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The Changes in the Biochemical Composition of Total Protein and Lipid in the Ovary, Liver Tissues and the Serum During the Process of the Ovarian Maturation in Female the Grass Carp, *Ctenopharyngodon idella*

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ABSTRACT

The grass carp is a subtropical and temperate fish species, belonging to family Cyprinidae and genus Ctenopharyngodon. Rivers and lakes in Asia represent the native habitat of this species, especially the Amur River. In Egypt, this fish species is exotic and it is used as biological control. It has a short spawning season extending from May till the end of June. Remarkably, this fish species does not spawn naturally in the captivity state. Thus, for a successful spawning process, injection with hormones is necessary. The biochemical composition including the changes in the ovary during maturation process was addressed using different indices. The total protein content in the ovarian tissues varied with maturation stages, showing a significant difference (P<0.05). The value ranged from 60.65 ± 0.299g/ 100g dry weight in immature stage and increased to 66.44 ± 0.420g/ 100g dry weight in maturing stage. On the other hand, lipid content in the ovary with maturation process fluctuated from the lowest value (5.77 ± 0.070g/ 100g) of dry weight in immature stage to the highest value (12.77 ± 0.070g/ 100g) of dry weight. The level of cholesterol during the ripe stage of ovary is attributed to the available cholesterol consumed in the synthesis of steroid hormones. All these items coordinateare important for maturation, covering the need of broodstock for sufficient proteins and lipids that must be supplied in fish food .

INTRODUCTION

The grass carp, *Ctenopharyngodon idella* belongs to family Cyprinidae **(Chitton & Muoneke, 1992)**. Grass carp is a subtropical and temperate fish species. The native habitat covers the rivers and lakes in eastern Asia, specially Amur River. Grass carp is cultured throughout the world for two reasons. It represents a cheap source for protein production, and it is used as a biological control. In America, the grass carp has been used to control the aquatic weeds such as *Hydrilla verticillata* **(Fish Base 2004)**. While in Egypt, the spread of nuisance of aquatic plants causes serious problems in the River Nile and its canals. For this reason, the grass carp is introduced in Egypt as a biological control agent for aquatic plants. The main problem of culturing grass carp as an exotic

fish in Egyptian fauna is failing to spawn naturally **(sheha, 2020)**. The study on biochemical composition and changes in biological indices associated with maturation in the ovary is important. The variation of total proteins and lipid contents in the ovary and liver tissues play an important role in gonadal maturation. Additionally, the role of cholestrol was not neglected; it was addressed **(Shankar & kulkarmi , 2007)** in *Notopterus notopterus*, and the serum cholosterol was high during the breeding period compared to the resting period .The increase in cholestrol during the breeding season may be due to an increase in cortisol synthesis needed for the gonad maturation .

The present study aimed to determine the changes in the biochemical composition of total protein and lipid contents in the ovarian and liver tissues during the different maturation stages. In addition, the study addressed the role of cholesterol which plays an important role in the estradiol hormone (E_2) for completing the process successfully to attain the final oocyte maturation.

MATERIALS AND METHODS

Fish collection

The grass carp *Ctenopharyngodon idella* fish specimens were collected from a floating cage in Damietta branch of the River Nile near Faraskour City and El -Serw fish farm. Fish specimens were obtained from January to December 2021, using special nets from floating cages. The fish were monthly caught to ensure the possible changes in the gonad of female. Fish collected ranged from 53.2 to 72cm in length and 2350 to 4754g in weight.

Gonadosomatic index (GSI)

The females were weighed (g) and the fish individual was dissected, and the ovary was weighed(g). The gonad was described histologically and classified according to the different developmental stages into six stages: 1- chromatin nucleolus stage; 2- early and late perinucleolus stage; 3-yolk vesical stage; 4- yolk granules; stage 5- ripe stage, and 6- atretic stage. The classification was described according to **Yamamoto *et al.* (1956)** and **Mousa (1994)**. The gonadosomatic index (GSI) was calculated according to previous studies **(Elgamal, 2001; Yomeda *et al.*, 2001; Ahmet *et al.*, 2004)** using the following equation:

$$GSI = \frac{\text{Gonad weight (g)}}{\text{Gutted weight (g)}} \times 100$$

For the measurement of the oocyte diameter, the oocytes were put in a solution of 40% formalin in 0.6 % of Nacl. They were measured with an ocular micrometer under stereomicroscope.

Hepatosomatic index (HSI)

Both the liver and gutted body cavity were weighed (g) and the hepatosomatic index was calculated using the following equation, following the guidance of several authors **(Assem, 1995; Andrade *et al.*, 2003):**

$$HSI = \frac{\text{liver weight (g)}}{\text{Gutted weight (g)}} \times 100$$

Blood collection

Blood samples were collected from the heart in small tubes and left outside the refrigerator for15 minutes. They were centrifuged for 20 minutes at 4000 RPM, and then the serum was carefully collected in eppendrof tubes and stored at-20˚ C. The level of proteins was measured applying the Biluret method. The cholestrol was measured by pars Azmune kits. Total lipid was assessed by photometry. Immuno tech kits were used for estradiol (E_2) determination.

