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EFFECT OF ARTIFICIAL DIETS ON GROWTH PERFORMANCE, BODY COMPOSITION AND GONAD MATURATION OF MULLET (*LIZA RAMADA*)

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ABSTRACT: *This study was conducted to evaluate four different diets (fish oil FO, Palm oil PO, sunflower oil SO and mixed diet of three oils as 2% FO : 2%PO : 2%SO) on growth performance, body composition and gonad maturation of Liza ramada with an initial weight of 5.31±0.31g. Hundred fish were randomly distributed in twelve cement ponds with a volume of 2m^3 each and fed for 120 days at a rate of 3% live body weight (BW) twice daily. The results showed significant differences (P<0.05) between diets. The highest growth performance, feed utilization and hepatosomatic index were obtained with the fish fed fish oil (FO) and Mixed diets, without significance difference between them. However, the fish fed palm oil (PO) and sunflower oil (SO) recorded less growth performance. Differences in certain fatty acid composition were detected but levels of saturated, mono-saturated and n-3 fatty acids recorded increased in each FO and mixed diets without significant differences between them. On the other hand, n-6 showed significantly increased in both palm oil (PO) and sunflower oil (SO) groups. The essential fatty acids (EFAs): arachidonic acid (ARA), ecosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were different across treatment groups suggesting that Liza ramada can affected with different oil sources. In the second trial the mixed diet was fed to the broodstock fish in earthen ponds. The broodstock fish showed an increased in growth performance, feed efficiency, heptosomatic and gonadsomatic indices of Liza ramada. The implications of the results are discussed in terms of oil type effects, diet costs and gonad histology of Liza ramada broodstock.*

KEYWORDS: Liza Ramada, Broodstock, Fish Oil, Palm Oil , Sunflower Oil, Growth Performance, Feed Utilization, Body Composition, Fatty Acids, Gonad Histology.

INTRODUCTION

Marine fish were introduced to lake Qaroun, an agriculture waste water recipient at EL-Fayoum province, since the early 20s. Mullet species were the first marine fishes introduced there and no evidence on natural propagation of Muglidea family was observed. Consequently, mullet fry must be transplanted regularly to the lake to maintain the local stock. The operation of mullet fry transplantation to lake Qaroun was conducted on a commercial scale since the early 30s. Collection and transport of mullet species fry on a commercial scale was known and applied since more than 90 years to lake Qaroun to substitute the declining of Nile fish biomass in this lake. Mullet fry collection from different coastal regions of Mediterranean Sea and can be introduced directly to the lake or near shore pens and enclosures. This system was associated with great losses of fry and a true figure of released fingerlings cannot be obtained [1]. The total catch of the lake in 2014 was 441 tones and the Muglidea family represented by 1283 tones [2]. Mullet is an economically important euryhaline and eurythermal species contributing to sizable fisheries of estuarine and

coastal regions including Egypt [3]. Owing to omnivorous feed habit of grazing on plant detritus and microflora, it is an ecologically important species feeding at the lowest trophic level and suitable for mono or polyculture.

Liza ramada is an alternative species for farming in sea, brackish and fresh water [4]. It is considered as an excellent candidate for aquaculture because of the rapid growth rate, ability to efficiently utilize a wide range of natural and artificial foods, tolerance of wide range of environmental condition and resistance to disease and stresses. Thin lipped mullet, *L. ramada* is an economically important species of fish found in Egypt. However, its supply is almost dependent on the wild. Recently, number of wild thin lipped mullet has been gradually declining. All farming in Egypt is carried out using the fries which increase fears of a further decline in this resource and sharp rise in price. Therefore, the establishment of a method of artificial propagation for thin lipped mullet is needed to support the supply [5].

Fish oil has been the focus of much attention in human health studies due to its large amount of essential n-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition, fish oil is also the main source of lipid used in the formulation of commercial aquafeeds for marine fish species. However, high dependency on marine fish oil as a major lipid source in aquafeeds has raised major concerns of sustainability for aquaculture in the future due to limited supply of fish oil and expanding global nature of aquaculture production [6]. On the other hand, continuous increase of world vegetable oil production elevated the interest in inclusion of vegetable oils for aquafeeds to partially replace and reduce the dependency on fish oil. Many studies have successfully used vegetable oils such as soybean oil, rapeseed oil, sunflower oil, corn oil and palm oil as replacements of fish oil in aquafeeds. Palm oil (PMO), the largest production with the cheapest price in the world vegetable oil market, has been successfully used as a significant substitute of fish oil in diets for several fish species including red sea bream [6], red hybrid tilapia [7] and rainbow trout [8]. A number of recent studies suggest that, dietary vegetable oil (VO) inclusion does not result in reduced growth performance or feed conversion in Atlantic salmon *Salmo salar* [9], rainbow trout *Oncorhynchus mykiss* [10], gilthead sea bream *Sparus aurata* [11] and European sea bass *Dicentrarchus labrax* [12]. However, at levels above 50% VO inclusion, significant accumulation of fatty acids derived from VO especially 18:2n-6, reduction of EPA (20:5n-3) and DHA (22:6n-3) occurs in fish tissues [9,11,12,13]. Recently the benefits of increased EPA C20:5n-3 and DHA C22:6n-3 intake have been shown for a wide range of life style disorders with an associated inflammatory pathology as well as improving the symptoms of certain neurological disorders, they play a key role in the prevention and management of coronary heart diseases, hypertension, diabetes, pharmaceutics and production of feed for rearing fish species. Due to the proven benefits of n_3-HUFA, numerous governmental and non-governmental organizations across the world currently advise increased fish intake as a means of improving the health of their citizens [14, 15].

Studies on reproduction biology of fish have started since the early decades of the 20th century [16]. An understanding of the reproductive biology of a species is a central aspect for providing sound scientific advice for fisheries management.

Reproductive biology plays a large part in determining productivity and therefore a population's resiliency to exploitation by fisheries or to perturbation caused by other human activities [17]. The reproductive biology of Mugil species were studied by many authors [18] for *Mugil cephalus* [19], for *Mugil liza* [20], for *Mugil seheli* and *Mugil capito* in Lake Timsah [21], for *Mugil auratus* [22], for *Liza abu* [23], for *Liza ramada* [24] and for *Liza aurata* [25] to determine the breeding season and its character as important factor. The reproduction cycle of *Mugil capito* was studied by [23] to evaluate the sexual maturity, length at first sexual maturity, gonadosomatic index and the analysis of egg diameters. They observed that, *Mugil capito* has a prolonged fractional breeding season, five periods of oocyte maturation and asynchronous specie.

