Aijun *et al*., 2005). The percentage and type of ingredients in supplementary feed determines the growth of fishes (Glencross *et al*., 2007). The development of fisheries sector and highest percentage of yield from freshwater resources can be achieved by providing supplementary feed. The main aim of this present investigation is to study the importance of nutrition on the gonadal development of male *Cyprinus carpio*.

2. MATERIALS AND METHODS:

2.1 PREPARATION OF SUPPLEMENTARY DIET:

The ingredients given in Table.1 were used for feed formulation for *C. carpio*. Three types of feed (control feed C, feed E1 and feed E2) were prepared with different ratio of fish oil (0%, 2.5% and 5%). The formulation contained GOC (20%), wheat bran (10%), Soy flour (15%), spirulina (5%), fish oil (5%), tapioca flour (4%), mineral mixture (1%), maize flour (4%), vitamin E (0.5%), salt (0.5%). The control feed C containing 0% fish oil and 40% de oiled rice bran. The experimental feed E1 was prepared with 2.5% fish oil and 37.5% de oiled rice bran. The experimental feed E2 was prepared with 5% fish oil and 35% de oiled rice bran. The ratio of other ingredients same in the three types of experimental feed.

Table 1. Composition of experimental fish feeds.

S.NO	Ingredients	% inclusion		
		C	E1	E2
1	GOC	20	20	20
2	De oiled Rice bran	40	37..5	35
3	Wheat bran	10	10	10
4	Soy bean meal	15	15	15
5	Spirulina	5	5	5
6	Fish oil	0	2.5	5
7	Tapioca flour	4	4	4
8	Mineral mixture	1	1	1
9	Maize flour	4	4	4
10	Vitamin E	0.5	0.5	0.5
11	Salt	0.5	0.5	0.5
	Total	100	100	100

C- Control feed; E1 – Experimental feed 1; E2 – Experimental feed 2

2.2 FATTY ACID ANALYSIS OF SUPPLEMENTARY DIET:

Fatty acids were analyzed by the method of Folch *et al*. (1957).

2.3 EXPERIMENTAL FISH:

The experimental fish *C. carpio* size ranged from 300±50g collected from Tamil Nadu Fisheries Development Corporation, Aliyar Nagar, Pollachi, Coimbatore District, Tamil Nadu, India. The fishes were stocked in cultured tanks for 10 days of acclimatization. Then the fishes were divided into three groups (Group I, Group II and Group III) based on the feed given. Each group containing 100 fishes each. Triplicate group maintained for each group. Group I fishes allowed to fed control feed C and Group II fishes fed with experimental feed E1 and Group III fishes allowed to fed with experimental feed E2. Feed was given at 2% of their body weight daily twice. The experiment was carried out from January to December, 2013.

2.4 GONADAL WEIGHT AND GSI:

Three fishes were randomly collected from three groups of experimental fishes and the paired testis from each fish were carefully removed, washed, cleaned with distilled water, dried with the help of blotting paper and then weighed nearest to 0.1g. Gonadal weight was taken at the end of every month throughout the experimental period from January to December, 2013.

GSI of experimental group of fishes was calculated by using the following formula (Qasim, 1973).

$$GSI = \frac{\text{Weight of the Gonad}}{\text{Weight of the fish}} \times 100$$

3. RESULTS:

Fatty acid analysis of the three experimental feeds was given in Table.2. There are 11 fatty acids identified in the three types of experimental feeds. The fatty acids analysed ranged from (C14:0) to (C22:6, n-3). Fatty acid analysis showed higher variations in three experimental feeds control feed C, feed E1 and feed E2. The three experimental feeds (control feed C, feed E1, feed E2) have five saturated fatty acids (SFA) such as myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0). All saturated fatty acids found in three experimental feeds are significantly ($P<0.05$) different.

Oleic acid (C18:1 n-9) and palmitoleic acid (C16:1 n-9) are the monounsaturated fatty acids (MUFA) identified in all experimental feeds. Oleic acid identified as the predominant fatty acid with higher percentage in all experimental feeds (control feed C, feed E1 and feed E2) compared to other fatty acids. Total MUFA were found to be higher in feed E2 (42.31%) followed by feed E1 (39.94%) and control feed C (34.48%). MUFA in three types of experimental feeds are significantly ($P<0.05$) different.

