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European Journal of Experimental Biology, 2014, 4(1):117-124

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Study on the effects of different dietary fats on ovarian tissue of striped murrel (*Channa striatus*)

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ABSTRACT

Different fats in the diets were used to examine their impact on the ovarian tissues of striped murrel (Channa striatus). The juveniles of the test fish were acclimatized on the diet prepared from the same feed ingredients as that of experimental diets excepting different fat sources used in the experiment. The fishes were subsequently fed with seven experimental diets (F1, F2, F3, F4, F5, F6 and a control, F7 of natural foodstuffs, NATFO). F1 (L3HUF) contained 0.5% n-3 fatty acid& 7.5% saturated oil; F2 (H3HUF) 1.0% n-3 fatty acid& 7.0% saturated oil; F3 (MUSOL) 8.0% mustard oil; F4 (LINOL) 8.0% linseed oil; F5 (MIXOL) 4.0% mustard oil and 4.0 % linseed oil; F6 (SATOL) 8.0% saturated oil. Ovaries of 3 fishes from each treatment were excised and processed for routine histological evaluation. The cellular changes in ovarian tissues, following dietary fat incorporation, were assessed under light microscopy. The ovaries of all the fishes fed different experimental diets had more or less similar architecture of the ovarian follicles which indicated that the addition of 8% fats of these ingredients was unharmful to the fish. However, there was significant difference in the fecundity condition of the fish with these diets. The highest fecundity was observed with F-2 followed by F-1, F-4, F-3, F-5, F-7 and F-6. It was concluded that addition of various experimental fats has a significant positive development in the ovarian tissues in this species and linseed oil and mixed oil could be safely used for better and/ or higher follicular development and fecundity.

Key words: Dietary fat, ovary, histology, *Channa striatus*, Saul

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INTRODUCTION

The striped murrel, *Channa striatus*, is a highly priced freshwater fish of the Indian sub-continent and south-east Asia. They are in great demand as food fish due to their good flavour [1], few spines, medicinal importance [2–6] and air-breathing nature [4] that facilitate high density culture and easy transportation in live state to the retail markets. Murrel adapts well to low oxygen condition and hence can be cultured in high stocking densities. The culture of *C. striatus* is widely popular in Thailand and on limited scale in India, Philippines and Taiwan [4, 7-9] due

to non-availability of seed. The striped murrel breeds in ponds and rivers a little prior to or with the onset of monsoons [7, 10, 12] and their spawning season extends to the last monsoons [13]. It is a batchs pawner and breeds two or three times in a season, the breeding season extends from February–March to October–November and lay floating egg in a nest made up of leafy vegetables [13]. Both parents guard the eggs and fry [11]. Parmeshwaran and Murugesan [14] attempted captive breeding of striped murrel in India when they injected carppituitary glands to both male and female with varying success. Marimuthu *et al*. [15-17] successfully carried out spawning of striped murrel in a net enclosure fixed in a pond and in cement tanks using Ovatide (sGnRH).

The maturation in this fish starts with the development of ovarian follicle as an aggregate of ova and epithelial cells. These follicles start as oogonia, or mother cells, which are regularly generated in the germinal epithelium. The oogonia are composed of cells which forms and produce oocytes. The epithelial cells grow as the ovum grows and are distinctly detached from each other by a gradually thickening hyaline capsule. These are main source for nourishing the ovum and secreting the yolk. In many species, several generations of ovum may be recorded in different developmental stages at a time.

