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Original article

Effect of dietary supplementation of fatty acids and vitamins on the breeding performance of the carp *Catla catla*

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Abstract — Five isonitrogenous diets (~33% crude protein) were fed to the brood female carp, *Catla catla* (weighing 3.0 to 5.5 kg), for a period of 93 days in order to observe their breeding performance in earthen ponds. Diet-I (control) contained only basic ingredients like rice bran, groundnut oil cake, roasted soybean meal, fish meal and mineral mixture; diet-II contained added vitamins; diet-III contained added vitamins and vegetable oil (rich in n-6 polyunsaturated fatty acids, PUFA); diet-IV contained added vitamins and fish oil (rich in n-3 PUFA); and diet-V contained added vitamins and a mixture of vegetable and fish oils. The results showed that nutritional quality of the diet considerably influenced breeding performance in the species. The total number of matured females was the highest in the diet-V group and maturity was advanced by 35 days in this group compared to the control. In diet-III and diet-V groups, all the maturated females bred fully and the relative fecundity was increased significantly in diet III, IV and V. The maximum (73.4%) fertilisation rate was observed in the diet-V group, followed by 61.3%, 56.8%, 49% and 22.7% in diet-I, diet-IV, diet-III and diet-II groups respectively. Most of the eggs in the diet-II treatment group remained immature. The various data thus obtained suggest that dietary supplementation of both n-3 and n-6 PUFA, is essential to improve gonadal maturation, breeding performance and spawn recovery in the *Catla* female broodstock.

diet / fatty acid / broodstock / breeding performance / *Catla*

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1. INTRODUCTION

In India, freshwater aquaculture is largely of finfish, especially carp oriented and in recent years, interest and importance have grown due to success in controlled breeding, opening up possibilities of year round availability of carp fry (in season and out of season) adequate to cater to the needs of fish farmers [18]. So far, information on the effects of broodstock nutrition with regard to reproductive performances and the egg quality of fish species of economic importance like Indian major carps is scarce. Farmers are therefore constrained in employing a variety of husbandry and feeding regimes for maintenance of broodstock to maximise fecundity and egg viability. Despite relative paucity of work on broodstock nutrition, the nutritional status of broodstock is known to have a profound effect on the reproductive performance and quality of offspring in several species. Studies performed on nile tilapia, turbot, lake trout, goldfish and yellow tail [16, 19, 25, 26, 28, 29, 36, 46, 47] have demonstrated that incorporation of essential nutrients into the developing eggs depends on the availability of these nutrients in the female broodstock and consequently on the dietary input in the period preceding gonadal maturity. In this context, supply of dietary n-3 highly unsaturated fatty acids (HUFA) is known to improve broodstock performance [27]. Supplementation of HUFA to the turbot broodstock diet resulted in a significant increase in egg diameter and oil globule diameter [26]; in red seabream, a deficiency dramatically decreased hatchability and increased abnormalities in eggs and larvae, and a dose-response of reproductive success to dietary n-3 HUFA levels was demonstrated [47, 48]. This underlines the impact of such fatty acid supplementation during the maturation processes of fish. A general point which can be derived from the literature is that eggs usually contain substantial amounts of (n-3) polyunsaturated fatty acids (PUFA). This is related to the importance of these fatty acids as essential constituents of the cell membrane phospholipids particularly phosphatidylcholine. Since during embryogenesis and larval development, proliferation and differentiation of cells occur, it is vital that a sufficient supply of these fatty acids be available to broodstock. In fish eggs, the n-3 HUFA, docosahexaenoic acid, DHA (22:6 n-3) is mainly found in phosphatidylcholine and the developing fish larva incorporates it in neural cell membranes of the eye and brain tissues for its normal development, which largely constitute the larval body mass [3, 43]. Another important function of PUFAs, in particular of arachidonic acid, ARA (20:4 n-6) is the role as precursors of eicosanoids [34]. In fish, ARA is preferentially incorporated into phosphatidylinositol (PI) which performs an important role in signal transduction in the cell membrane. A strong correlation has been found between dietary phosphatidylinositol concentration and larval development in the common carp [14].

Although a considerable amount of information on the aspects of breeding habits and reproduction in captivity is available [6, 18], very little information on the influence of essential dietary constituents like PUFA and vitamins on the reproductive success in *Catla catla* is available. Currently, there is a high demand for stockable fry of this preferred species of the Indian major carp (IMC) due to its faster growth rate and amenable to culture in different freshwater ecosystems [31]. This paper reports the results of a pond experiment to elucidate the possible effects of dietary supplementation of PUFAs and vitamins on egg and larval quality of this species.

