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Effects of various dietary fats on the cellular structure of ovary in threatened Asian catfish (*Clarias batrachus*, Linnaeus, 1758)

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ABSTRACT

Various fats in the diets were mixed to assess their impact on the cellular structures of ovarian tissues of threatened Asian catfish (Clarias batrachus). Six treatments diets (FISOL, BETAL, SOYAL, LINOL, MIXOL, SATOL and NATFO) with 10% dietary fat containing Fish oil, Tallow, Soybean oil, Linseed oil, Mixed oil (i.e. containing in 1:1:1:1 ratio of Fish oil, Tallow, Soybean oil, Linseed oil), Vegetable oil and minced chicken meat as natural food were used as F1,F2,F3,F4,F5,F6 and F7 respectively, each having three replications, and stocked @ 30 fish with initial av. wt. 55.83 ± 3.14g in plastic pools(300 l cap.).The fishes were fed vigorously for 12-weeks with formulated diets and after the termination of experiment, the ovaries of 3 fishes from each treatment were excised and processed for routine histological assessment in ovarian tissues under light microscopy. The ovaries of all the fishes fed different experimental diets had almost similar cellular architecture of the ovarian tissues except some more granulation and dense cytoplasm which indicated that the addition of 10% fats as these ingredients were not harmful to the fish. It was inferred that addition of various fats has a significant positive impact in the ovarian tissues in this species and linseed oil, soybean oil and mixed oil could be safely used for better and/ or better follicular growth and development of ovarian tissues.

Key words: Dietary fat, ovary, histology, *Clarias batrachus*, Asian catfish, threatened

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INTRODUCTION

The Asian catfish *(Clarias batrachus)* is a premium freshwater fish of the south-east Asia and Indian sub-continent as well. They are in very high demand as food fish due to their good flavor and medicinal values. The catfishes fishes diets are rich in proteins and lipids at the time of attaining maturity which need to be supplied through artificial diets when reared in captivity. India has big potential source for the production of cheaper and easily

available plant sources e.g. oil cakes etc. which are rich in essential fatty acids and can be used as a source of lipid in carnivorous fish diet not only for growth purposes but also for ovary development through inclusion of these rich ingredients in the diet. Agro-based by-products, like oil cakes etc. can be used as source of lipid and EFA. Thus, composition of plant origin fatty acids, as good source of HUFA, can be used for carnivore fishes nutrition as a replacement of animal lipid source. In many studies on dietary supplementation fat in feed had been reported on changes in various tissues of *Clarias batrachus* like liver [1], kidney[2], intestine [3]. Similarly, the cellular changes in various organs like liver [4], kidney [5], intestine [6], gill[7], in *Channa striatus*. On feeding various lipids in the diet the fatty acid profile of *Channa striatus*[8] and *Clarias batrachus*[9] had been reported. The growth performances, survival on adding the various lipid in *Channa striatus* grow-out and fingerling [10,11] and *Clarias batrachus*[12] also been well reported. Srivastava *et al*. [13] had on the impact of live and artificial feed on Breeding and Larval Rearing of Asian Catfish, *Clarias batrachus*.

The development of ovarian tissues and maturation in this cat fish initiate with the development of ovarian follicles as an aggregate of epithelial cells and ova. These follicles start as mother cells or, oogonia, which are generated in the germinal epithelium wall. The epithelial cells also grow as the ovum grows and are detached from each other by a thickening of hyaline capsule. These are main source for feeding/ nourishing the ovum and secreting the yolk to ova. In many other species, several stages of ovum may be exhibited in different stages of development at the same time. The basic data on dietary requirements of nutrient components such as protein etc. is a prerequisite requirement for the feed formulation of balanced feed for the fish especially during pre-breeding season. Saroware*t al*. [14] and have reported the effects of different diets on growth and survival of *C. striatus*. Influence of dietary lipid/protein ratio requirement has been reported in *C. striatus* [15]. Dayal *et al*.[16] have reported the impact of dietary fats on the development of ovarian tissues of *Channa striatus*. 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 Asian catfish (*Clarias batrachus*).Although time-consuming and expensive, histology is considered the most precise technique, and is a powerful technique for the evaluation of fish maturity stages [17,18].The parameter for study was the estimation of changes in histology of ovary.