The basic information on dietary requirements of nutrient components such as protein and energy is a prerequisite for the formulation of balanced diet for the fishes particularly during maturation season. The carnivorous fishes particularly diets rich in proteins and lipids at the time of attaining maturity which need to be met out from artificial diets when cultured in captive conditions. India has huge potential for the production of cheaper plant sources e.g. de-oiled cakes like linseed oil cake etc. (rich in essential fatty acid,, omega-3 HUFA) which can be utilized as source of lipid in carnivorous fish nutrition not only for growth purposes but also for gonadal maturation through dietary manipulations. Recycling of agro-based by-products, like mustard oil cake, linseed oil cake etc. can be used in place of animal oils as source of lipid and EFA. Thus, the fatty acid composition of these plant origin are good source of HUFA which can be utilized for carnivore fishes nutrition as a supplement. in place of animal lipid source.. Sarowar *et al*. [18] have studied the impacts of different diets on growth and survival of *C. striatus* grow-outs. Influence of dietary lipid/protein ratio requirement has been studied in *C. striatus* [19]. The present study was taken up to evaluate the impact of dietary lipids on the ovarian growth by utilizing various agro-based dietary lipid ingredients by the striped murrel, *C. striatus*. The parameter for study were the estimation of fecundity, maturation condition of ovary and histology.

MATERIALS AND METHODS

Procurement of Test Fish

Wild striped murrels, *C. striatus* (Avg. weight 388–407 g, Photo-1) were collected from different sites of Unnao and Barabanki districts of Uttar Pradesh, India and were stocked in ponds at NBFGR farm facility 3 months prior to experiments. They were fed with laboratory made feed(Table-1) *ad libitum* during acclimation period.

Photo-1 Brood fish of *Channa striatus*

Feed Preparation and Feeding

Six type of feeds were formulated having similar feed ingredients in same quantities as given in Table-1 excepting different source of fat namely low level of highly unsaturated fatty acid (L3HUF, F1); high level of highly unsaturated fatty acid (H3HUF, F2); mustard oil (MUSOL, F3); linseed oil (LINOL, F4); mixed oil (MIXOL, F5); saturated fat (SATOL, F6) and a control (NATFO, F7) comprising of natural food stuffs (Table 2). F1, contained 0.5% n-3 fatty acid and 7.5% saturated oil; F2, 1.0% n-3 fatty acid and 7.0% saturated oil; F3, 8.0% mustard oil; F4,

8.0% linseed oil; F5, 4.0% mustard oil and 4% linseed oil; F6, 8% saturated oils. (Table-2). In order to evaluate the effect of different oil sources on the ovary of *C. striatus*, the experiment was conducted in indoor condition in 14 (7 types of feed, 2 replicates) rectangular plastic pools of 1200litre capacity, each filled-up with 800 litre borewell water stocked with 10 juveniles having an initial average weight 273.6±90g to 325.4±41g. The tanks were provided aeration from a portable aerator round the clock. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours *ad libitum* per day. Rearing tanks were cleaned every second day and about half of the water was replaced with fresh bore-well water to reduce the nitrogenous waste accumulated as debris and faecal matters.

Table-1 Feed composition of *Channa striatus*broodstock during acclimatization

Sr. No.	Ingredients	%	Approx. Ratio (w/w)
1	Goat intestine	66	33
2	Wheat flour	24	12
3	Soybean meal	8	4
4	Vitamin + Mineral Mix[a]	2	1
	Composition		
	Protein	39.01	
	Carbohydrate	23.22	
	Fat	12.10	
	Ash	11.23	
	Fibre	4.55	
	Gross Energy (K.cal/100g)	353.78	

[a]vitamin and mineral composition (per 100 g): vitamin A 70,000 IU, vitamin D3 7,000 IU, vitamin E 25 mg, nicotinamide 100 mg, cobalt15 mg, copper 120 mg, iodine 32.5 mg, iron 150 mg, magnesium600 mg, manganese 150 mg, potassium 10 mg, selenium 1 mg, sodium 0.59 mg, sulphur (%) 0.72, zinc 96 mg, calcium (%) 25.50,phosphorus (%) 12.5. From Agrivet Farm Care Division, Glaxo-SmithKline Pharmaceuticals Limited (Mfg. by Sunder chemicals Pvt.Ltd., Chennai)