Estradiol were determined by ELISA kits according to **Bayunova *et al.* (2002)**, and their values were measured according to Roche Hetachi Cobas C_{311}.

Protein content

The protein content of liver and ovary was estimated according to **Lowry *et al*. (1951)**.

Total lipied content

The total lipid content of the liver and ovary was determined according to **Branes and Blackstock (1973)**.

Histological observation

The ovaries of different developmental stages were separated and then removed from the body cavity. Small pieces of ovary were cut and fixed in 10% of neutral buffered fomaline, then ascending in grades of ethyl alcohol. They were cleared in xylene and mounted in molten paraplast parafin (56 -58˚ C). Serial sections were selected and stained by the following techniques:

1. Harris haematoxyline and eosim according to **Elgamal (1997)**.

2- Mercury bromophenol blue method for detection of protein according to **Mazia (1953)** and **Sheha (2020)**.

Statistical analysis

Data were statistically analyzed using one- way ANOVA, followed by Tuky multiple range test for multiple comparisons; $P < 0.05$ is considered a significant difference. Statistical analysis was performed with the SPSS Software program.

RESULTS

Morphological and Histological observations in the ovary

Morphological observation on the ovary

The ovary of female grass carp *C. idella* is a paired elongated organ of two longitudinal strands comprised dorsoventral, adhering to the wall of the abdominal cavity and located above and on both sides of the swim bladder. The two lobes of the ovary are nearly equal in size, and the two strands are connected in short duct and ended with genital opening as in Fig. (1A).

Generally, the morphology of ovary varies with progress of sexual state of female along the annual cycle. During the progress of maturation, the color, size and blood supply of ovary varied. The color of the ovary changed from transparent in immature stage to yellow in the ripe stage, while it appeared between reddish to yellowish in their atretic stage, as shown in Figs. (1B, D, E and F). According to the morphological changes of ovary, the developmental stages could be classified into five stages as follows:

Stage 1 (Immature stage). In this stage, the ovary was colorles The ovary could not be distinguished from the testis, during immature stage. The blood supply couldn't be detected with naked eyes during this stage, as presented in Fig. (1A, E).

Stage 2 (Maturing stage). In this stage, the ovary was reddish in color, and the size of ovary increased in thickness. (Fig. 1B, F).

Stage 3 (Ripe stage). The ovary occupied most of the body cavity space, and the ovary was large, opaque and appeared yellow in color. The eggs were loaded with yolk, and the eggs were clearly visible with the naked eyes. The blood supply of the ovary could be observed through the developing of blood capillary (Fig. 1C, G).

Stage 4 (Atretic stage). The size of the ovary decreased to a notable degree, and the ovary was flaccid, flabby and yellowish in color. Some ovaries appeared in translucent, and opaque residual eggs were visible with the naked eyes, The ovary during this stage was still rich in blood supply, similar to the ripe stage (Fig. 1D, H).