Dietary lipids and in particular polyunsaturated fatty acids (PUFAs) play a critical role in the successful production of high quality gametes and eggs of marine fish [26,27]. While a large proportion of dietary lipids are catabolized to fuel reproductive processes, they also deposited into gametes, especially as yolk reserve in the oocytes [28]. Yolk fatty acid composition directly affects the optimal development of the embryo and yolk-sac larvae by providing docosahexaenoic acid (DHA), essential in neural and visual development, as well as eicosapentaenoic acid (EPA) and arachidonic acid (ARA), which serve as precursors of eicosanoids involved in the modulation of neural hypothalamic and immune functions [29,30,31]. ARA is a key PUFA for fish reproduction through the production of prostaglandins that stimulates ovarian and testicular steroidogenesis, final oocyte maturation, ovulation and milt production and act as pheromones to influence sexual behavior [32,33].

The aim of this study was to determine the effect of different sources of oils (Fish oil, palm oil and sunflower oil) as a single or mixed source on growth performance, feed utilization and body composition of reared mullet (*Liza ramada*). It's also to investigate the gonad maturation and histological characters of *liza ramada* broodstock.

MATERIALS AND METHODS

Fish culture and experimental diets

The present study was conducted using the research facilities of the experimental station at Shakshouk, Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF). The system contained two water pumps and two upstream sandy filter units at a point between the water source and tanks. Each pump was drowning the drain water from collection cement pond to the storage tanks and forced it to the rearing tanks in open system. On the same trend, the drain water supply the earthen ponds through cement channel. Physicochemical characteristics of water tanks and earthen ponds were examined every two weeks according to [34].

First trial

The first trial was aimed to evaluate different sources of oil (fish oil, palm oil and sunflower oil) fed to *Liza ramada* fingerlings by using 12 cement ponds with a volume of $2m^3$. Four

diets were formulated in order to test the optimum source of oil in feeding of *Liza ramada*. The first diet considered to be the control with fish oil (FO). The second diet use palm oil (PO). The third diet contains sunflower oil (SO). The fourth diet use a mixed of 2:2:2% from fish oil, palm oil and sunflower oil, respectively. 100 *Liza ramada* with mean initial weight of 5.31±0.31g were randomly distributed in the experimental ponds after two weeks of acclimatization period. All diets were processed into dry sinking pelleted form, using California pelleting machine with 2mm diameter. The experimental diets were fed at 3% rate of live body weight (BW) twice daily at 10.00 a.m and 16.00 p.m. The experimental treatments were triplicated and lasted 120 days after start.

Second trial

The second trial was conducted in three earthen ponds with a volume of 100 m^3 and 1.5 depth. Each pond had 30 broodstock with an initial weight of 200±1.8g. The best diet in the first trial was used as applied diet for feeding of broodstock of *Liza ramada* as 3% of body weight for a period of seven months from September 2014 to March 2015. Fish samples were collected monthly from fish ponds to record each gutted weight (gw) and total length (cm). The fish were dissected to determine sex, maturity stages and gonadosomatic index (GSI). A known weight (about 0.1 gm.) of ovary was preserved in 4% neutral formalin. Fixed part of gonads were washed in 70% ethyl alcohol for two days prior dehydration, then cleared and embedded in paraffin wax sections of 3-6 µm. thick were stained with eosin and hematoxylin [35].

Growth performance

The following growth performance and other experimental parameters were calculated as follows:

- Specific growth rate = 100 × (Ln final weight - Ln initial weight) / 120.

- Condition factor = (wet weight) / (total length^{-3}) × 100.

- Feed conversion = (feed given per fish) / (weight gain per fish).

- Protein efficiency ratio = (weight gain per fish) / (protein intake per fish).

- Net protein utilization = 100 (Final body protein-initial body protein/ protein intake).

-Hepatosomatic index (HSI) = (liver weight) / (fish weight) ×100.

-Gonadosomatic index (GSI) = (gonad weight) / (gutted weight) ×100.

Chemical analysis

The experimental diets and fish carcass were dried for subsequent protein (kieldahl), ether extract (Soxhlet) and moisture analysis, according to AOAC [36] methods. Protein levels were calculated by multiplying the total nitrogen (N) with 6.25. Nitrogen free extract was calculated based on the difference between the dry matter content minus protein, fat and ash content according to [36].

Lipid analysis

Total lipid in samples was extracted after homogenisation, using an ultraturrax tissue disrupter (Fisher Scientific, Loughborough, UK), in ten volumes of chloroform–methanol (2:1, v/v) containing 0·01% butylated hydroxytoluene as antioxidant, basically according to [37] and essentially as described by [38]. Fatty acid methyl esters were prepared from aliquots of total lipids by acid-catalysed transmethylation for 16 h at 508°C, using tricosanoic acid (23 : 0) as internal standard [38]. Fatty acid methyl esters were extracted and purified as described previously [39] and were separated using a Hewlett-Packard 5890A series II gas chromatograph (Hewlett-Packard, Barcelona, Spain) equipped with a chemically bonded (PEG) supelcowax-10 fused silica wall coated capillary column (30m £ 0·32mm i.d.; Supelco Inc., Bellefonte, PA, USA), using an 'on column' injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 508°C to 1808°C at 258°C/min and then to a final temperature of 2358°C at 38°C/ min, with the final temperature maintained for 10 min. Individual fatty acid methyl esters were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard Chem. Station Software.

Gross energy (MJ Kg^{-1} diet) was calculated according to [40]) using the following calorific values:23.9, 39.8 and 17.6 KJ Kg^{-1} diet for protein, ether extract and nitrogen free extract, respectively. The metabolizable energy contents of the experimental diets were calculated as 18.9, 35.7 and 14.7 KJ Kg^{-1} diet for protein, lipid and nitrogen free extract, respectively according to [41].

Statistical analysis

One way analysis of variance (ANOVA) was applied to test the effect of different dietary protein levels on various growth parameters, nutrient utilization and chemical composition of experimental fish according to [42]. Duncan multiple range test was used to detect the significant differences between the means of treatments [43]. All analysis were performed using SAS (version 6, 2004 SAS Institute, Cary, NC, USA), [44].

RESULTS

Physicochemical characteristics of ponds water

Water physicochemical characteristic revealed that water temperature, pH, dissolved oxygen, salinity and unionized ammonia are within the optimum ranges for rearing *Liza ramada* according to [4]. Similar physicochemical condition was observed in all ponds of the present study as presented in (Table 1).