Histological study

After 12-weeks of feeding trials with seven feed combinations (Table-2), the fishes were sacrificed. The ovary from control(F7,NATFO) and experimental fishes (feed with different fats (F1 to F6) were excised and fixed in 4 % formaldehyde and processed by standard histological techniques ([20] i.e., kept in aqueous Bouin's fluid for 24-hr and washed for 8-hr in running tap water. The organs were routinely processed (dehydrated in ethanol series, embedded in paraffin, serially sectioned at 6µ). Sections of the ovary tissue were stained with Haematoxylin and Eosin (HE). Histological slides were observed under microscope (Labomed, Model: Digi 2) for assessment of the maturity condition.

Table: 2 Ingredients composition (w/w) of feeds for *Channa striatus.*

Feed Ingredients	F-1 (L3HUF)	F-2 (H3HUF)	F-3 (MUSOL)	F-4 (LINOL)	F-5 (MIXOL)	F-6 (SATOL)	F-7 (NATFO)
Soybean meal	41.0	41.0	41.0	41.0	41.0	41.0	-
Starch Soluble	25.0	25.0	25.0	25.0	25.0	25.0	-
Casein	20.0	20.0	20.0	20.0	20.0	20.0	-
Carboxy Methyl Cellulose	2.0	2.0	2.0	2.0	2.0	2.0	-
Papain	0.5	0.5	0.5	0.5	0.5	0.5	-
Vitamin & Mineral Mix.	3.5	3.5	3.5	3.5	3.5	3.5	-
Omega – 3 HUFA	0.5	1.0	-	-	-	-	-
Saturated Oil	7.5	7.0	-	-	-	8.0	-
Mustard Oil	-	-	8.0	-	4.0	-	-
Linseed Oil	-	-	-	8.0	4.0	-	-
Live Fish/ NATFO	-	-	-	-	-	-	100.0

L3HUF = Low Omega – 3 HUFA; H3HUF = High Omega – 3 HUFA; MUSOL = Mustard Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Mustard Oil : Linseed Oil :: 1 : 1 w/w) ; SATOL = Saturated Oil; NATFO = Natural Food

RESULTS AND DISCUSSION

The experimental feeds had an impact on ovary condition and the fecundity of *C. striatus*. The size of ovary with different types of feeds and the fecundity is given in Fig1-7 and Table-3. The fecundity was recorded as 53796±3452 with F-1, 66576±2756 with F-2, 10893±4531 with F-3, 52497±2587 with F-4, 9440±5276 with F-5, 4720±2329 with F-6 and 7703±2368 with F-7 . The H/E sections of ovary of control fish fed with natural feedstuffs (NATFO, F7) showed normal architecture of ovary tissue with normal follicles and granulation (Fig 8). F-1, fed fishes showing normal follicles with granulation (Fig 9); F-2, showing normal follicles with dense granulation(Fig

10); F-3, fed fishes showing normal follicles with granulation and spacing between follicles(Fig 11); F-4, showing huge numbers of follicles with granulation(Fig 12); F-5, showing huge numbers of follicles with granulation and different stages of ovary(Fig 13) and F-6, fed fishes showing huge numbers of follicles with granulation and spaces(Fig 14). Ovarian tissues of C. *striatus* fed with natural food (NATFO, F7) showing normal follicles. The results exhibited comparatively massive changes in the ovary tissue by having larger number of follicles with granulation and with linseed oil and mixed oil enriched feeds. The results also exhibited increased granulation and numbers of follicles and better effects towards fecundity, granulation etc., after addition of various dietary fats in the feed in comparison to control.

In all the treatments, the most of the cellular features were the presence of well-organized architecture of the ovary and cellular structures which indicated that all types of oils tested in the study were not harmful rather beneficial to striped murrel, *C. striatus* at 8% supplementation. Hence these fat ingredients may be used either alone or in combination in the diet of this species which did not create significant changes in ovary structure but also immensely improved fecundity.