MATERIALS AND METHODS

Feed Preparation and Feeding

Six treatments diets with 10% dietary fat (FISOL, BETAL, SOYAL, LINOL, MIXOL, SATOL and NATFO) containing Fish oil, Tallow, Soybean oil, Linseed oil, Mixed oil (i.e. containing in 1:1:1:1 ratio of Fish oil, tallow, Soybean oil, Linseed oil), Vegetable oil and minced chicken meat as natural food were used as F1, F2, F3, F4, F5, F6 and F7 respectively (Table-1), each having three replications, and stocking @ 20 fish per pool.

Fish rearing

Clarias batrachus (av. weight 55.83 $\pm$ 3.14 g) were hatchery bred at National Bureau of Fish Genetic Resources (NBFGR), Lucknow and reared in the wet laboratory. The fishes were acclimated to laboratory conditions in a 1500 L capacity Fibre Reinforced Plastic (FRP) tank, feeding on crushed/crumbled pelleted feed containing a minimum of 500 g per kg crude protein for one week. Further, fishes were accustomed to aerated, 300 L capacity plastic pools with two - thirds filled with bore well water and covered with plastic covers. Four hundred twenty (Replicate 3 X Feed 7 X Fish 30) fishes were randomly sampled and distributed into twenty-one plastic pools containing about 200 L of water. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours *ad libitum*.

Histological study

After 12-weeks of feeding trials with seven feed combinations (Table-1), 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 ([19] 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: 1 Ingredients composition (w/w) of feeds for *Clarias batrachus*

Feed / Ingredients	F-1 FISOL	F-2 BETAL	F-3 SOYOL	F-4 LINOL	F-5 MIXOL	F-6 SATOL	F-7 NATFO
Soybean meal	35.0	35.0	35.0	35.0	35.0	35.0	-
Starch Soluble	29.0	29.0	29.0	29.0	29.0	29.0	-
Casein	19.5	19.5	19.5	19.5	19.5	19.5	-
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.	4.0	4.0	4.0	4.0	4.0	4.0	-
Fish Oil	10.0	-	-	-	2.5	-	-
Tallow	-	10.0	-	-	2.5	-	-
Soybean Oil	-	-	10.0	-	2.5	-	
Linseed Oil	-	-	-	10.0	2.5	-	-
Saturated Oil	-	-	-	-	-	10.0	-
Live Fish/ Natural Food(minced chicken meat)	-	-	-	-	-	-	100.0

FISOL = Fish Oil; BETAL = Tallow; SOYOL = Soybean Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Fish Oil : Tallow : Soybean Oil : Linseed Oil :: 1 : 1 :1 :1 w/w) ; SATOL = Saturated Oil; NATFO = Natural Food

vitamin and mineral composition (per 100 g): vitamin A 70,000 IU, vitamin D3 7,000 IU, vitamin E 25 mg, nicotinamide 100 mg, cobalt 15 mg, copper 120 mg, iodine 32.5 mg, iron 150 mg, magnesium 600 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).

RESULTS AND DISCUSSION

The feeds tested in this experimentation didn't have negative impact on survival, growth and condition on *C. batrachus*. The experimental feeds had an impact on ovary condition. The H/E sections of ovary of control fish fed with natural feedstuffs (NATFO, F7) showed normal architecture of ovarian tissue with normal follicles and granulation in cells (Fig 1). F-1, fed fishes showing follicles with normal granulation (Fig 2,3); F-2, showing normal follicles with more dense granulation(Fig 4,5); F-3, fed fishes showing normal follicles with normal granulation and spaces between the follicles(Fig 6,7); F-4, showing more numbers of follicles with normal and optimized level of granulation(Fig 8,9); F-5, showing improvised cellular structure and increased numbers of follicles with granulation and different stages of ovarian tissue(Fig 10,11) and F-6, fed fishes showing increased numbers of follicles with spaces and dense granulation (Fig 12,13). Ovarian tissues of *C. batrachus* fed with natural food (NATFO, F7) showing normal follicles and cellular structure. The results exhibited comparatively more changes in the ovarian tissue by increasing larger numbers of follicles with increased granulation when fishes fed with linseed oil and mixed oil incorporated feeds. The results also exhibited more and dense granulation of follicles when added with various fats in the feed and the changes were more pronounced than in control.