2. MATERIALS AND METHODS

2.1. Fish and pond management

Catla catla (about two years old) of both sexes weighing 3.0–5.5 kg having no external

symptoms of gonadal maturity were collected from a water hyacinth infested non-cultural pond (1 ha) and stocked in the ponds of Krishi Vigyan Kendra Instructional Fish Farm at Kausalyaganga (Lat. 20°1′06″–20°11′45″N, Long. 80°50′52″–85°51′35″E). The females were kept in ten identical ponds (0.05 ha, average water depth 2 metres) at 750–800 kg·ha^{-1}(10 fish/pond). All the males were stocked in a separate pond (0.4 ha, average water depth 1.5 metre) at 1500 kg·ha^{-1}. These ponds were prepared before stocking following routine broodstock pond management practices [18]. The pond water was replenished with 25–30% freshwater from a nearby stocking pond (1 ha) at fortnightly intervals during the first 45 days of the total 93-day experimental period. Temperature, dissolved oxygen, pH and ammonia of the experimental pond water varied between 27.4–32.7 °C, 5.5–6.8 ppm, 7.0–7.4 and 2.39–4.12 μg atom N·L^{-1} respectively during the experimental period.

2.2. Experimental diets and feeding regime

After 7 days of acclimatisation in the experimental ponds, the female broodstock of ten ponds (two replicates for each diet treatment) were fed with five different formulated diets (Tab. I). These five diets had identical crude protein content (about 33%); except diet-I (control), the other four diets (D-II to D-V) consisted of exogenous addition of vitamin supplements. Diets III, IV and V had different sources of oils; while D-III and D-IV had only vegetable oil and fish oil supplements respectively, D-V had a mixture of both vegetable oil and fish oil supplements. No oil supplementation was provided to D-II. All these diets were prepared into 5-mm diameter pellets and were fed at 3% body weight once daily in the morning in a basket throughout the experimental period. Similarly, males were fed at 3% body weight with a dough containing water soaked ground nut oil cake and rice

Table I. Ingredients and nutrients composition of different test diets for female *Catla* broodstock.

Composition	Diets				
	D-I	D-II	D-III	D-IV	D-V
Ingredients					
Rice bran	30	30	30	30	30
Ground nut oil cake	38	38	38	38	38
Roasted soybean meal	20	20	20	20	20
Fish meal	10	10	10	10	10
Mineral mixture[a]	2	2	2	2	2
Vegetable oil[b] (Soybean oil)	–	–	3	–	2.7
Fish oil[b]	–	–	–	0.3	0.3
Vitamin mixture[a]	–	0.2	0.2	0.2	0.2
Nutrients					
Crude protein (N × 6.25)	33.34	33.21	33.30	33.32	33.15
Crude lipid	11.65	11.72	14.67	12.13	14.51
Gross energy (kJ·g^{-1}) DM	16.57	16.70	20.13	16.82	19.88

[a] Composition of the mixtures remained the same as that described elsewhere [30].
[b] Vegetable oil, fish oil and vitamins were added as extra amounts (in kg) per 100 kg of the concerned diets.

bran mixture (1:1) once daily in a basket. The composition of ingredients / nutrients, and the fatty acid profile of the diets are given in Tables I and II, respectively.

2.3. Sampling and breeding protocol

The ponds were netted out and the fishes were examined individually for gonadal development and maturation through external symptoms at 15-day intervals initially up to 30 days and 7-day intervals afterwards until the end of the experiment. Breeding operations were undertaken as and when maturity of the broodstock was observed and they were allowed to breed within the conventional breeding hapa in pond conditions (0.4 ha, depth 2.0 metres, water temperature 28 °C, dissolved oxygen 8.0 ppm and pH 7.2). Only the fully matured female broodstock showing external symptoms of ripening, viz. bulging and softness of the abdomen, and pinkish appearance of the vent, were selected for the breeding operation. Matured males with free milt oozing upon gentle hand pressure were selected for breeding. Males and females were kept in the ratio of 2:1 in the breeding hapa after injecting them with a single dose of ovaprim (Salmon GnRH+ Domperidone, Syndel lab, Canada) at 0.5 $mL \cdot kg^{-1}$ for females and 0.2 $mL \cdot kg^{-1}$ for males. The brood fishes were handled with utmost care during the operation to minimise physical stress. The females were considered as fully bred when no eggs came out through the vent after pressing the abdomen with the hand from the upper to lower region. The eggs were

Table II. Selected fatty acid composition (%) of the formulated diets. Data are mean of three determinations for each diet lipid.