Table-3 Fecundity of *Channa striatus* on feeding different dietary fats

S.N.	Feed	Fecundity (per kg body weight)
1	F-1 (L3HUF)	53796±3452
2	F-2 (H3HUF)	66576±2756
3	F-3 (MUSOL)	10893±4531
4	F-4 (LINOL)	52497±2587
5	F-5 (MIXOL)	9440±5276
6	F-6 (SATOL)	4720±2329
7	F-7 (NATFO)	7703±2368

Figure-8 Ovary of *C. striatus* fed with Natural feed (NATFO, F7, control) showing normal follicles(H/E 40X)
Figure-9 Ovary of *C. striatus* fed with low unsaturated fatty acid (L3HUF) (F1) showing normal follicles with granulation (H/E 40X)
Figure-10 Ovary of *C. striatus* fed with high unsaturated fatty acid (H3HUF) (F2) showing normal follicles with dense granulation (H/E 40X)
Figure-11 Ovary of *C. striatus* fed fishes with F-3 (MUSOL) showing normal follicles with granulation and spacing between follicles (H/E 40X)
Figure-12 Ovary of *C. striatus* fed with linseed oil (LINOL, F4) showing huge nos. of follicles with granulation (H/E 40X)
Figure-13 Ovary of *C. striatus* fed with mixed oil (MIXOL, F5) showing huge nos. of follicles with granulation and different stages of ovary (H/E 40X)
Figure-14 Ovary of *C. striatus* fed with saturated oil (SATOL, F6) F-6, showing huge nos. of follicles with granulation and spaces (H/E 40X)

A few workers have attempted captive breeding of striped murrels with varying success. Alikunhi [11] and Parameshwaran and Murugesan [14] attempted breeding of this species by using pituitary gland. Haniffa *et al.* [21], Selvaraj and Francis [22] induced bred this fish using sGnRH and HCG respectively. Haniffa *et al.* [23]also made a comparative study on induced breeding of this fish using pituitary gland, HCG, LHRH-a & Pimozide andovaprim. Marimuthu *et al.* [15] used Ovatide for breeding in hapa inside the pond. In teleosts, ovarian development and the ultimate production of mature eggs is a highly complex process, timed and modulated by various environmental and endocrine pathways such that young's are produced only at times when fry survival is optimal – usually when food availability is at its highest. Ovarian development in teleosts is broadly divided into seven distinct developmental stages based upon the biochemical properties and histological morphology of the nucleus, cytoplasm and follicular layer: oogonial proliferation, oogenesis, folliculogenesis, cortical alveolar formation, vitellogenesis, final maturation and ovulation [24,25]. Even within an individual oocyte however, there are likely to be periods in which these distinct phases overlap [26].Many authors observed the effect of pesticide on ovary. Also, Kumar and Pant [27] found that 2-4 month exposure of an Indian teleost to lead caused disappearance of oocytes in the ovaries. In addition, Magar and Bias [28] investigated histopathological effects of sublethal concentration of insecticide which widely used in agriculture in the ovary of fresh water teleost, *Channa punctatus*. They observed complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shapes.

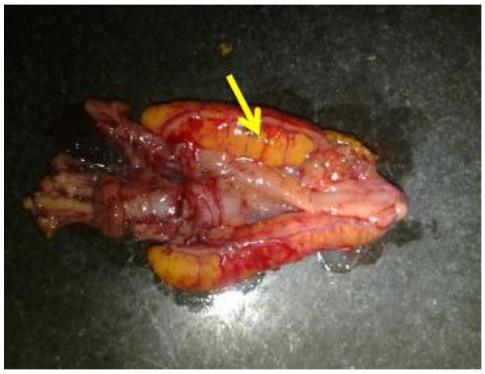

Fig.-1 Control Ovary of *C. striatus* fed with natural food (F7).