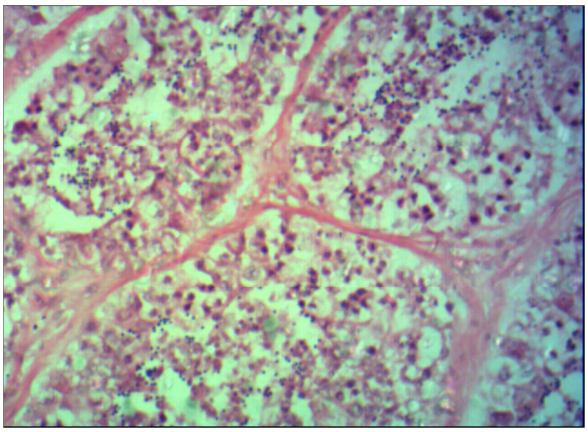

Fig-1. Control Ovary histology of *C. batrachus* (F7) 40X (H/E)

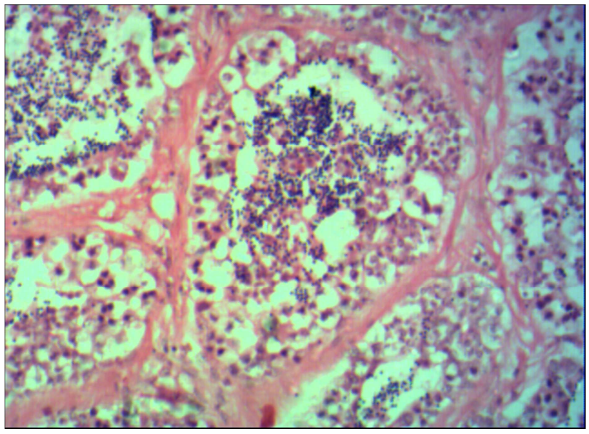

Fig-2. Ovary histology of *C. batrachus* fed with FISOL(F1) 40X (H/E)

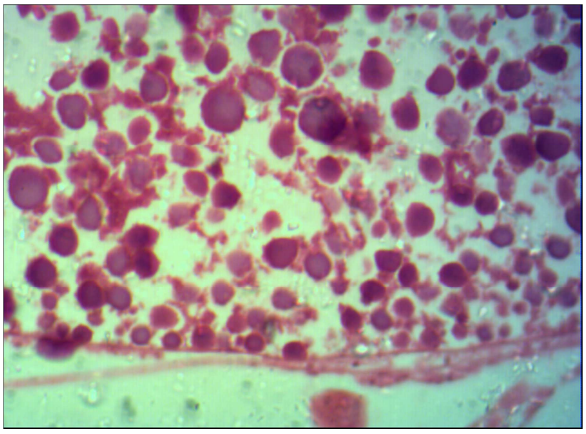

Fig-3 Ovary histology of *C. batrachus* fed with FISOL(F1) 100X (H/E)

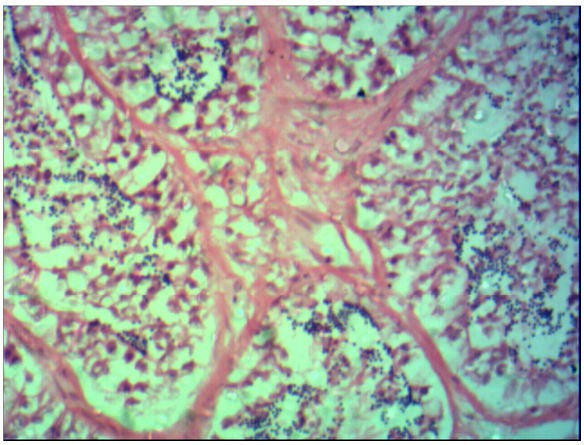

Fig-4. Ovary histology of *C. batrachus* fed with BETAL(F2) 40X (H/E)