Fatty acids	Diets				
	D-I	D-II	D-III	D-IV	D-V
14:0	6.1	6.4	2.4	3.2	1.5
16:0	21.1	20.9	14.3	16.5	14.7
16:1 (n-7)	10.7	9.6	4.6	2.1	3.5
18:0	7.8	7.1	5.9	7.7	5.5
18:1 (n-9)	46.3	45.8	40.8	25.7	30.3
18:2 (n-6)	2.8	3.7	25.7	5.8	20.8
18:3 (n-3)	0.2	0.2	0.5	3.6	2.5
18:4 (n-3)	–	–	0.1	2.5	1.8
20:1 (n-9)	3.2	2.8	1.7	0.6	1.2
20:4 (n-6)	0.2	0.4	3.0	0.7	3.6
20:5 (n-3)	0.1	0.3	0.1	11.7	5.2
22:5 (n-3)	–	–	–	6.2	3.9
24:5 (n-3)	–	–	–	2.2	0.9
22:6 (n-3)	–	–	–	5.5	2.7
Total n-3	0.3	0.5	0.7	31.7	17.0
Total n-6	3.0	4.1	28.7	6.5	24.4
Total PUFA	3.3	4.6	29.4	38.2	41.4
n-3/n-6	0.1	0.12	0.02	4.87	0.7
DHA/EPA	–	–	–	0.47	0.52
ARA/EPA	2.0	1.33	30.0	0.06	0.7

hatched in hatching hapa fixed in the pond (average water temperature 28 °C), distributing the eggs at 3 L/hapa.

2.4. Analytical methods

The formulated diets were analysed for crude protein, total lipid and gross energy content. Crude protein (N × 6.25) content was estimated in a Kjeltech system and crude lipid was extracted with chloroform and methanol 2:1 (v/v) and estimated gravimetrically [12]. Gross energy was estimated using a Parr bomb calorimeter. The extracted lipid was subjected to an acid catalysed transmethylation reaction for 16 h at 50 °C using 1 mL toluene and 2 mL 1% H_2SO_4 (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography as reported earlier [30]. FAME were separated and quantified using a Pye-Unicam gas chromatograph equipped with a flame ionisation detector (250 °C). Nitrogen was used as the carrier gas. Fatty acids were identified by comparing their positions and retention time with those of a known mixture of standard fatty acid methyl esters (Sigma, Chemical Co., USA) under identical conditions. Statistical analysis of the breeding performance data, viz. breeding response, relative fecundity, fertilisation, water hardened egg diameter, individual weight of the water hardened eggs and relative spawn recovery, were performed using analysis of variance [37].

3. RESULTS

During the experimental period of 93 days, total percentage of matured females among the initial broodstock population was increased ($P < 0.05$) in D-V and decreased ($P < 0.05$) in D-II and D-IV as compared to the control, whereas in the D-III group it was comparable to that of the control (Fig. 1). Among the five different diet treatments, the first fully matured female was observed on the 37th day in D-V, on the 44th day in

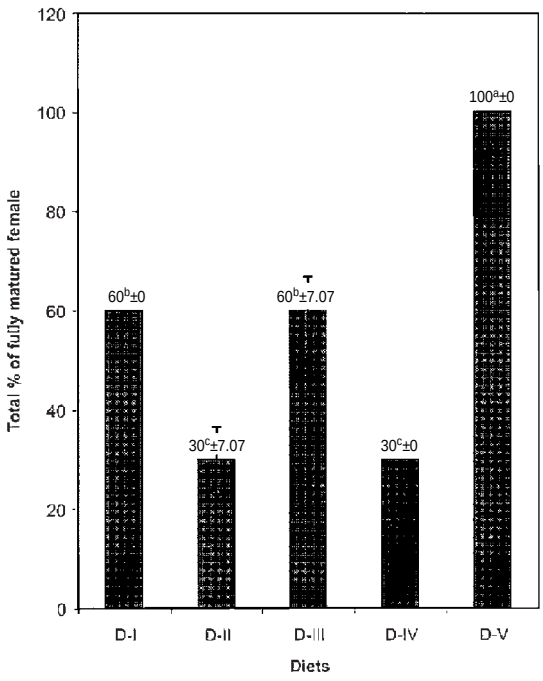

Figure 1. Total percentage of fully matured females out of the total initial population in different diets during the experimental period.