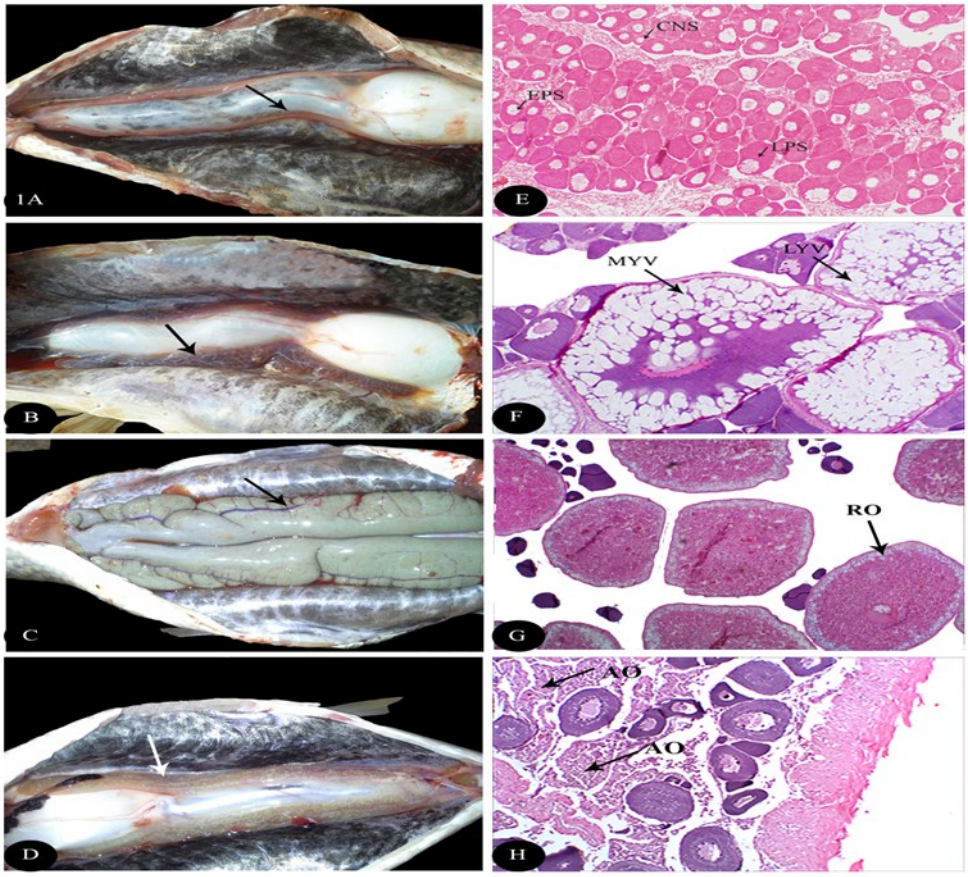

Fig. 1 (A-D). Photomacrographic of ovaries showing morphological appearance of the ovary during maturation stages (X5) **A.** Immature ovary appears transparent strand; **B.** Maturing elongated ovary appears orange in color; **C.** Ripe stage, the ovary appears yellow in color and **D.** Atretic stage, some residual eggs were visible with the naked eyes **Fig. 1 (E-H).** Photomicrographic of ovaries showing histological structure during maturation stages. (X400) **E.** General structure of ovigerous lamella, and chromatin nucleous stage (CNS)), Early and late perinucleolus stage (EPS&LPS),; **F.** Ovary during maturing stage containing oocyte at mid yolk vesicle (MYV) and late yolk vesicle stages (LYV) (X400); **G.** Ripe ovary (RO) (H. and E., X400) and **H.** Ovary at atretic stage contain atretic oocyte (AO)

A- Gonadosomatic index (GSI) of female.

During the annual reproductive cycle of grass carp females, gonadosomatic index was calculated and recorded. The results showed that the weight of the ovaries related to the total of body weight varied according to their developmental stages. The annual reproductive cycle of female started with small GSI value from January (0.366 $\pm$ 0.025), and the value increased gradually reaching 0.478 $\pm$ 0.101 in March. The apparent increase of gonadosomatic index was recorded during April (8. 874 $\pm$ 5.466)and

continued in May, recording values of 17.645 ± 0.381 .The sharp decrease in the value of GSI was in June, and its value reached 8.289 ± 0.253. The gradual decrease was attained in July and reached 3.152 ± 0.22 till the end of the reproductive cycle (Table 1 & Fig. 2).

B- Hepatosomatic index (HSI) of female.

The hepatosomatic index (HSI) was used for determining the clear changes in the liver weight throughout the period of study. The value of HSI varied according to the maturity stage of the gonads. The average value of hepatosomatic (HSI) in females was gradually decreased from 3.681 ± 0.214 in January and reached 1.389± 0.098 in May then begin to increase gradually from June 1.695 ± 0.144 and reached the values of 3.331 ± 0.288 and 3.685 ± 0.168 in October and December, respectively. The results indicate that the maximum value of HSI was attained in December 3.685 ± 0.168, while the minimum average value was observed in May 1.389± 0.098 (Table 2 & Fig. 3).

C-Variation of total protein and lipid contents in the ovarian tissues

1- The total protein content in the ovarian tissue

At maturation, protein content in the ovarian tissues varied with the maturation stage and showed a significant difference (P<0.05). The values varied from 60. 65 ± 0.299g/ 100g of dry weight in immature stage, and then increased to record values of 66.84+ 0.420g/ 100g of dry weigh in maturing stage. The value of protein in the ovary was increased in ripe stage to reaching 70.88 ± 0.273g/ 100g dry weight (Table 3 & Fig. 4a). Variation of the ovarian protein was related to weight of the ovaries. The average value of GSI in immature stage was 3.5%, and the lowest value in the tissues of ovarian protein recorded 60.65 ± 0.299g/ 100g of dry weight. The high value of protein content in the ovary during the ripe stage coincided with the highest value of GSI 14.790 %. The total protein content reached its highest value with 70.88 ± 0.273g/ 100g of dry weight in the ripe stage. The protein contents decreased to 63 ± 0.110g/ 100gm of dry weight, and the GSI decreased and recorded 63.79 ± 0.110 g/100g of dry weight, the GSI recorded the lowest value 3.09% in the atretic stage (Table 3 & Fig. 4b).

2-The relationship between the total protein content in the ovary and hepatosematic index (HSI)

An inverse relationship was recorded between the protein concentration in the ovary and the average value of HSI. Indirect relationship, HSI attained the lowest value (1.64%) during exogenous vitellogenesis in the ripe stage. protein in the ovary in immature recorded the lowest value (60. 65 ±0.299g/ 100) of dry weigh while the value of HSI peaked of 3.23 (Table 3 & Fig. 4c)

3- The protein contents in the liver tissues

The total protein contents in the liver tissues fluctuated with the progress in the gonadal maturation and showed a significant difference (P<0.05). The protein value in immature stage of was recorded as 62.84 ± 0.543g/ 100g of dry weight. And then increased to reached its maximum value of 70.03 ± 0.155g/ 100g of dry weight in maturing stage. During the ripe stage, the value of protein content was sharply dropped and reached 60.72 ± 0.70g/ 100g of dry weight. During the atretic stage, the fluctuation in the liver protein content was accompanied with the variation value in the ovarian weight (Table 4 & Fig. 4c).