Chemical composition of diets

Results of the chemical composition of diets are shown in (Table 2). All the test diets contained almost identical concentrations of dry matter, crude protein, crude lipid, NFE, CF, ash, growth energy and metabolizable energy. The experimental diets were differentially in their source of oils. The dietary fatty acid compositions were influenced by the fatty acid compositions of each source of oil, where the FO and mixed diets showed high n-3 fatty acids compared with PO and SO diets (Table3). For instance, high n-6 fatty acids were found in

each PO and SO, respectively. In the same trend the percent of n-3/n-6 represented high values in FO and mixed diets than PO and SO diets.

Growth performance

First trial

As presented in (Table 4), averages of initial weights ranged between 5.26 to 5.38 g/fish with insignificant differences among the dietary groups indicating the random distribution of the experimental fish among treatment groups. The results of the first feeding trial are shown in (Table 4). Fish in all dietary treatments survived well during the trial (98%), indicating that the tested diets had no effects on *liza ramada* survival rates, thus all mortalities were due to accidental factors during the samples collection every two weeks to adjust the feed amounts. Concerning growth performance parameters (Table 4) the highest final weights of *liza ramada* fish ($P<0.05$) were recorded by Mix and FO groups, without significance difference between each other. However, PO and SO diets recorded lower ($P<0.05$) final weight compared to the other diets. The same trend was observed with total gain, daily gain, specific growth rate and condition factor, where FO and Mix groups recorded higher values ($P<0.05$) compared to the other groups. As can be seen in the same table, average amounts of feed consumed were found to be 85,78,80 and 87g for fish fed on FO,PO,SO and Mix diets respectively, which indicate slight increases in feed consumption in fish fed on Mix diet compared to the PO and SO diets. On the other hand. FCR,PER,NPU and HSI showed a significant differences in the values ($P<0.05$) between diets.

Second trial (Broodstook)

As presented in (Table 5) the growth parameters, feed efficiency and hepatosomatic index (HSI) of mixed diet showed a good utilization of nutrients and oil type fed to broodstock of *Liza ramada*.

Reproductive Biology of *L. ramada* broodstock fed mixed diet

Maturity stages

Ovary of *Liza ramada* consists of two lobes of almost equal size, lying below the alimentary canal along the body cavity. In the present study, the maturity of gonads can be divided into the maturity stages by appearance (shape, color, and size of gonads) taking into the generalized scales of [45]. These stages are as follows:

Immature stage

The ovaries appear thin and thread like, translucent and colorless. Sexes usually can not be distinguished. As development progresses, the ovary increases in size occupying 1/3 of the body cavity. More rounded, translucent and eggs can't evident to the naked eye.

Maturation stage

The ovary increases in size occupying half of the body cavity.Thickening, pale yellowish, eggs small but visible to naked eye.

Nearly ripe stage

The ovary increases in size occupying more than half but less than two third of the body cavity and it fill most of body cavity. The opaqueness of the ovary is mainly due to the beginning of vitellogenesis. The color is yellow and large eggs can be seen.

Ripe stage

The ovary occupy two third of the body cavity. The color is yellow. Opaque eggs are large and extruded by gentle pressure on abdominal wall.

Spawning stage

Ovaries show somewhat shrinkage due to discharge of a considerable amount of eggs. Ovary is typically orange yellow in color and occupy almost the whole body cavity and hence the belly of the female seems swollen and eggs can be evacuated by a slight pressing on belly.

Spent stage

Ovaries reduced in size. They are thin and flaccid, congested, sometimes contain transparent small residual eggs with few large opaque-eggs can be seen.

Monthly changes in the ovary

The histological examination of the ovaries of *Liza ramada* during the period of prespawning, spawning and post spawning season revealed the following observation. The oocyte of chromatin nucleolus stage, early perinucleolus stage and late perinucleolus stage are represented in the ovaries throughout the experiential period.

During September, the ovary of *Liza ramada* was in immature stage. The value of GSI in this period is very low with an average 0.54 ± 0.23. all of the oocytes are in very early development stages. Most of the ovary in this period have transparent ova group that are chromatin nucleolus stage, early perinucleolus stage and late perinucleolus stage (Photo 1 &2). Transparent ova diameters ranged from 160µ*m* to 240 µ*m*. During October and November, the ovary of *Liza ramada* was at nearly ripe stage. The average GSI in these months increased to 5.99 ± 2.50 and 10.70 ± 3.80 respectively. The ovaries contained oocyte of more advanced cytoplasmic growth in addition to the above-mentioned oocyte. Moreover the ovaries in this period displayed different stages of developmental oocytes. Most ovaries of fishes contain a relatively large number of oocytes at primary, secondary and tertiary yolk stages in addition to few numbers of perinucleolus oocytes (Photo 3). The oocytes increased in diameter and reached 500 µ*m.* In December and January, ovaries at ripe stage which characterized chiefly by the migration of nucleus towards the animal pole. They displayed different stages of development. They contain a numerous number of mature and tertiary yolk deposition stages, in addition to few numbers atresia and small number of early stage of cytoplsmic growth were appeared as a new generation for next breeding season. The egg diameter increased and ranged from 600 µ*m.*to 680 µ*m.* The average GSI in these months are very high (14.34 ± 2.70) and (8.25 ± 2.3) due to the increased number of oocyte at yolk deposition stage and mature stage (Photo 4). In February and March, ovaries were in atresia stage. At this period unovaluated (residual) eggs undergo atresia. The majority oocytes are found in atritic stage and early stages of cytoplasmic growth (Photo 5, 6). The egg diameter decreased drastically and ranged from 160 µ*m.*to 400 µ*m.* The average GSI in this month

rapidly decreased to 1.84 ± 1.43 and 0.95 ± 0.60. The reproductive cycle is obscured since the fish doesn't meet the suitable conditions to spawn.

Gonadosomatic index (GSI)

The monthly variation of GSI values of female and male broodstock fish were presented in Fig.(1). The average GSI of female was the lowest value, equal to 0.54 ± 0.23 during the immature stage, while. GSI value increased suddenly during the maturation period to 5.998 ± 2.50 then increased to reach 10.70 ± 3.80 during the nearly ripe stage, then reached to the peak value to 14.34 ± 2.70 in ripe stage in December. The GSI values decreased suddenly to 1.84 ± 1.43 and continue the atresia period in February. The minimum GSI values were recorded in spent stage which was 0.95 ± 0.60 in March. On the other hand, the mean GSI of the male was represented in Fig. (1), it can be showed that in September, the GSI value was 0.26 ± 0.12. Then, in November it increased gradually and reach the maximum value (11.65± 0.91). After that, the mean GSI decreased gradually to 4.76 ± 0.01 in January. Then the mean GSI decreased suddenly and recorded the minimum values which was 0.76 ± 0.01 in March. From Fig. (1) it is cleared that, the GSI for male have nearly former the monthly maturation trend of female.