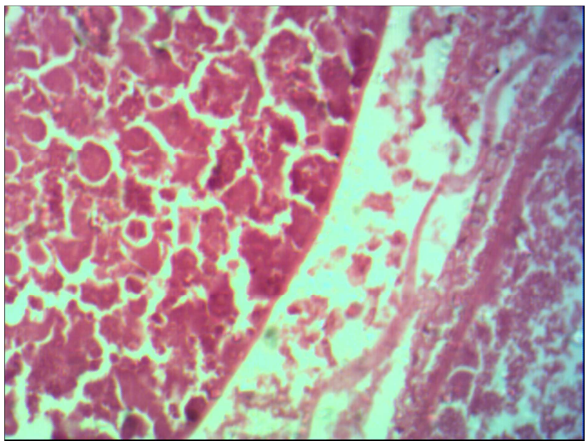

Fig-5 Ovary histology of *C. batrachus* fed with BETAL(F2)100X (H/E)

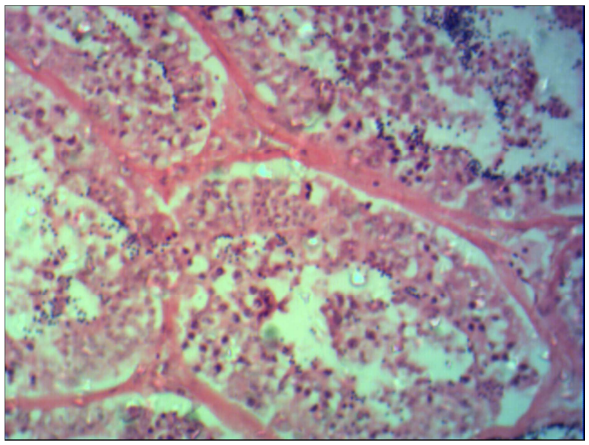

Fig-6. Ovary histology of *C. batrachus* fed with SOYAL(F3) 40X (H/E)

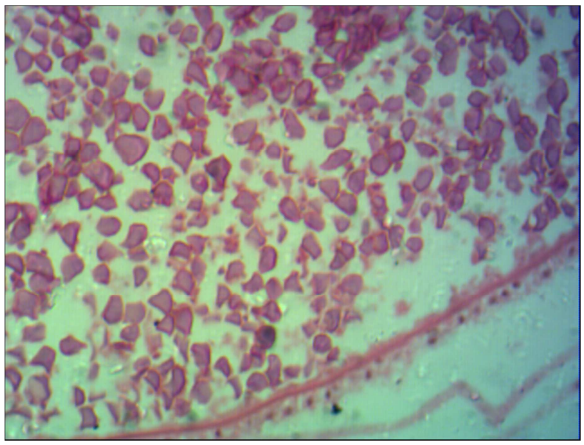

Fig-7 Ovary histology of *C. batrachus* fed with SOYAL(F3)100X (H/E)

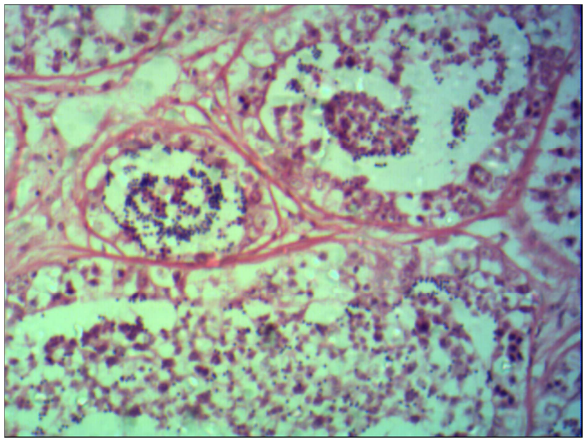

Fig-8. Ovary histology of *C. batrachus* fed with LINOL (F4) 40X (H/E)

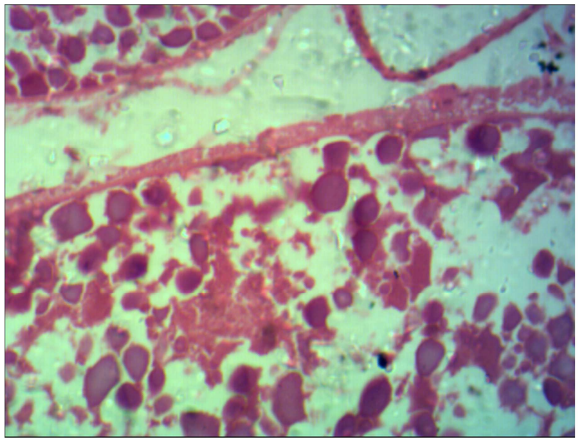

Fig-9. Ovary histology of *C. batrachus* fed with LINOL(F4)100X (H/E)