4- Lipid content in the ovarian tissues

The lipid content in the ovary with maturation stage fluctuated from the lowest value 5.77± 0.070g/ 100g of dry weight in immature stage to increase with a steady state reaching a value of 12.77 ± 0.111g/ 100 of dry weight in maturing stage. A heavy accumulation of lipid content in the ovary tissue was found in ripe stage, reached peak value (21.75 ± 0.337g/ 100g) of dry weight. A sharply decrease of lipid content was recorded (14.8 ± 0.279g/ 100g of dry weight) during the atretic stage. The fluctuation of lipid content in the ovary was accompanied with maturation and GSI value. 14.79, while the lowest lipid content in the ovary was recorded in immature stage and the GSI recorded 3.5%; however, the lowest value of GSI 3.09% was attained in atretic stage (Table 3 & Fig. 5).

5-The relationship between the total lipid in liver and the GSI

During the immature stage, the total lipid content in the liver tissue was 14.15 ± 0.106g/ 100g of dry weight, then the value increased gradually to peak at 15 .97 ±0.657 g/100g of dry weight in maturing stage. The total lipid content in liver sharply decreased to 7.22 ± 0.077g/ 100g of dry weight in ripe stage, and significant differences were calculated (P<0.05). During the atretic stage, the value of lipid in liver was 12.53± 0.045 g/100g of dry weight. The fluctuation of total lipid content in the liver was recorded in an inverse relation with variation of GSI. At the same time, the lipid content in the liver was the lowest value 7.22± 0.077 g /100g dry weight during the ripe stage. The value of GSI attained a peaked value of 14.79%, and HSI reached the lowest value (1. 64 %) in the ripe stage. The value of total lipid content in liver increased during the atretic stage, while the value of GSI declined from 14.79% during the ripe stage to the lowest value (3.09 %) during the atretic stage. The value of HSI decreased in atretic stage, while the value of lipid returned to increase (12.53± 0.045g/ 100g of dry weight), as shown in Table (4) and Fig.(6).

6- The serum total protein in relation to maturation

The serum protein was at its lowest value during the immature stage 0.4 ± 0.012 g/dL, and then it increased gradually to record a value of 1.3 ± 0.021 during the maturing stage. The highest value of protein content in serum was observed in the ripe stage at a value of 2.45 ± 0.086g/ dL, then this value was sharply declined during the spent stage and recorded 0.67 ± 0.031 as observed in Table (5a) and Fig.(7a) .

7- The serum total lipid in relation to maturation

The serum lipid during the immature stage ovary was recorded (314±3.8 mg/dL) with the acceleration of the maturation process; the value of lipid elevated to reach 343± 4.4 mg/dL during the maturing ovary stage. The maximum value was recorded during the ripe stage with a value of 374 ± 5.7mg/ dL. This value of lipid declined to 234± 1.6mg/ dL during the atretic stage in the ovary, as shown in Table (5) and Fig. (7b).

8- Average of cholestrol in serumin relation to maturation

At the onset of ovary maturation, the cholesterol level in serum was at its minimum value (77± 3.3mg/ dL) during the immature stage. The value of cholesterol level peaked in the serum during the mature stage and was measured 249 ± 1.2mg/ dL. With the ovary developing towards the ripe stage, the level of chotesteral in the serum decreased sharply to record 114 ± 5.1mg/ dL and then recorded 85 ± 4 mg/dL in the atretic stage, as shown in Table (5b) and Fig. (8).

9 -The Average of 17- β Estradiol in the serum in relation to maturation

At the onset of ovarian maturation, the level of estradiol (E_2) in serum was 524 ± 3.265 Pg/ml in immature stage. This level of estradiol (E_2) was declined and reached 381±1.885 pg/ml in maturing stage. In ripe stage, this value of estradiol in serum was moderately increased and recorded 471± 3.091pg/ ml. In atretic stage, the value of (E_2) in serum decreased and reached the minimum value (108 ±1.699pg/ ml) (Table 5b & Fig. 8).

Table (1): Monthly variation of the gonadosomatic index (GSI) of females grass carp, *C. idella* during the period from January to December, 2021.