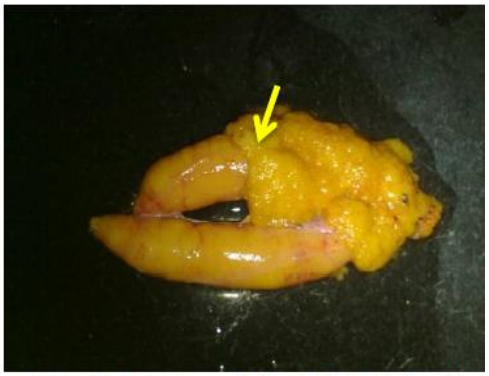

Fig.-2 Ovary of *C. striatus* fed with L3HUF (F-1).

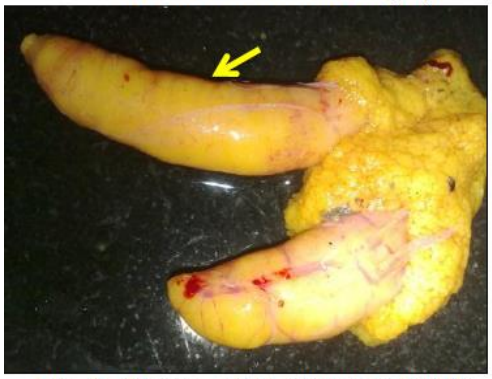

Fig.-3 Ovary of *C. striatus* fed with H3HUF (F-2).

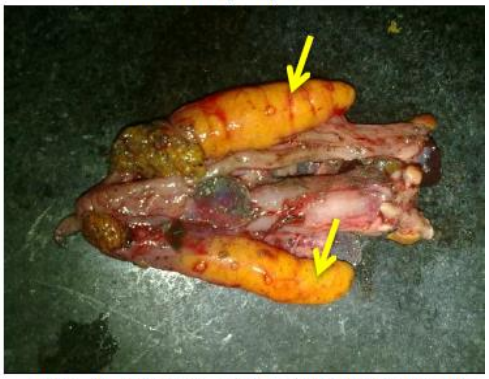

Fig.-4 Ovary of *C. striatus* fed with MUSOL (F-3).

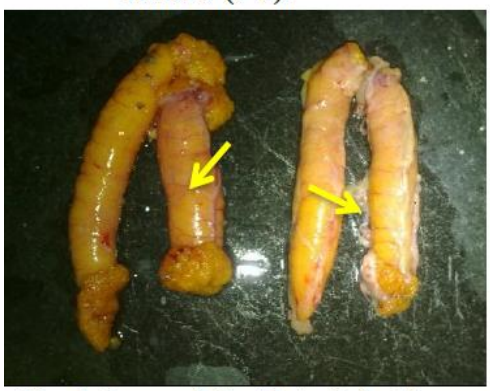

Fig.-5 Ovary of *C. striatus* fed with LINOL (F4).

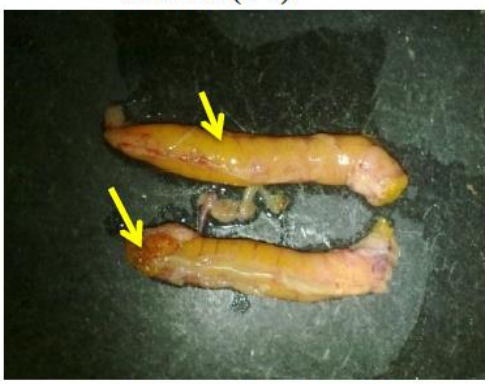

Fig.-6 Ovary of *C. striatus* fed with MIXOL (F-5).

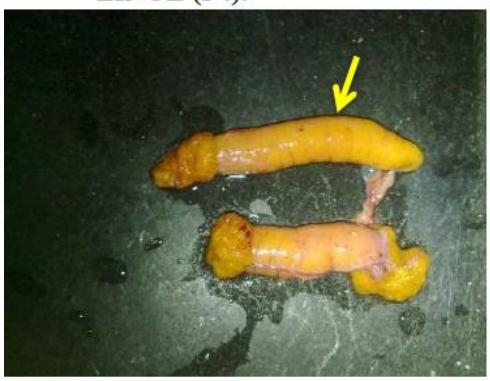

Fig.-7 Ovary of *C. striatus* fed with SATOL (F-6).