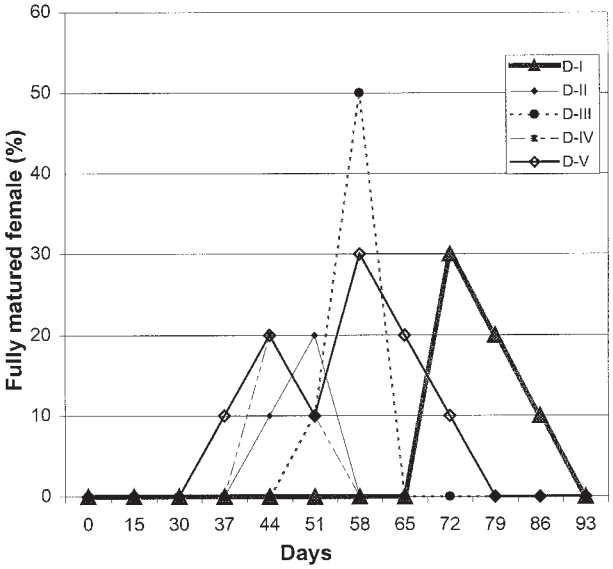

Figure 2. Percentage of fully matured females out of the total initial population in different diets on different days.

D-II and D-IV, on the 51st day in D-III and on the 72nd day in D-I (control) groups (Fig. 2). Therefore the maturity of the fishes was 28, 21, 28 and 35 days earlier in D-II, D-III, D-IV and D-V diet groups respectively as compared to the control. But D-V fishes were mature 7, 14 and 7 days earlier respectively as compared to those of D-II, D-III and D-IV groups. For early maturation of fish, the order of effectiveness of the diet with different nutrient supplementation was D-V (with vegetable oil and fish oil) followed by D-II (with vitamin mixture), D-IV (with fish oil) and D-III (with vegetable oil) respectively. The span of fully matured fish observed was the highest (35 days) in D-V (37–72 days), intermediate (14 days) in D-I (72–86 days) and lowest (7 days) in D-II (44–51 days), D-IV (44–51 days) and D-III (51-58 days) groups (Fig. 2).

The details of spawning performances (viz. breeding response, relative fecundity, fertilisation percentage, average diameter and weight of fully swollen water hardened eggs, and number of spawn recovered) of the species fed with different diets are presented in Table III. Data thus obtained showed that the breeding response (measured in terms of the egg laying capacity) was better and increased in D-III and D-V but decreased in D-II as compared to the control. Relative fecundity was increased significantly ($P < 0.05$) in all oil supplemented diet treatments (D-III, D-IV and D-V) as compared to the control; in D-II it was comparable with the control. This increase was 66.7, 47.8 and 81.2 percent in D-III, D-IV and D-V groups, respectively. The fertilisation percentage, diameter and weight of the water hardened eggs were significantly lower ($P < 0.05$) in D-II groups. In other diet groups (D-III, D-IV and D-V), these parameters were comparable with those in the control. Most of the eggs of the D-II group remained immature and were unable to imbibe water fully. The number of spawn recovered per kg female body weight was significantly increased ($P < 0.05$) in the D-IV and D-V groups, and decreased ($P < 0.05$) in D-II as compared to the control (D-I). In D-III it was comparable with the control. While comparing D-IV and D-V groups, the relative spawn recovery

Table III. Breeding performances of female *Catla* fed with different formulated diets.

Parameters	Diets				
	D-I	D-II	D-III	D-IV	D-V
Breeding response* (%)					
Fully bred (%)	50	–	100	67	100
Partially bred (%)	50	100	–	33	–
Relative fecundity** ($RF \times 10^5$)					
Mean (S.E.)	0.69^{d} (0.04)	0.86^{cd} (0.06)	1.15^{ab} (0.09)	1.02^{bc} (0.12)	1.25^{a} (0.04)
Fertilisation*** (%)					
Mean (S.E.)	61.3^{ab} (5.18)	22.7^{c} (3.29)	49.0^{b} (2.04)	56.8^{b} (7.90)	73.4^{a} (3.02)
Water hardened egg diameter (mm)					
Mean (S.E.)	4.36^{a} (2.0)	2.66^{b} (0.43)	4.26^{a} (0.39)	4.83^{a} (0.34)	4.97^{a} (0.14)
Individual weight of the water hardened egg (mg)					
Mean (S.E.)	41^{ab} (2.0)	28^{c} (3.61)	39^{b} (3.06)	42^{ab} (2.08)	48^{a} (3.06)
Relative spawn recovery**** ($RSR \times 10^5$)					
Mean (S.E.)	0.19^{c} (0.04)	0.003^{d} (0.01)	0.31^{bc} (0.03)	0.39^{b} (0.05)	0.75^{a} (0.05)

^{a,b,c,d} Values with different superscripts in a row differ significantly ($P < 0.05$).