Months	Min.	Max.	Average± SD
Jan.2021	0.345	0.624	0.3664±0.025
Feb	0.436	0.728	0.5663±0.091
Mar	0.345	0.624	0.4785±0.101
Apr	2.812	16.467	8.874±0.4668
May	16.228	19.542	17.645±0.381
Jun	8.025	8.803	8.289±0..253
Jul	2.861	3.49	3.152±0.22
Aug	0.827	0.92	0.868±0.033
Sep	0.207	0.3	0.24±0.039
Oct	0.129	0.22	0.161±0.034
Nov	0.156	0.22	0.184±0.023
Dec.2021	0.16	0.257	0.208±0.036

Each result represent the main STD (N=10)

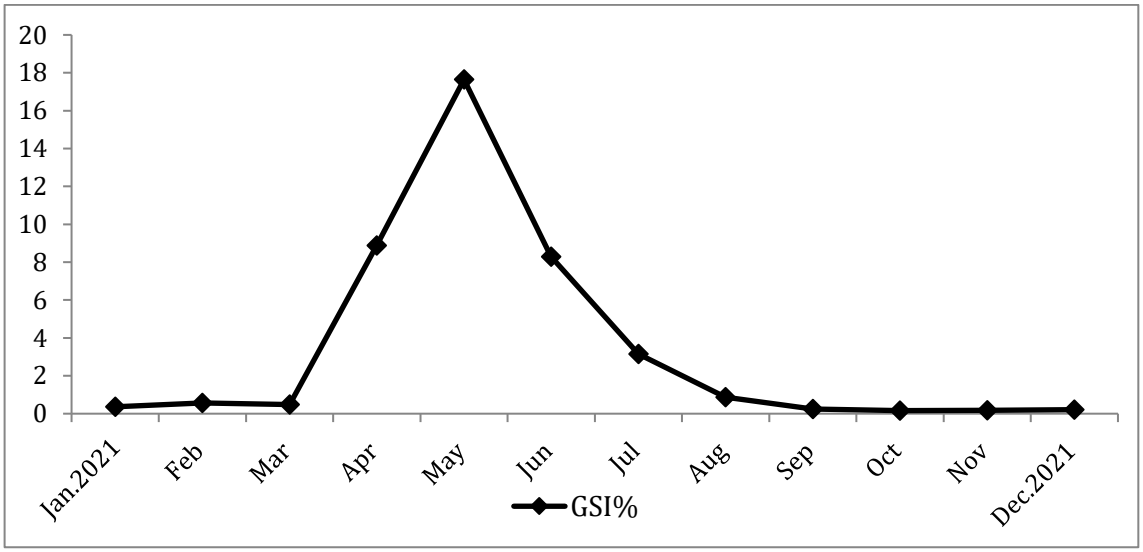

Fig. (2): Monthly variation of the gonadosomatic index (GSI) of females grass carp, *C. idella* during the period from January to December, 2021

Table (2): Monthly variation of the Hepatsomatic index (HSI) of females grass carp, *C. idella* during the period from January to December, 2021 .

Months	Min.	Min.	Max.	Average± SD
Jan.2021	3.41	3.41	4.1	3.681±0.214
Feb	1.978	1.978	3.87	3.035±0.678
Mar	4.235	4.235	2	2.736±0.776
Apr	1.025	1.025	2.506	1.859±0.517
May	1.217	1.217	1.53	1.389±0.098
Jun	1.475	1.475	1.9	1.695±0.144
Jul	1.65	1.65	1.9	1.746±0.086
Aug	2.49	2.49	2.8	2.62±0.096
Sep	2.754	2.754	2.94	2.855±0.06
Oct	3.057	3.057	3.95	3.331±0.288
Nov	3.201	3.201	6.3	3.395±0.132
Dec.2021	3.4	3.4	3.91	3.685±0.168

Each result represent the main STD (N=10)

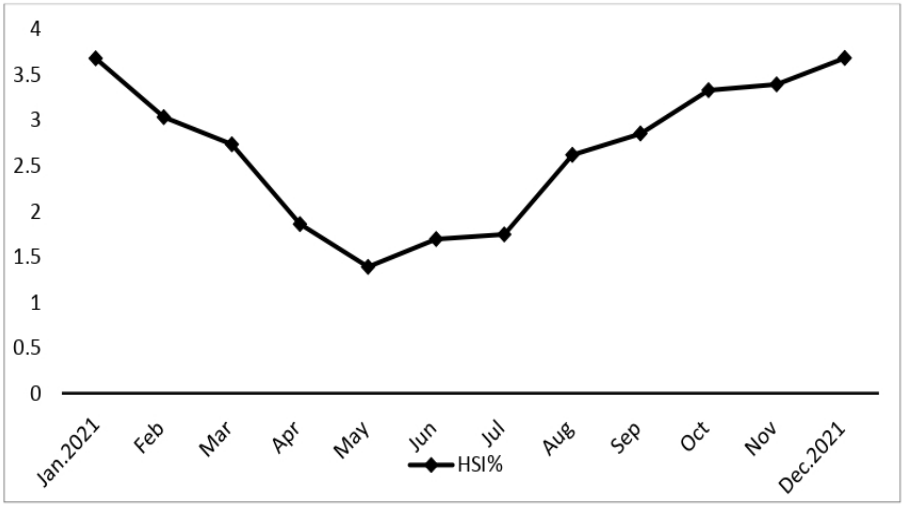

Fig. (3): Monthly variation of the hepatosomatic index (HSI) of females grass carp, *C. idella* during the period from January to December, 2021.