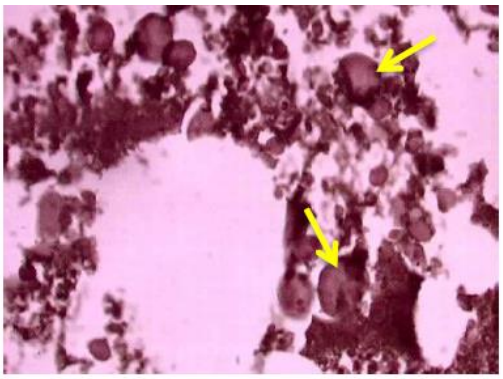

Fig.-8 Control Ovary histology of *C. striatus* fed with NATFO (F7).

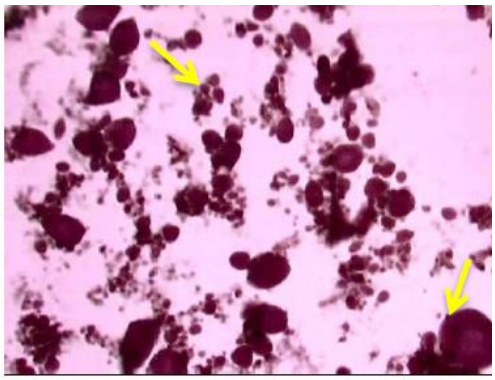

Fig.-9 Ovary histology of *C. striatus* fed with L3HUF (F-1).

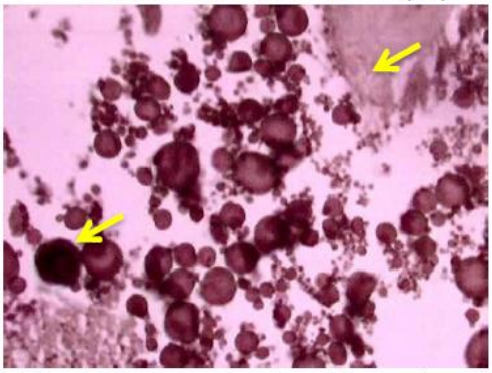

Fig.-10 Ovary histology of *C. striatus* fed with H3HUF (F-2).

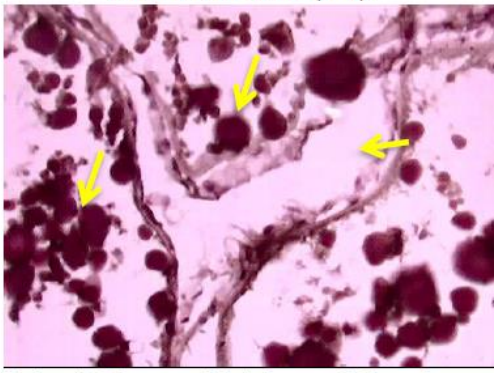

Fig.-11 Ovary histology of *C. striatus* fed with MUSOL (F-3).

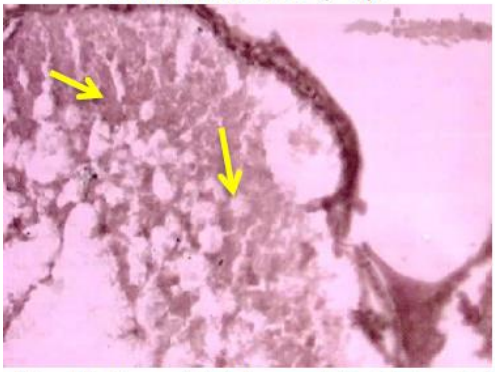

Fig.-12 Ovary histology of *C. striatus* fed with LINOL (F4).