Proximate composition of fish body and tissues fatty acid composition

Analytical results of whole body composition are shown in (Table 6). The results showed that dietary oil was a significant factor for fat and protein contents of fish whole body. Fish fed PO and SO had lower crude fat content of whole body than other treatments. However, protein and ash contents of whole body in all groups were not significantly different. On the other hand, the proximate composition of broodstock recorded an increase in their lipid and slightly decreased in their protein

contents compared with the growing fish in the first trial. Fatty acid compositions of muscle were reflected by dietary fatty acid compositions (Table 7).

Table (7) showed also that, the saturated mono-saturated and n-3 fatty acids recorded increased in each FO and Mix diets without significant differences between them. On the other hand, a decreased in these fatty acids were found in plant oils (PO and SO). However, n-6 showed a significantly increased in both palm oil (PO) and sunflower oil (SO) groups. As can be seen in the same table the Mix[1] diet of broodstock represented an increased in n-3/n-6 ratio compared with other groups. In the same trend, the ovary of broodstock recorded an increase in n-3 PUFAs.

DISCUSSION

In the laced decide many research effort has been conducted towards the use of plant oils as alternative sources to replace fish oil. The suitability of this replacement in terms of growth performance and feed efficiency is highly variable among fish species and experimental conditions. The present study detected that, all diets contain the recommended values from protein and essential fatty acids especially n-3 and n-6 required for this species [46].

The growth performance in present results revealed that, the mullet can use alternative source of mixed vegetable oils (VO) in their diets without decrease in growth performance where, the mixed diet showed the highest performance with the two trials. The similar results were reported with juvenile mullet *Liza ramada* [47]. In the same trend, a number of recent studies suggest that dietary vegetable oil (VO) inclusion does not result in reduced growth performance or feed conversion in Atlantic salmon *Salmo salar* [9,13], rainbow trout *Oncorhynchus mykiss* [10], gilthead sea bream *Sparus aurata* [11] and European sea bass *Dicentrarchus labrax* [12].

The results of feed utilization from (FCR, PER and NPU) and hepatosomatic index (HSI) were better in the groups fed FO and mixed diet compared with the SO and PO diets. The results cleared the complementary effect of oil sources in mixed diet in the two trials. These results are in agreement with the results obtained by [46] in the same species and other species such as Atlantic salmon and sea bream [9,10].

The reproductive biology of *Liza ramada* broodstock fed the mixed diet during the experiential period as maturity stages and GSI are discussed here in order to clarify the actual mode of reproduction. In the present study, monthly analysis of the maturity stages indicated that, immature and maturing oocytes were found throughout the season. The oocytes in December and January developed and reach ripe stage. While from February, the absorption process begins and the ovaries were in atresia stage. Similar results were also reported by [48,49] on *Liza ramada* and by [50] on *Mugil cephalus*. However, the presence of more than one batch of eggs in the ovary of *Liza ramada* indicates that, this species has a prolonged spawning season as indicated by [51]. Present results are in good agreement with those recorded by [49] on *Liza ramada*. In the present study, the monthly distribution of gonadosomatic index values indicated that, the peak value of GSI was attained in November, December and January then it decreased sharply from February and March. In agreement with our results about the GSI, there are one peak from many species during the spawning period previously recorded by [48,49,52] on *Liza ramada*, [25] on *Liza aurata* and [50,53,54] on *Mugil cephalus*. The present study clear also that, the ovary of *Liza ramada* showed three batches of eggs. The first batch includes the minute and immature eggs. The minute ones have a round shape while the second batch includes the large eggs, which are yellow in color. This batch represents the oocyte stock. However, the occurrence of these small eggs together with large ones in the ovary do not always indicate fractional spawning and in many fish, the small eggs remain in the ovary after spawning and are gradually reabsorbed [55]. The third batch includes the largest ova, which are transparent and yolky. From the previous observation it is obvious that the gonads develop to reach full degree of ripeness and the egg converted to atresia and hence, no ovulation takes place without induced spawning. The certain level and correct balance in Mix[1] diet between EPA, DHA and ARA seem to be important for successful reproduction and can be use as indices to evaluate the physiological condition and egg quality of *Liza ramada* broodstock. This finding is agreement with previous results which recorded that ARA stimulate ovarian and testicular steroidogenesis, triggering oocyte maturation in females and milt production in males of marine and freshwater fish [56,57, 58].

The amount of lipids and fatty acid composition in fish is known to be influenced by various factors in different species or within a species, such as geographical region, season, feeding habits and diet, age, sex, spawning period etc. Total lipid and fatty acid composition data are important for nutritionists and food scientists to help them in dietary formulations, nutrient labeling, processing and fish quality. In the present study, the oil type in the tested diet showed a significant effect on carcass contents of *Liza ramada*, where high lipid contents in broodstock were recorded. Comparable results in body and fatty acids proximate composition were recorded in mullet *Mugil cephalus* [59].

Broodstock fish employed in this study did not mobilize muscle fat in response to sexual maturation because of the adequate oil sources incorporated in the diet. The primary adipose tissue stores that support gonadogenesis in mullet include visceral fat, dorsal fat, and intramuscular fat associated with red and white muscle. This finding is agree with the previous work of [60] in rainbow trout. Several studies have shown that visceral fat is mobilized first to supply energy for gonadogenesis [60, 61, 62]. Fish will also mobilize intramuscular fat as a secondary energy source to support gonadogenesis when visceral reserves are low. This effect of gonadogenesis is evidenced by less visceral adipose tissue in broodstock fish. As shown in present work, the fatty acids contents in ovary of broodstok seem to be similar with the grower fish in the first trials and the wild analysis of mullet *M. cephlus* recorded by [59]. The results also revealed that the major saturated, mono-saturated and poly-unsaturated especially (20:5n-3, EPA, 22:6n-3, DHA and 20:4n-6, ARA) acids were highly compared with the present results in the same species, where agree with the previous results of [63] in trout and [64] in *Aractric char*. Among the PUFA, EPA, DHA and ARA have been shown to play pivotal role in regulation of oocyte maturation and ovulation [64]. In the same trend, the high levels of the major fatty acids mainly MUFA found in the common snook shows their importance as energy store for embryonic development [65].