Four polyunsaturated fatty acids (PUFA) were recorded in control feed C, feed E1 and feed E2 and they are linoleic acid (C18:2, n-6), linolenic acid (C18:3, n-3), eicosapentaenoic acid (EPA) (C20:5, n-3) and docosahexaenoic acid (DHA) (C22:6, n-3). The PUFA, EPA found to be higher level in feed E2 (0.90%) compared to control feed C (0.28%) feed E1 (0.45%). Another PUFA is DHA which is totally absent in control feed C and feed E1 contain 0.71%

and feed E2 contain 0.98%. Highest percentage of DHA was found in feed E2 compared to feed E1. The total PUFA was high in feed E2 (27.26%) compared to control feed C (20.17%) and feed E1 (25.44%). All polyunsaturated fatty acids in three types of feed (control feed C, feed E1 and feed E2) are significantly ($P<0.05$) different. EPA/DHA ratio showed higher values in feed E2 (0.92%) than feed E1 (0.63). The total n-3 PUFA and the total n-6 PUFA was high in feed E2 compared to feed E1 and control feed C.

Table 2. Fatty acid composition of experimental feeds.

Fatty acid	Structure	C (%)	E1 (%)	E2 (%)
Myristic acid	C14:0	1.80 ± 0.11^{a}	0.85 ± 0.07^{b}	2.11 ± 0.14^{c}
Palmitic acid	C16:0	22.37 ± 0.19^{a}	19.91 ± 0.16^{b}	16.77 ± 0.29^{c}
Stearic acid	C18:0	1.58 ± 0.10^{a}	5.43 ± 0.15^{b}	2.55 ± 0.13^{c}
Arachidic acid	C20:0	0.88 ± 0.08^{a}	0.66 ± 0.06^{b}	3.75 ± 0.16^{c}
Behenic acid	C22:0	0.84 ± 0.06^{a}	1.33 ± 0.12^{b}	1.65 ± 0.23^{c}
∑SFA		27.47	28.18	26.83
Oleic acid	C18:1 n-9	31.2 ± 0.26^{a}	31.46 ± 0.28^{b}	34.90 ± 0.36^{c}
Palmitoleic acid	C16:1 n-9	3.28 ± 0.14^{a}	8.48 ± 0.19^{b}	7.41 ± 0.22^{c}
∑MUFA		34.48	39.94	42.31
Linoleic acid	C18:2 n-6	19.09 ± 0.18^{a}	23.8 ± 0.20^{b}	24.32 ± 0.21^{c}
Linolenic acid	C18:3 n-3	0.8 ± 0.03^{a}	0.48 ± 0.03^{b}	1.06 ± 0.09^{c}
EPA	C20:5 n-3	0.28 ± 0.05^{a}	0.45 ± 0.02^{b}	0.90 ± 0.08^{c}
DHA	C22:6 n-3	-	0.71 ± 0.07^{a}	0.98 ± 0.07^{b}
∑PUFA		20.17	25.44	27.26
∑n-3		1.08	1.64	2.94
∑n-6		19.09	23.8	24.32
n3/n6		0.057	0.069	0.12
EPA/DHA		0	0.63	0.92

C – Control feed; E1- Experimental feed 1; E2- Experimental feed 2

Values are mean of three triplicates ± SD

[a-c] Mean values with in a row no common superscript differ significantly at 5% by DMRT

The monthly observation of gonad weight of three groups of experimental fishes was shown in Table 3. The weight of the gonads followed regular cyclic changes that were related with the reproductive activities of fish. The gonadal weight ranged from 21.25g to 79.74g in Group I fishes and 23.41g to 96.65g in Group II fishes and 23.98g to 110.59g in Group III fishes. The weight of the gonad increased from January to August, 2013 and then it gradually reduced in their size from September to October, 2013. This was observed in all experimental groups of fishes. The Table.3 also illustrates that fishes fed with feed E2 contain maximum gonad weight compared to fishes fed with feed E1 and fishes fed with control feed C throughout the experimental period. The maximum gonad weight (79.74gm, 96.65gm, 110.59gm) was recorded in the month of August, 2013 in all group of experimental fishes (feed C, feed E1 and feed E2) respectively. The gonad weight observed during the period of this experiment from January to December, 2013 was statistically ($P<0.05$) significant in all experimental group of fishes.

Table 3. Month-wise changes in gonadal weight of male C. *carpio* fed with three types of diets (C, E1 and E2) during the experimental period from January to December, 2013.