Table (3): Total protein, total lipid, HSI and GSI in ovarian tissue during maturation stage females of grass carp, *C. idella*

Stage	T-protein g/100g in dry weight of ovary			T-lipid g/100g in dry weight of ovary			Average GSI	Average HSI
	T-protein	Max.	Min.	T-lipid	Max.	Min.	%	%
Immature	60.65±0.299*	60.77	60.05	5.77±0.070*	5.92	5.63	3.5	3.23
Maturing	66.84±0.420*	67.52	66.17	12.77±0.111*	13.07	12.63	4.68	2.18
Ripe	70.88±0.273*	71.44	70.8	21.75±0.337*	22.46	21.05	14.79	1.64
Atretic	63.79±0.110*	64.01	63.58	14.8±0.279*	15.28	14.33	3.097	2.15

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

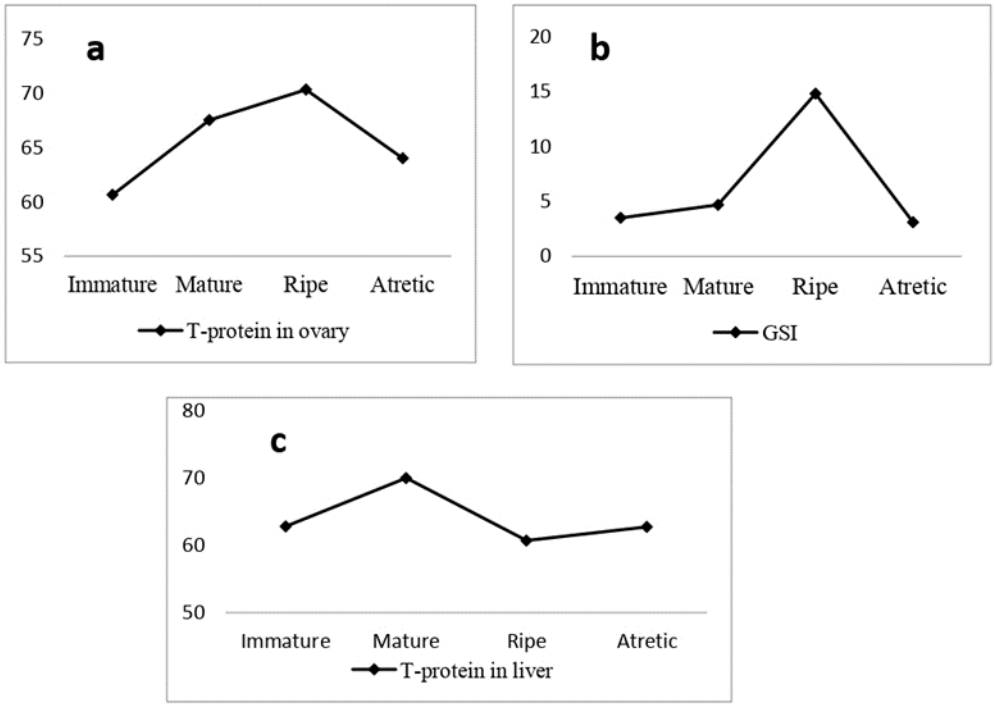

Fig. (4): Relationship between concentrations of total protein content (g/100g) in ovarian and liver tissue and GSI during maturation stage of females grass carp, *C. idel*

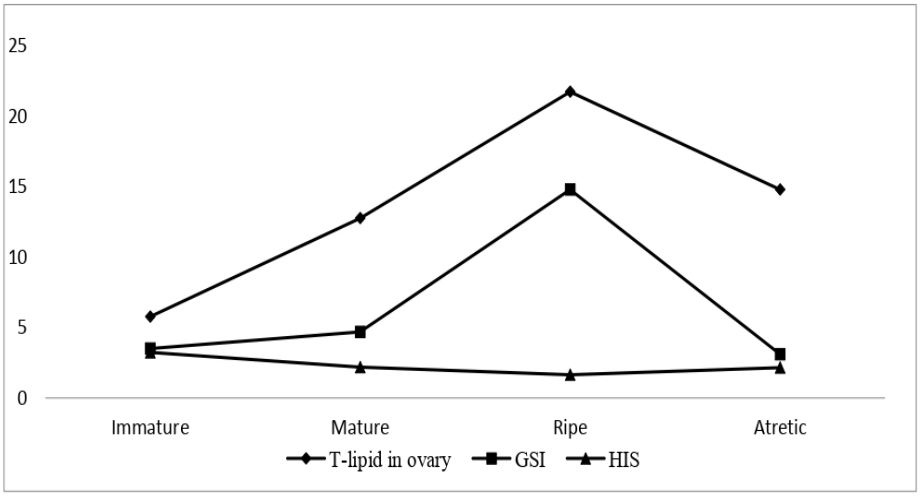

Fig. (5): Relationship between concentrations of total lipid (g/100g) in ovarian tissue and GSI during maturation stage of females grass carp, *C. idella*.