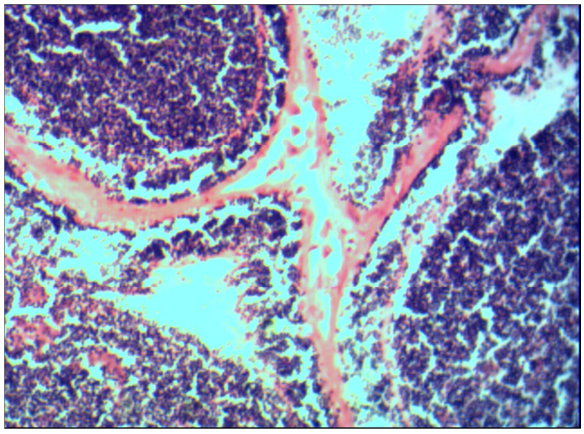

Fig-10. Ovary histology of *C. batrachus* fed with MIXOL(F5) 40X (H/E)

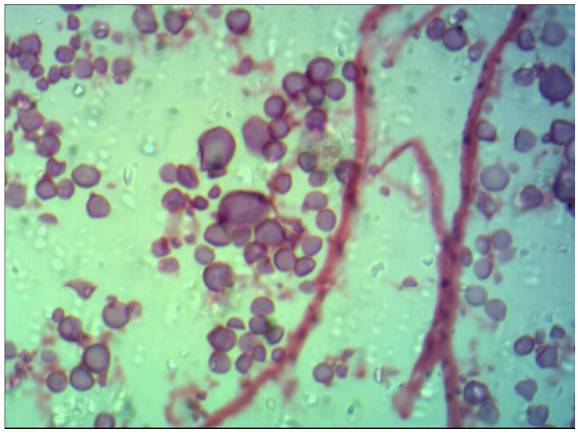

Fig-11Ovary histology of *C. batrachus* fed with MIXOL(F5)100X(H/E)

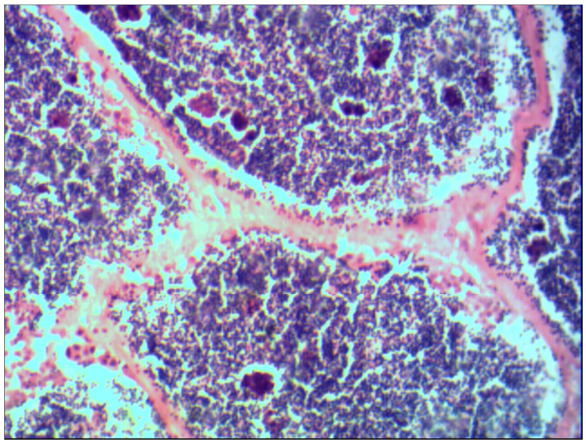

Fig-12. Ovary histology of *C. batrachus* fed with SATOL(F6) 40X (H/E)

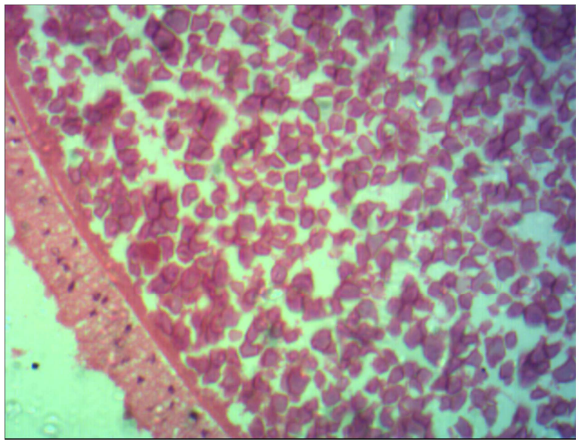

Fig-13.Ovary histology of *C. batrachus* fed with SATOL(F6) 100X(H/E)

Figure-1 Ovary of *C. batrachus* fed with Natural feed (NATFO, F7, control) showed normal architecture of ovarian tissue with normal follicles and granulation in cells(H/E 40X).