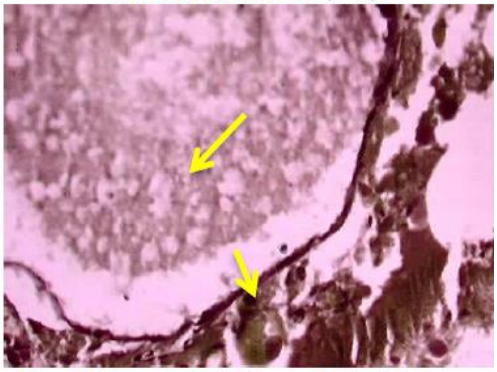

Fig.-13 Ovary histology of *C. striatus* Fed with MIXOL (F-5).

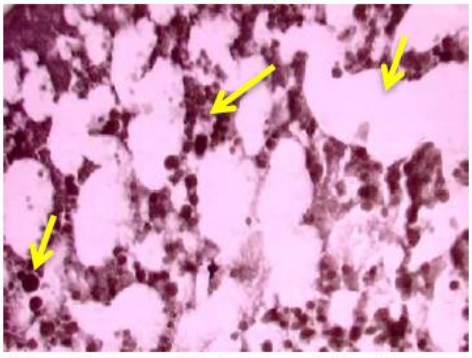

Fig.-14 Ovary histology of *C. striatus* fed with SATOL (F-6).

Dayal and co-workers [29] have reported influence of different sources of dietary lipid on the growth, feed efficiency and survival of snakehead *C.striatus* grow-out. The ovarian morphology in the present study resembles basically that described in the bass, *Ecetrarcuslabrax* [30] and that of bullhead catfish; *Ictalorusnebulosus*[31]. A characteristic Balbiani's body as that described by Beams and Kessel[32] and Mayer *etal*.[30] was clearly demonstrated in medium-sized follicles. Exogenous factors known to play regulatory roles in the reproductive physiology of tilapia include light intensity [33-36], water temperature [37-39], lunar cycles [40], maternal size and age[41-43], food ration size [44-46],dietary protein level [47-53] and dietary lipid level [54,55]. In other teleports that spawn pelagic eggs, especially marine perciformes, full-grown oocytes contain oil droplets which occupy up to half or more of ooplasm volume [56. These oil droplets coalesce into one or two large oil globules during maturation (see later discussion). In contrast with the Vg-associated lipids, oil globules contain mainly neutral lipids such as triglycerides and wax or steryl esters. These neutral lipids are rich in monoun saturated fatty acids that, in fishes, preferentially serve as metabolic energy reserves[57]. Thus, it appears that vitellogenin transports mainly structural lipids and essential fatty acids into growing oocytes to support embryo growth, whereas neutral lipids are taken up by other means and stored as isolated oil inclusions for meeting the energy demands during embryogenesis. In overall, this study showed that *C. striatus* performed optimally on diets containing LINOL, MUSOL, H3HUF and MIXOL with respect to gonadal maturation. It can be deduced that out of six types of fats used in the present study, the essential fatty acid (H3HUF, F2) is considered best as a feed substitute in the artificial diets. The other fats have shown very mild to moderate level of changes in the ovarian tissue at 8% addition in the diet in a 12-week trial. Therefore, these fats may be used in combination with other fats to cut down the feed price and without effecting the survival and growth of *C. striatus*. Therefore, it is recommended that various fats used in the present study in to the diet of *C. striatus* could be used for improved growth performance and better nutrient utilization in the development of ovarian tissue. However, further study aimed at increasing the concentration of the long chain polyunsaturated fatty acid in the fillet.

CONCLUSION

The observations, in the present study, suggest that manipulation with different dietary fat sources in the feed has direct relation with fecundity and cellular level modifications in the ovary of *C. striatus* brood fish.

Acknowledgements
Authors are highly grateful to the Director, NBFGR, Lucknow for providing facilities to conduct this research work.

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