Table (4): Total protein, total lipid and HSI and GSI level in liver tissue during maturation stage of female grass carp, *C. idella*

Stage	protein g/100g dry weight of liver			T-lipid g/100g in dry weight of liver			Average GSI	Average HSI
	T-protein	Max.	Min.	%	Max.	Min.	%	%
Immature	62.84±0.543	63.08	62.44	14.15±0.106*	14.28	14.02	3.5	3.23
Maturing	70.03±0.155*	70.25	69.85	15.97±0.657*	16.78	15.17	4.68	2.18
Ripe	60.72±0.709*	61.299	59.72	7.22±0.077*	7.32	7.13	14.79	1.64
Atretic	62.76±0.261	63.08	62.44	12.53±0.045*	12.59	12.48	3.097	2.15

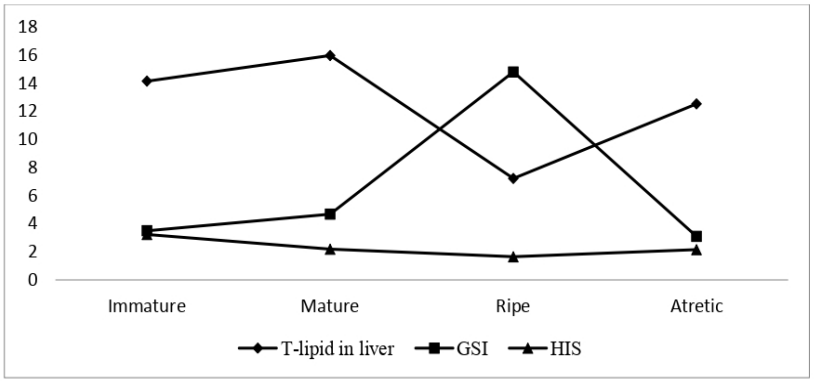

Fig. (6): Relationship between concentrations of total lipid content (g/100g) in liver tissue, HSI and GSI during maturation stage of females grass carp, *C. idella.*

Table (5A) Variation of Protein, lipid in the serum during maturation stage of female grass carp, *C. idella.*

Stage	Serum protein g/dl			Serum Lipid mg/dl		
	Main Protein ±SD	Max.	Min.	Main Protein ±SD	Max.	Min.
Immature	*0.4±0.012	0.42	0.38	*314±3.8	320	308
Maturing	*1.3±0.021	1.36	1.24	*343±4.4	3.5	333.8
Ripe	*2.45±0.086	2.59	2.38	*374±5.7	383	366
Atretic	*0.67±0.031	0.71	0.64	*234±1.6	240	229

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

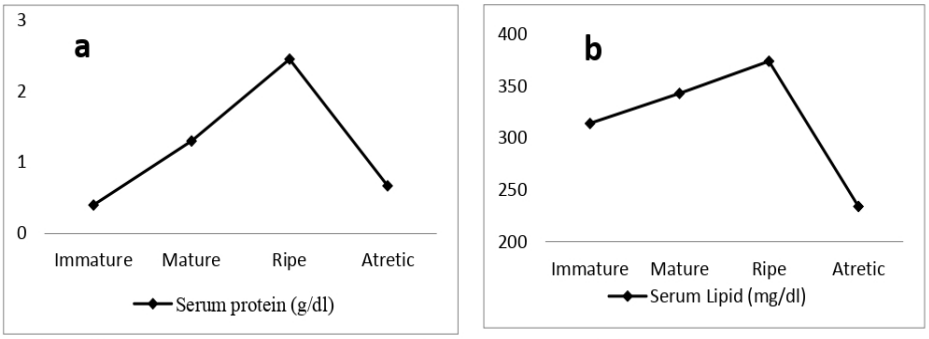

Fig. (7): Variation of total cholesterol lipid and protein (mg/dl) concentrations in serum during maturation stage of females grass carp, *C. idella*.

Table (5b) Variation of Cholesterol and Serum estradiol during maturation stage of female grass carp, *C. idella.*

Stage	Cholesterol mg/dl			Serum estradiol (pg/ml)		
	Cholesterol ±SD	Max.	Min.	Average ± SD	Max.	Min.
Immature	*77±3.3	83	71	*524±3.265	528	520
Maturing	*249±1.2	255	245	*381±1.885	384	379
Ripe	*114±5.1	120	108	*471±3.091	475	468
Atretic	85±4	88	79	*108±1.699	110	106

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

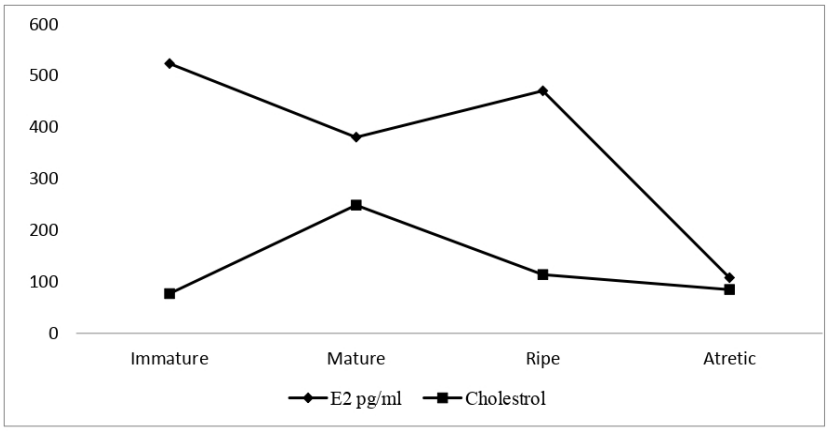

Fig. (8): Relationship between concentrations of total cholesterol in serum mg/dl and estradiol (E2) during maturation stage of females grass carp, *C. idella*.