Months	Control	E1	E2
January	21.25 ± 0.14[a]	23.41 ± 0.18[b]	23.98 ± 0.21[c]
February	25.34 ± 0.16[a]	28.69 ± 0.23[b]	29.94 ± 0.26[c]
March	26.53 ± 0.22[a]	32.27 ± 0.30[b]	37.34 ± 0.41[c]
April	32.48 ± 0.27[a]	41.62 ± 0.35[b]	48.74 ± 0.39[c]
May	43.27 ± 0.33[a]	54.61 ± 0.42[b]	62.37 ± 0.54[c]
June	49.83 ± 0.34[a]	65.82 ± 0.45[b]	71.28 ± 0.60[c]
July	67.91 ± 0.40[a]	80.12 ± 0.44[b]	89.36 ± 0.53[c]
August	79.74 ± 0.63[a]	96.65 ± 0.63[b]	110.59 ± 1.20[c]
September	65.44 ± 0.75[a]	83.73 ± 0.82[b]	98.2 ± 0.79[c]
October	60.16 ± 0.56[a]	78.58 ± 0.63[b]	92.28 ± 0.76[c]
November	67.27 ± 0.57[a]	83.48 ± 0.65[b]	98.52 ± 0.82[c]
December	72.51 ± 0.94[a]	89.37 ± 0.73[b]	105.31 ± 0.96[c]

Values are mean ± SD (n=5)
[a-c] Mean values with in a row no common superscript differ significantly at 5% by DMRT

The monthly observation of GSI of all experimental groups of fishes was given in Figure 1. The GSI of three groups of experimental fishes ranged from 5.87% to 8.46 in Group I fishes fed with control feed C and 6.34% to 8.63% in Group II fishes fed with feed E1 and 6.44% to 8.85% in Group III fishes fed with feed E2. In the present investigation, the cyclic changes in Gonado-Somatic Index of the three experimental groups of fishes were observed. The GSI value increased from January to August, 2013 in three groups of experimental fishes (Group I, Group II and Group III). The GSI showed its peak value in the month of August, 2013 in three groups of experimental fishes during the experimental study. Then the value of GSI was decreased from September to October, 2013 and again it was slightly increased in November, 2013 and December, 2013. The Figure.1 clearly illustrates that GSI was maximum in Group III fishes compared to Group I and Group II fishes.

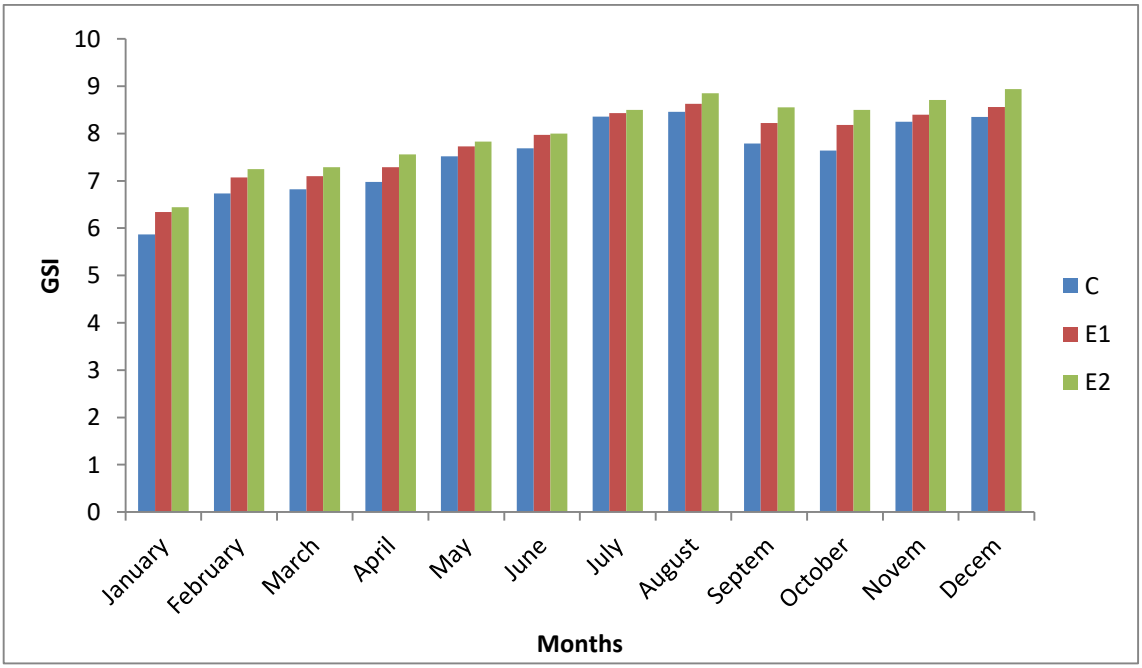

Figure 1. Month-wise changes in GSI of *C. carpio* male fed with three types of diet (C, E1 and E2) during the experimental period from January to December, 2013.