Deficiency or excess of n-3 highly unsaturated fatty acids has been found to depress egg quality of several species [66]. n-3/n-6 fatty acids play an important structural role as components of phospholipids in fish bio-membranes and are associated with the membrane fluidity and correct physiological functions in marine fish [67]. It was also noticed that, the ratio of n-3/ n-6 in the present trial was 2.9% is agree with the previous results in snook eggs recorded by [68] and high than 0.39% in fertilized eggs of *Liza ramada*. However, poor hatching rates and survival were observed in sea bream [69,70] linked to fatty acids content and also recorded a negative effect on egg quality of *Liza ramada*.

The present study concluded that, the incorporation of vegetable oils with the detected values of n-3/n-6 ratio enhance the reproductive biology, fish quality for consumer and decrease the costs of diet fed to *Liza ramad*. It's also cleared that, this species is a good candidate for aquaculture but the shortage supply of fries from the wild resources lead to further research conducting with the artificial propagation of this specie.

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APPENDIX

Table1:Averages physicochemical characteristics of ponds water during experimental period.

Parameters	Diets			
	FO	PO	SO	Mix
Temperature ^{o}C	28.6±0.5	28.4 ±0.6	27.8 ±0.8	28.4 ±0.7
Salinity (g/l)	5.6±0.2	5.4±0.1	5.5±0.2	5.6±0.2
pH	8.2 ±0.15	8.1± 0.12	8.3±0.14	8.2±0.15
Dissolved oxygen (mg/l)	7.5±0.11	7.6±0.12	7.5±0.14	7.6±0.16
Unionized ammonia (mg/l)	0.031± 0.001	0.034±0.001	0.033±0.001	0.032±0.001

Table2: Formulation and chemical composition of the experimental diets.

Ingredients	Diets			
	FO	PO	SO	MO
Fish meal	10	10	10	10
Poultry-by-product meal	20	20	20	20
Corn gluten meal	20	20	20	20
Wheat bran	35	35	35	35
Yellow corn	7	7	7	7
Fish oil	6	---	---	2
Palm oil	---	6	---	2
Sunflower oil	---	---	6	2
Vitamin / Mineral Mix[1]	2	2	2	2
Total	100	100	100	100
Chemical composition (%D.M.)				
Dry matter	92 .5	92.5	92.5	92.5
Crude protein	30.23	30.23	30.23	30.23
Ether extract	17.92	17.92	17.92	17.92
Nitrogen free extract	34.39	34.39	34.39	34.39
Fiber	4.58	4.58	4.58	4.58
Ash	12.88	12.88	12.88	12.88
Gross energy(MJ kg^{-1} diet)[2]	20.49	20.49	20.49	20.49
ME (MJ kg^{-1}diet)[3]	17.15	17.15	17.15	17.15

1-Vitamin-mineral premix supplied the following (g Kg^{-1} mixture); retinyl acetate 0.67; ascorbic acid 120; cholecolciferol 0.1; tocopheryl acetate 34.2; menodione 22; thiamin 5.6; riboflavin 12; pyridoxine 4.5; calcioum panthothenate 14.1; p-aminobenzoic acid 40; cyanocobalamin 0.03; niacin 30; biotin 0.1; choline chloride 350; folic acid 1.5; inositol 50;canthaxanthin 10;butylated hydroxytoluene 1.5; butylated hydroxyanisol 1.5.; CaHPO4,2H2O 29.5; Ca (H2PO4)2 H2 O 217; NaHCO3 94.5; Na2SeO35H2O 0.011; Kci 100; Nacl 172.4; Ki 0.2; Mgcl2 63.7; MgSO4 34.3; MnSO4 2; FeSO4H2O 10; CuSO4 5H2O 0.4; ZnSO4 10.

2-Lozano *et al*., (2007).

3-Jobling (1994).

Table3: Fatty acid composition (% total fatty acid) of experimental diets (Mean±S.E. n=3).

Fatty acids	Diets			
	FO	PO	SO	Mix
14:0	5.4^{b}±0.2	2.4^{c}±0.1	2.2^{c}±0.4	8.3^{a}±0.5
16:0	15.2^{c}±0.1	30.8^{a}±0.1	28.2^{a}±0.2	25.4^{b}±0.1
18:0	3.6^{b}±0.3	4.5^{a}±0.2	4.1^{a}±0.1	4.1^{a}±0.2
Σ- saturated	24.2^{c}±0.2	37.7^{a}±0.1	34.5^{b}±0.2	37.8^{a}±0.2
16:1n-7	7.2^{a}±0.2	3.4^{c}±0.1	3.6^{c}±0.2	5.0^{b}±0.3
18:1n-9	14.6^{d}±0,3	26.2^{a}±0.1	22.6^{b}±0.1	21.1^{c}±0.2
Σ-mono-unsaturated	21.8^{c}±0.2	29.6^{a}±0.1	26.2^{b}±0.1	26.1^{b}±0.2
18:2n-6	2.4^{c}±0.1	7.2^{a}±0.4	6.8^{a}±0.1	5.5^{b}±0.1
20:2n-6	0.4^{b}±0.1	0.8^{a}±0.1	0.6^{b}±0.1	0.5^{b}±0.1
20:4n-6	1.2^{a}±0.1	0.8^{b}±0.1	0.6^{c}±0.1	0.9^{b}±0.1
Σ-n-6fatty acids	4.0^{c}±0.1	8.8^{a}±0.1	8.0^{a}±0.1	6.9^{b}±0.1
18-3-n3	1.7^{a}±0.1	0.4^{c}±0.1	0.2^{c} ±0.1	0.7^{b}±0.1
18-4n-3	0.4^{a}±0.1	0.5^{a}±0.1	0.4^{a}±0.1	0.4^{a}±0.1
20-5n-3	8.6^{a}±0.2	3.8^{c}±0.1	3.5^{c}±0.3	6.1^{b}±0.2
22-5n-3	1.1^{a} ±0.2	0.8^{a}±0.3	0.7^{a}±0.2	0.9^{a}±0.1
22-6n-3	9.5^{a}±0.4	4.1^{c}±0.2	3.8^{c}±0.3	6.2^{b}±0.4
Σ-n-3fatty acids	21.3^{a}±0.2	9.6^{c}±0.2	8.6^{c}±0.2	14.3^{b}±0.2
EPA:DHA[1]	0.9^{a}	0.92^{a}	0.92^{a}	0.98^{a}
n-3:n-6 ratio	5.32^{a}	1.09^{c}	1.07^{c}	2.07^{b}

Means in the same raw with different super script letters are significantly different (P<0.05).
[1]EPA: DHA=20:5n−3 concentrations/22:6n−3 concentrations

Table 4: Growth performance mean values (Mean±S.E. n=20) of mullet *Liza ramada* fed on different experimental diets for 120 days(First trial).