DISCUSSION

The annual fluctuation of steroids hormones' concentration in relation to the maturation stage in the gonads of several teleosts species has been studied. These studies postulated that the annual variation of steroids hormones concentration is closely related to factors such as fish species, temperature, sex and length of day **(Pavlidis *et al.*, 2000)**. On the other hand, **Abbasi *et al.* (2008)** added that, seasonal variation of steroid hormones can be related to feeding habits, reproductive cycle and growth rate of fish. 17-B estradiol (E_2) is the main of ovarian steroid hormone in teleost fishes, which is produced by hepatic yolk precursor as stated in the study of **Jerez *et al.* (2006)**. **Erdouan *et al.* (2002)** found that, in case of *Capoeta umbla*, the seasonal changes in both serum lipids and steroid hormones are associated with the reproductive activity. The present results showed that, (E_2) concentration in the serum of females *Ctenopharyngodon idella* at the beginning of maturation maintained a high level and then decreased in the mature stage. This finding coincides with that of **Heidari *et al.* (2010)**. The level of serum cholesterol was recorded the lowest during the immature stage, and with the progress of maturation, the value elevated to record the highest level value in the maturing stage. These results concur with those of **Nasari *et al.* (2014)** in case of *Acanthopagrus latus*. The period of gonad maturation usually was depending on the action of steroid hormone. The decrease in the cholesterol level during the ripe stage can be explained with the available cholesterol consumed in the synthesis of steroid hormone. This conclusion was also reported in the study of **Shankar and kulkarmi (2007)** who suggested the conversion of cholesterol into steroid hormone as gonadal maturation progressed.

In the present study, with the exogenous accumulation of protein and lipid (during proliferation period) and with acceleration of vitellogenesis, E_2 returned to increase again to form a peak during the ripe stage. Our results are confirmed by previous authors **(De Vlaming *et al.*, 1980; Smith & Haley, 1988; Alvarado *et al.*, 2015)**. The results of the present study on grass carp showed that the level of E_2 increase with the progress of exogenous accumulation of protein and lipid in the ovary towards the ripe stage, with a peaked value of 471pg/ ml, and then declined to reach the minimum level in serum 108pg/ ml during the atretic stage. These findings agree with those of previous studies **(Mayer *et al.*, 1990; Schulz *et al.*, 2010; Alvarado *et al.*, 2015)**. In case of female grass carp under study, the peak of (E_2), and GSI are consistent in the ripe stage and declined in the atretic stage. In addition, **Sabet *et al* (2009)** found that the (E_2) and testosterone (T) reached their higher values in April, coinciding with the peak of GSI.

In the present study, the increase in cytoplasmic mass during the protoplasmic growth period chromatin nucleolus stage and perinucleolus stage was mainly due to the accumulation of non-yolky materials **(Sheha, 2011)**. At the same time, the values of protein tissue and serum cholesterol were the lowest. During trophoblastic accumulation period (yolk vesicle stage and yolk granules stages), the mainly cytoplasmic mass is attributed to the accumulation of lipids and proteins. During this stage, the content in the ovarian tissues and the level of serum lipid increased during the mature ovary and peaked in the ripe stage. These results are confirmed with applying bromophenol blue stain that reacted with total protein. As vitellogenesis started, the cell size reached the maximum size, GSI peaked and protein content in ovarian tissue and the level lipid in serum attained highest value. The yolk déposited in the cytoplasm appeared as small rod

granules and showed deeply stained with PAS reaction and bromophenol blue reaction that ensures accumulated polysaccharides and protein materials **(Sheha, 2020)**. An inverse relationship was attained between hepatosomatic index (HSI) and protein and lipid content in the ovarian tissue since during less developed stage (immature and maturing stages), the HSI attained the maximum value and decreased with maturation; the protein and lipid content of ovarian tissues showed inverse fluctuation. Thus, the protein that accumulated in the ova cytoplasm secreted in liver tissue and transported in blood stream and deposited in the ovary **(Tyler & Sumpter, 1996; pawlowski *et al*., 2000; sheha, 2020)**.

CONCLUSION

It can be concluded that, the fluctuation in ovarian protein levels were accompanied with the variation in the weight of the ovary. The value of GSI in grass carp was 3.5% in immature stage. While, the peak of protein content in the ovary was during the ripe stage with gonadosomatic (GSI) of 14.79 %. The lipid content in the ripe stage reached a peak value of 21.75±0.337g/ 100g of dry weight. On the other hand, the cholesterol level in the serum during the ripe stage was consumed in the synthesis of steroid hormone. Present result cooperate to give a preif picture gonadal maturation as described in the present species .

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