Figure-2 & 3 Ovary of *C. batrachus* fed with fish oil (FESOL) (F1) showing normal follicles with granulation (H/E 40X and 100X)

Figure-4 & 5 Ovary of *C. batrachus* fed fishes with tallow (BETAL, F-2) showing normal follicles with more dense granulation(H/E 40X and 100X).

Figure-6 & 7 Ovary of *C. batrachus* fed with soybean oil (SOYAL, F3) fed fishes showing normal follicles with normal granulation and spaces between the follicles(H/E 40X and 100X).

Figure-8 & 9 Ovary of *C. batrachus* fed with Linseed oil (LINOL, F4) showing more numbers of follicles with normal and optimized level of granulation(H/E 40X and 100X).

Figure-10 & 11 Ovary of *C. batrachus* fed with mixed (MIXOL, F5) showing improvised cellular structure and increased numbers of follicles with granulation and different stages of ovarian tissue(H/E 40X and 100X).

Figure-12& 13 Ovary of *C. Batrachus* fed with saturated oil (SATOL, F6) showing increased numbers of follicles with spaces and dense granulation(H/E 40X and 100X).

In all the feeding trials, the cellular features were showing well-organized cellular structures of the ovarian tissues which exhibited that different fats used in this research study were not showing deleterious effects whereas, showing better and/ or rather positive to striped catfish, *Clarias batrachus* fed at 10%inclusion. Hence, these various dietary fat ingredients can be used either in combination or individually in the fish diet of this fish which do not exhibit accountable changes in ovarian tissue however, considerably improved granulations in the tissue.

Ovarian development in fish is classified into seven distinct developmental stages based upon the histological morphology and biochemical properties and follicle layer [20, 21, 22]. Kumar and Pant [23] reported that an Indian teleost showed reduction of oocyes in the ovaries on exposure to external factors. They recorded total loss of normal ovary, necrosis, enlarged ovarian follicles, and disrupted ova with abnormal structures. Dayal and co-workers [10, 11, 16] have reported influence of different sources of dietary lipid on the growth, feed efficiency and survival of snakehead *C. striatus* and reproductive performances, including cellular changes in ovary of grow-out. The ovarian morphology in the present study looks alike basically that exhibited in the bass, *Ecetrarcuslabrax* [24], bullhead catfish; *Ictalurusne bulosus*[25]. Beams and Kessel[26] and Mayer *etal*.[24] demonstrated in medium follicles. Exogenous influence are well known to play more regulatory roles in the reproductive performances and physiology of tilapia include intensity of light [27-30], ambient water thermal condition [31-33],lunar cycles [34], maternal body and their age[35-37], food size [38-40],dietary protein inclusion levels [41-47] and lipid levels [48, 49]. In many other teleosts full-grown oocytes contain oil [50]. These oil droplets coalesce into one or two large oil during maturation. Such neutral lipids are rich in unsaturated fatty acids that, in fishes, preferentially work for metabolic energy stores[51]. Thus, it is exhibited that vitellogenin transports mainly lipids and essential fatty acids into growing oocytes to support growth, whereas neutral lipids are taken up by other stored oil for the supplementation for meeting the energy requirements during embryogenesis. In overall, this study showed that *C. batrachus* performed optimally on diets containing fats has a significant positive impact in the ovarian tissues in this species and linseed oil, soybean oil and mixed oil with respect to ovary maturation. It can be concluded that out of all the types of fats used in this research study, the linseed oil, soybean oil and mixed oil are considered best as a dietary substitution in the diets. The other fats have shown moderate level of changes in the ovarian component at 10% addition in the diet for 12-week feeding trial. Therefore, these fats can be used in mixed form with other fats to reduce the feed price without effecting the growth of *C. batrachus*. Therefore, it is recommended that various fats used in the present study in to the diet of *C. striatus* could be used for enhanced growth and improved nutrient utilization for the better development of ovary. The results suggest that manipulation with different dietary fat sources in the feed has some direct relation with cellular changes in the ovary of *C. batrachus* brood fish. Further studies are required to assess and quantify the yolk and/or vitellogenin contents

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