Parameters	Diets			
	FO	PO	SO	Mix
Initial aveg. weight (g/fish)	5.34^{a}±0.32	5.36^{a}±0.32	5.22^{a}±0.32	5.35^{a}±0.32
Final aveg. weight (g/fish)	50.8^{b}±2.47	40.6^{c}±1.8	38.5^{d}±1.6	53.0^{a}±2.4
Total gain (g/fish)	45.46^{a}±1.4	35.24^{b}±1.8	33.22^{b}±2.2	47.65^{a}±1.6
Average daily gain (g/fish/day)	0.37^{a}±0.1	0.29^{b}±0.1	0.27^{b}±0.1	0.39^{a}±0.1
Specific growth rate	1.87^{a}±0.2	1.69^{b}±0.2	1.66^{b}±0.2	1.91^{a}±0.2
Condition factor (g/cm^{-3})	1.82^{a}±0.2	1.48^{b}±0.3	1.41^{b}±0.3	1.93^{a}±0.1
Survival rate %	98	98	98	98
Feed consumed (g/ fish)	85.0	78.0	80.0	87.0
Feed conversion ratio	1.86^{a}±0.3	2.21^{b}±0.1	2.4^{b}±0.3	1.82^{a}±0.2
Protein efficiency ratio(PER)	1.76^{a}±0.3	1.49^{b}±0.2	1.37^{b}±0.4	1.81^{a}±0.2
Protein productive value (PPV%)	32.51^{a}±2.4	26.43^{b}±2.8	23.84^{c}±3.2	32.69^{a}±2.8
HSI (%)	1.68^{a}±0.2	1.42^{b}±0.2	1.40^{b}±0.1	1.72^{a}±0.2

Means in the same row with different superscript letters are significantly different (P<0.05).

Table 5: Growth performance mean values (Mean ± S.E. n=10) of broodstook mullet *Liza ramada* fed on mixed diet for 210 days (Second trial).

Parameters	Mixed diets
Initial aveg. Weight (g/fish)	200.5±1.8
Final aveg. Weight (g/fish)	360.4±1.6
Total gain (g/fish)	114.9±1.2
Specific growth rate	3.5±0.2
Condition factor (g/cm^{-3})	2.17±0.3
Survival rate %	99
Feed consumed (g/ fish)*	180.0
Feed conversion ratio	1.56±0.2
Protein efficiency ratio(PER)	2.11±0.1
Protein Productive Value (PPV%)	36.15

Table 6:Carcass analysis of *Liza ramada* fed on the experimental diets ,Mean±S.E., n=3 (%w/w basis).

Items	Diets					
	Initial	FO	PO	SO	Mix	Mix[1]
Dry matter	27.3	29.9^{a}	26.8^{a}	25.9^{a}	29.1^{a}	31.4
Protein	18.6	18.4^{a}	17.8^{a}	17.5^{a}	18.1^{a}	16.5
Lipid	5.2	7.8^{a}	5.6^{a}	5.2^{a}	7.4^{a}	10.2
Ash	3.5	3.7^{a}	3.4^{a}	3.2^{a}	3.6^{a}	5.3

Means in the same raw with different super script letters are significantly different (P<0.05).

Table7:Muscle fatty acid composition (% total fatty acid) of mullet *Liza ramada* fed different experimental diets (Mean±S.E., n=3).

Fatty acids	Diets				
	FO	PO	SO	Mix	Mix[1]
14:0	8.5^{a}±0.1	7.5^{b}±0.3	7.4^{b}±0.2	8.3^{a}±0.5	8.4^{a}±0.3
16:0	25.6^{a}±0.4	22.8^{b}±0.2	22.2^{b}±0.4	25.4^{a}±0.1	25.6^{a}±0.2
18:0	3.9^{a}±0.2	3.7^{a}±0.4	3.6^{a}±0.1	3.8^{a}±0.2	3.5^{a}±0.2
Σ- saturated	38.0^{a}±0.2	34.0^{b}±0.3	33.2^{b}±0.2	37.5^{a}±0.2	37.59±0.2
16:1n-7	9.8^{a}±0.1	7.6^{b}±0.2	6.8^{b}±0.3	9.3^{a}±0.5	9.5^{a}±0.3
18:1n-9	11.5^{a}±0,3	10.2^{b}±0.4	9.8^{b}±0.2	11.2^{a}±0.4	11.1^{a}±0.4
20:1n-7	2.4^{a}±0.2	2.1^{a}±0.4	2.0^{a}±0.2	2.3^{a}±0.1	2.4^{a}±0.1
20:1n-9	4.6^{a}±0.2	3.4^{b} ±0.1	3.2^{b}±0.2	4.4^{a}±0.4	4.5^{a}±0.2
Σ-mono-unsaturated	28.3^{a}±0.3	23.3^{b}±0.3	21.8^{b}±0.3	27.2^{a}±0.4	27.5^{a}±0.1
18:2n-6	2.1^{c}±0.2	6.5^{a}±0.1	6.1^{a}±0.3	4.2^{b}±0.2	4.4^{b}±0.1
20:4n-6	3.5^{a}±0.1	3.1^{a}±0.2	3.2^{a}±0.1	3.4^{a}±0.3	3.6^{a}±0.2
Σ-n-6fatty acids	5.6^{c}±0.1	9.6^{a}±0.1	9.3^{a}±0.2	7.6^{b}±0,2	8.0^{b}±0.1
18-3n-3	1.2^{b}±0.1	1.1^{b}±0.2	1.3^{b} ±0.3	1.0^{b}±0.2	1.2^{a}±0.1
18-4n-3	2.8^{a}±0.2	1.7^{b}±01	1.8^{b}±0.1	2.4^{a}±0.4	2.5^{a}±0.3
20-5n-3	7.6^{b}±0.3	5.6^{c}±0.1	5.4^{c}±0.2	7.2^{b}±0.2	7.6^{a}±0.1
22-5n-3	3.2^{b} ±0.2	2.8^{c}±0.3	2.4^{c}±0.2	3.0^{b}±0.1	4.3^{a}±0.2

22-6n-3	4.8^{a}±0.2	3.6^{c}±0.3	3.2^{c}±0.1	4.1^{b}±0.2	4.2^{a}±0.3
Σ-n-3fatty acids	19.6^{b}±0.2	14.8^{c}±0.2	14.1^{c}±0.2	17.7^{b}±0.2	19.8^{a}±0.2
EPA-DHA[1]	1.58^{a}	1.55^{a}	1.68^{a}	1.75^{a}	1.8
n-3: n-6 ratio	3.5	1.54	1.51	2.32	2.47

Means in the same raw with different super script letters are significantly different (P<0.05).
[1]EPA: DHA=20:5n−3 concentrations/22:6n−3 concentrations.