* Breeding response (egg laying capacity) % $= \dfrac{\text{Number of females actually bred (Full/partial)}}{\text{Total number of females kept for breeding}} \times 100$

** Relative fecundity (RF) $= \dfrac{\text{Total number of eggs produced}}{\text{Total body weight of the females actually bred (kg)}}$

*** Fertilisation (%) $= \dfrac{\text{Number of fertilised (eyed) eggs}}{\text{Total number of eggs produced}} \times 100$

**** Relative spawn recovery (RSR) $= \dfrac{\text{Total number of spawn produced}}{\text{Total body weight of the females actually bred (kg)}}$

was significantly higher ($P < 0.05$) in D-V as compared to D-IV. Spawn recovery in the D-I group was considerably low on the contrary to its relatively higher fertilisation rate.

4. DISCUSSION

The results from this experiment have shown that in female *Catla* broodstock, although maturity was advanced in all treated groups (D-II to D-V) with the highest value in D-V, the entire population of each diet treatment did not mature (Fig. 1) within the experimental period except in D-V. This result revealed that gonadal maturity was influenced largely by the dietary oil sources from both vegetable and fish origins with n-6 and n-3 PUFAs, respectively. The increased pattern of breeding response, relative fecundity and relative spawn recovery (Tab. III) in all three oil supplemented groups indicated that both vegetable oil and fish oil supplementation act additively. They have a significant impact on breeding response and relative fecundity, and act synergistically on relative spawn recovery of fish as evidenced in D-V. Obviously, obtaining matured fish over a prolonged period, as observed in the D-V groups (Fig. 2),

offers scope in taking up breeding operation at different times according to the timely need of the farmers for spawn stocking. Moreover, the considerably higher fertilisation rate (20% over the control) in the D-V groups (Tab. III) facilitates the harvest of higher numbers of spawn.

In fish [36], 20:5(n-3) has a physiological role in modulating the formation of eicosanoids from 20:4(n-6) by competing with the enzyme systems converting 20:4(n-6) to eicosanoids. 20:4(n-6) is considered as the precursor of several eicosanoids [44] which are produced by the ovarian tissues [24] and play an important role in the ovulation [15, 32] process. It therefore seems that both these fatty acids, viz. 20:5(n-3) and 20:4(n-6) are required in sufficient quantities for an increased production of eicosanoids with a consequence of greater response in ovulation. Since either vegetable or fish oil alone supplied in the D-III and D-IV diets respectively were not able to supply both these fatty acids in sufficient quantities (Tab. II), both of these oil supplementations in the broodstock diet were considered necessary for efficient breeding response as evidenced in D-V. However, the greater breeding response in the D-III groups than in D-IV might be due to the presence of high amounts of both 18:2(n-6) and 20:4(n-6) and conversion of 18:2(n-6) to 20:4(n-6) PUFA [23, 40, 41] which has a stimulatory effect on the ovarian follicles in steroid production [28].

There is a preferential transfer of (n-3) PUFA from the adipose tissue reserve of brood fish into the egg lipids particularly phosphatidylcholine [20]. It has been observed that phosphatidylcholine is preferentially used to provide metabolic energy [11], implying a relatively large catabolism of (n-3) PUFA. The specific role of (n-3) PUFA, especially 22:6(n-3), in maintaining the structural and functional integrity in cell membranes, specially in the neural cell, is well known [29]. Moreover, the presence of 22:6(n-3) phosphatidylethanolamine as a major molecular species in the fish brain and eye suggests its importance during the development of the larvae [3].