4. DISCUSSION:

The most common dietary component in broodstock nutrition is lipids. Spawning behaviour in fish is controlled by lipids and essential fatty acids present in the diet (Dhert *et al*., 1991; Mazorra *et al*., 2003). Broodstock diet with lipid

and fatty acids modulate the pituitary as well as the spawning behaviour (Cerda *et al*., 1994). Broodstock performance enhanced by providing (n-3) HUFA enriched diet (Lie, 1993). Several constituents of spermatozoa and seminal plasma have been associated with broodstock nutrition. Phospholipids and cholesterol in spermatozoa plasma membrane associated with the type of feed given to male broodstock. In rainbow trout the phospholipid content of spermatozoa affected by brood- stock nutrition (Pustowka *et al*., 2000). Lipid composition in sperm plasma membrane and seminal plasma determines the structure and fertilizing ability of sperm. In addition to that lipids and cholesterol protects the sperm against environmental influences when it is introduced into water or extender solutions (Aramli *et al*., 2013). Fish oil used as a source of essential fatty acids in the present study. Different level of fish oil (0%, 2.5%, 5%) used to prepare different types of experimental feed for this study. Fish oil contains two important components, EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid). The EPA (20:5 n-3) and DHA (22:6 n-3) are principle fatty acids included in the omega-3 PUFA family. Omega-3 PUFA such as DHA and EPA involved in the production of eicosanoids (Kinsella *et al*., 1990a). Freshwater fishes cannot synthesize omega-3 fatty acids. So they must be present in the diet. EPA is also specifically play a main role for larval growth (Watanabe *et al*., 1989) and broodstock fertility.

In the present study the experimental fishes fed with feed E2 showed higher gonad weight throughout the experimental period significant at $P<0.05$ level from January, 2013 to December, 2013. The gonad weight noted in the present study was maximum in August, 2013 (110.59g) in fishes fed with feed E2 compared to fishes fed with feed C (79.74g) and feed E1 (96.65g). The gonadal weight of experimental fishes showed variation in different months of the year, 2013. The increased gonad weight of the fishes noticed due to the increase of spermatogonia and their active division to form spermatocytes. Then the gonad weight was reduced in the month of September and October, 2013. This is mainly due to the release of spermatozoa during breeding season (August, 2013) and the size of the testes was reduced this condition was referred as the resting stage (Dhriti Guha and Dilip Mukherjee, 1991). The male gonad fully attains maturity only during breeding season (July-August) in *L. rohita*. The gonad weight was reduced and GSI value falls in male *L. rohita* in post spawning period (Gunwant *et al*., 2014). The same result was observed in the present study.

GSI is used as an indicator of reproductive efficiency of fishes (Froese and Binohlam, 2000; Serajuddin and Ali, 2005; Ahirrao, 2008; Emmanuel and Melanie, 2009; Cakmak and Alp, 2010). The lowest GSI values were recorded in January, 2013 indicated that during this month the weight of the testes in male fish was minimum. As the weight of the fish increased the gonad weight also increased in the forthcoming months up to August, 2013 afterwards it was declined in the month of September and October, 2013. This was mainly due to reduction in size and gonad weight of the experimental fishes after spawning period. The peak value of GSI observed in the present study was 8.46% in fishes fed feed with C, 8.63% in fishes fed with feed E1 and 8.85% in fishes fed with feed E2 in August, 2013.

After then the GSI was slowly increased from November to December, 2013. On the basis of observed values of GSI in all groups of experimental fishes it can be concluded that in a reproductive life cycle of *C. carpio*, April to June, 2013 is the pre breeding season and July, to August, 2013 is the breeding season and September to October, 2013 is the post-breeding season. The GSI of eel fish showed peak value of 2.47% and 1.75% for females and males during breeding season (September) respectively (Uthaykumar, 2012).

5. CONCLUSION:

Nutrition is an important factor in fishes as that of other animals. Fish cannot synthesize essential aminoacids and essential fatty acids required for growth, survival and reproduction. Providing supplementary diet with balanced nutrition is best choice for increasing gonadal development and reproductive ability of fishes. The present study proved that supplementary diet with 5% fish oil improved gonadal growth in male *C. carpio*.

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