Table 8: Ovary fatty acid composition (% total fatty acid) of mullet broodstock *Liza ramada* fed different experimental diets for 210 days (Mean±S.E., n=3).

Fatty acids	Mix Diet
14:0	7.8±0.2
16:0	22.4±0.5
18:0	3.3±0.3
Σ- saturated	33.5±0.4
16:1n-7	8.6±0.2
18:1n-9	12.2±0,1
20:1n-7	2.6^{a}±0.4
20:1n-9	4.2±0.1
Σ-mono-unsaturated	27.6±0.2
18:2n-6	3.1±0.1
20:4n-6	3.2±0.2
Σ-n-6fatty acids	6.3±0.2
18-3n-3	1.1±0.1
18-4n-3	2.4±0.4
20-5n-3	7.4±0.2
22-5n-3	3.2 ±0.1
22-6n-3	4.2±0.1
Σ-n-3fatty acids	18.3±0.3
EPA-DHA[1]	2.0±0.0
n-3/n-6 ratio	2.9

Means in the same raw with different super script letters are significantly different (P<0.05).
[1]EPA:DHA=20:5n−3 concentrations/22:6n−3 concentrations

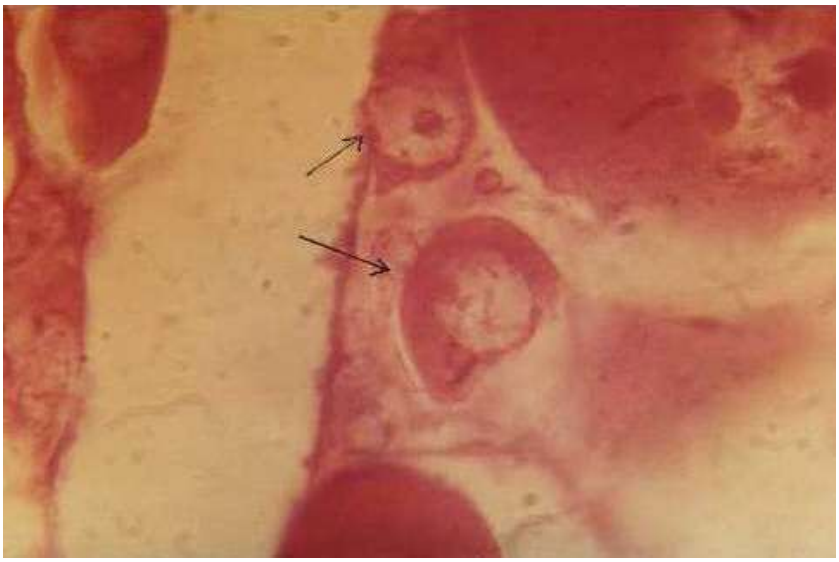

Photo (1). Cross section in an immature ovary of *Liza ramada* showing nests of chromatin-nucleolus stage (arrow) (X 1000).

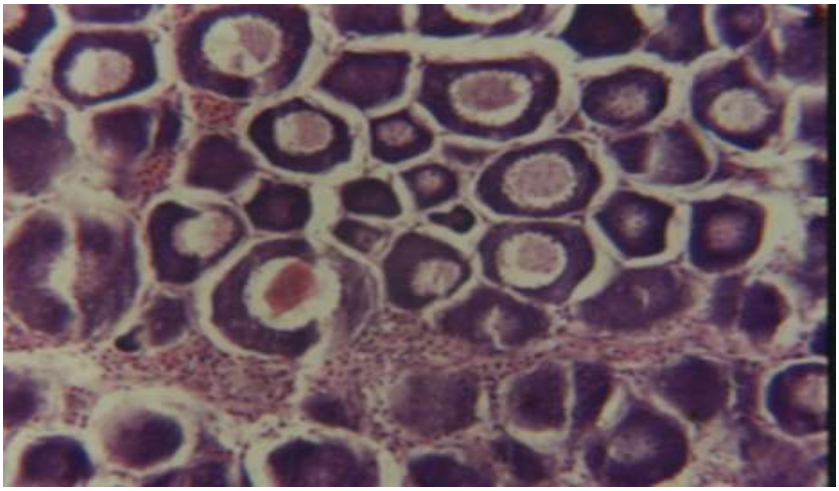

Photo (2). Cross section in an immature ovary of *Liza ramada* showing: (A) early perinucleolus stage; (B) late perinucleolus stage; (N) nucleus; (Chr) chromatin material (X 250).

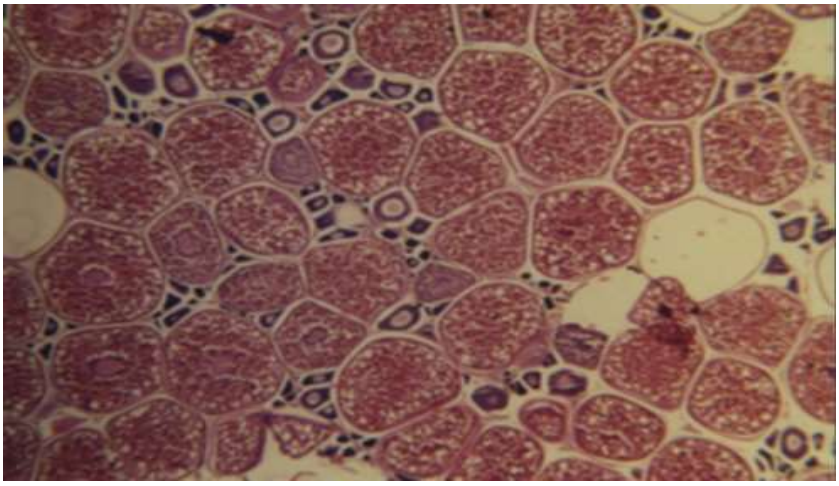

Photo (3). Cross section of the ovary of *Liza ramada* at nearly ripe stage showing: (A) late perinucleolus stage; (B) vacuolized stage; (C) different stages of 1 ^{ry}; 2 ^{ry} and 3 ^{ry} yolk deposition stages. Eosin haematoxylin (X 400).