In comparison with a fish oil supplemented diet (D-IV), a vegetable oil supplemented diet (D-III) contains much less (n-3) PUFA; even certain fatty acids with higher homologues like 22:5(n-3), 24:5(n-3) and 22:6(n-3) are entirely lacking. Freshwater fish receive an appreciable amount of 18:3(n-3) through dietary intake of natural fish food organisms [35] which predominantly have this fatty acid. In addition they are generally capable of converting this fatty acid to the higher homologues [22, 23, 30, 36, 40–42] such as 20:5(n-3) and 22:6(n-3). However, it has been reported [8] that the rainbow trout cannot convert 18:3(n-3) to 22:6(n-3) at significant rates and may not be able to fulfil their higher requirement of 22:6(n-3), particularly during gonadal maturation and their incorporation into the lipid of egg yolk. So, probably due to insufficient dietary supply of these fatty acids with consequent incorporation into the egg lipid, spawn recovery was not influenced in the D-III groups as compared to the control (D-I). By contrast, a significant increase of spawn recovery in D-IV and D-V groups might be due to the presence of sufficient amounts of these fatty acids in these diets (D-IV and D-V) obtained through the addition of fish oil. The significantly higher spawn recovery in D-V groups as compared to D-IV groups (92% over D-IV) probably further indicates that vegetable oil supplementation (rich in n-6 PUFA) in the diet is also necessary along with the addition of n-3 PUFA enriched fish oil for higher larval survival of this species. So, these fatty acids must be supplemented in the diet to harvest quality spawn [2].

The pronounced combined effect of (n-6) and (n-3) PUFA as essential nutrients in the brood fish diet on the breeding performances of *Catla* in D-V support our previous findings [33] as well as those of Acharia et al. [1]. A fairly good balance of (n-6) and (n-3) PUFA is present in the mature unfertilised eggs of *Catla* [33]. This suggests that a

proper balance in the diet of both of these PUFA which are critical during organogenesis in embryos and larvae is required in the broodstock diet for optimum reproductive success of fish [5]. It is now known [4, 7] that diets with an over high ratio of n-6/n-3 PUFA could exaggerate stress response in fish broodstock leading to cardiac pathologies. The involvement of essential fatty acids in broodstock fish and developing eggs and larvae and their fundamental involvement in stress reactions demands consideration of what constitutes an optimal or even desirable dietary ratio of n-6/n-3 PUFA in broodstock. It was previously demonstrated [30] that a combination of n-3 and n-6 fatty acids is important for normal growth and survival of *Catla* fry with a 1:1 mixture of sunflower oil and cod liver oil. Essential fatty acid deficiency has been reported to affect fecundity and hatching success in rainbow trout [49]. Efficacies of two diets containing corn oil and fish oil for rainbow trout reared at 8° and 18 °C were compared [10] and it was shown that a dietary n-3/n-6 ratio affects egg fatty acid composition. It was also pointed out [5] that in addition to maintaining a proper ratio of DHA/EPA in fish diets, the consideration of what constitutes a desirable EPA/ARA ratio should be of equal importance when considering the formulation of broodstock diets. Diet formulation containing DHA/EPA ratios greater than 1 and EPA/ARA ratios smaller than 3 as well as the exclusion of 18:2(n-6) rich vegetable oils were found to be beneficial in terms of larval quality and survival of sea bass [5].

The latest maturity and a very low spawn recovery against a quite high fertilisation rate in the control groups (D-I) might be due to either a deficiency of (n-3) and (n-6) PUFA or an improper balance of these fatty acids in the diet and a consequent deficiency in the egg lipids.

The reason why almost all the matured brood fish, as observed from external-symptoms in the D-II groups, showed poor breeding performances including the release of immature eggs which were unable to imbibe water is still unclear. The genetic variability among females and non-optimal feeding conditions might have caused the variation. The extent to which the supplementation of the broodstock diet with vitamins could induce egg maturation and its release, needs to be studied. In wild *Catla* broodstock, common experience is that there is a tendency for large amounts of fat to be deposited in association with the gonadal tissue leading to poor gonadal maturation and partial or even total failure of breeding response [21]. This, however remains to be investigated. In addition the interaction between vitamins and oil on breeding performances of fish also require comprehensive research investigation.

The breeding performances of the female broodstock depend on the levels and activity of the reproductive hormones, body energy status and final stage of egg maturity which can be accelerated in IMC through various ways like increased day length, photoperiod and temperature [13, 39, 45]; by administration of external hormone preparation [9, 38] and improved brood fish management practices [17]. However, the acceleration of gonadal maturation and obtaining quality fish spawn in sufficient quantities in *Catla* through dietary manipulation of both (n-3) and (n-6) PUFA by supplementing fish oil and vegetable oil respectively may be a very effective and easily practicable technique.

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