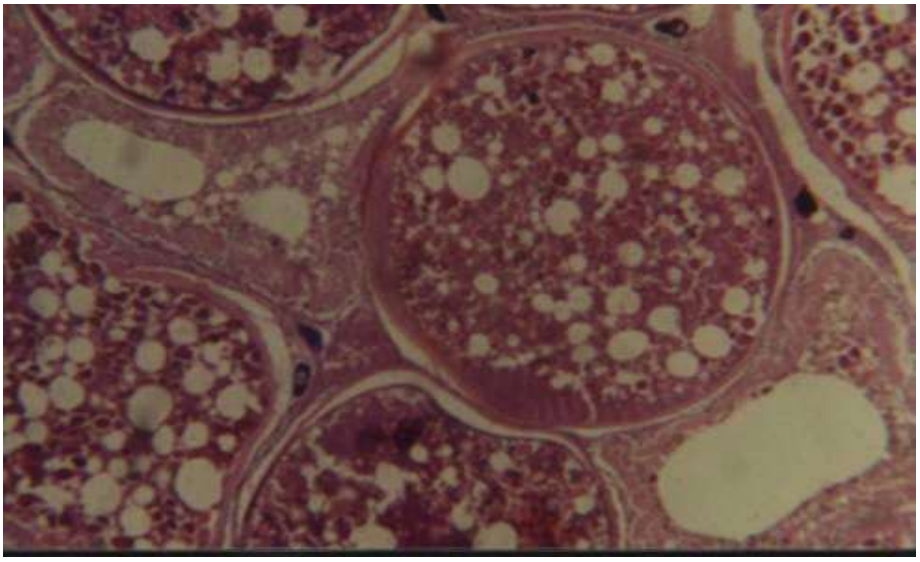

Photo (4).Cross section of the ripe ovary of *Liza ramada* showing mature oocyte (arrow); nucleus (N) at animal pole; lipid droplet (LD). (X 250).

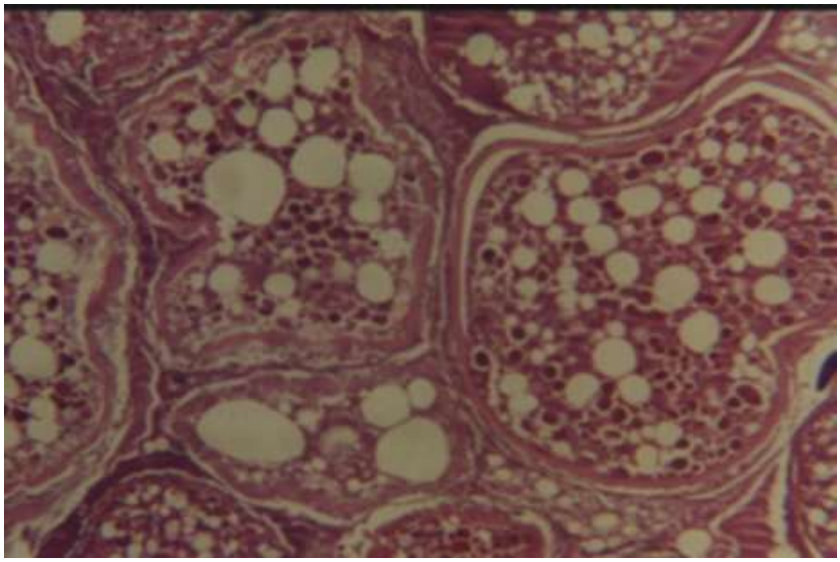

Photo (5). Cross section of the ovary of *Liza ramada* at atresia stage (arrow) showing the follicular wall become thick and irregularly folded. (X 400)

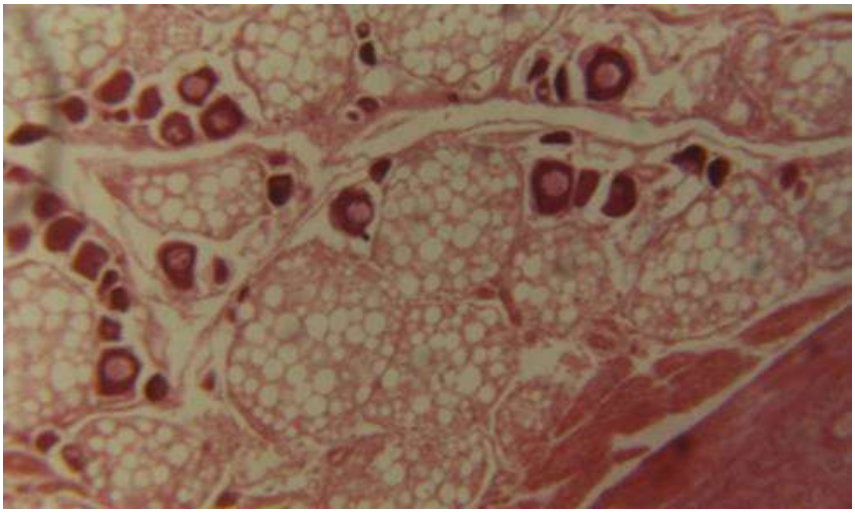

Photo (6). Cross section of the ovary of *Liza ramada* at atresia stage (arrow) showing the ooplasm appears liquefied having large vacuoles and atresia growth type. (X 400)

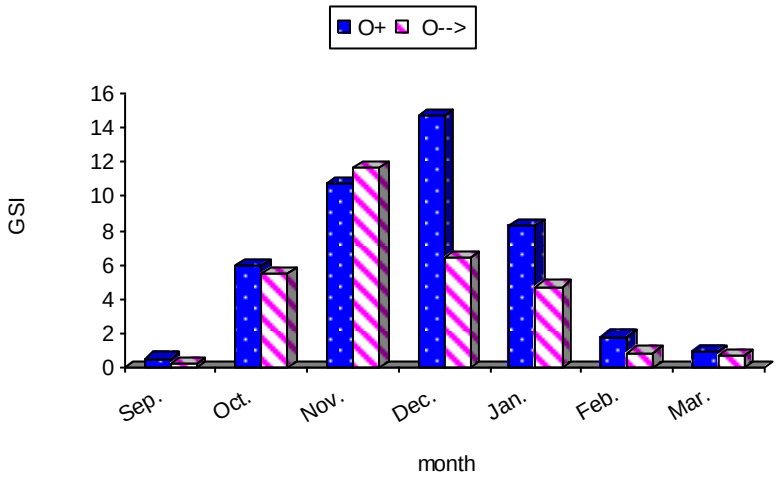

Fig.(1). Monthly variation in gonadosomatic index (GSI) value of female and male *Liza ramada* fed mixed diet.