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LIPID AND PROTEIN CONTENT IN RAINBOW TROUT IN RELATION TO GONADAL GROWTH

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ABSTRACT

The purpose of this study was to examine various lipid classes as well as the protein content in rainbow trout ovaries. A total of 80 samples of *Oncorhynchus mykiss* (Walbaum 1792) were collected from Kokernag and Verinag hatcheries (Jammu and Kashmir, India) between 2017 and 2019. The mature stage in female fish had the highest gonadosomatic index (GSI) and minimal GSI in the spent stage. Higher lipid content of the ovary was reported during the mature stage (36%), as compared to other pre-breeding stages. Similarly, lipid classes also showed fluctuation during the development of the ovary. The lipid classes, i.e. glycolipids, free fatty acids, cholesterol and triglycerides, had a higher content in the mature stage, while the phospholipid content was higher in the mature stage. The protein content was also higher in the mature ovary than in the other developmental stages. The study provides reference values for various biochemical parameters in rainbow trout that could be useful for population monitoring programs and for the development of diets and management methods for fish production under controlled conditions.

How to Cite

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INTRODUCTION

Rainbow trout *Oncorhynchus mykiss* is native to coastal watersheds of the eastern Pacific Ocean where it ranges from Baja California in the south to Kamchatka Peninsula and Alaska in the north (Stewart and Wakinson, 2004). At present, rainbow trout is farmed all over the world as a result of its introduction to every continent except Antarctica. Knowing the biochemical composition of fish gonads is very beneficial for assessing their nutritional content as well as their quality, environmental and physiological status.

Fish lipids are the primary source of energy for reproduction and large amounts of lipids are required for egg maturation and development during the reproduction of female fish (Goda et al., 2007; Ebrahimnezhadarabi et al., 2011). The changes in lipid content and categorization in the gonads correlate with fish maturity and spawning (Mourente and Odrizola, 1990). Lipid fluctuations occur especially in the ovary, testis and liver, leading to variability in gonadosomatic (GSI) and hepatosomatic indices (HSI) (Love, 1980). During reproduction period, higher energy expenditure is needed and the lipid reserve in the liver and muscles is then mobilized and moved to the gonad during fish maturation and spawning (Zaboukas et al., 2006; Sutharshiny and Sivashanthini, 2011; Singh et al., 2012).

Lipids are a diverse family of compounds classified into two groups: one is a polar lipid composed of phospholipids and the other is a non-polar lipid composed of triglycerides and cholesterol (Tochler, 2003). The primary lipid of cell membranes is a phospholipid, which is an essential component of egg yolk in fish (Johnson, 2009). Triglycerides have been found to be the main source of energy storage in fish and are stored in the liver and muscles. Cholesterol acts as a precursor to steroid hormones and also performs several important cellular functions in the testes (Scott, 1987; Sharpe et al., 2006). Lipids, such as phospholipids and cholesterol, are also primary units of cellular and subcellular membranes, and necessary for cellular differentiation (Schiopu et al., 2006; Liu et al., 2007).

Fatty acids (FA) form lipids and some of them such as dihomo-gamma-linolenic acid (20:3 n6, DHGLA), arachidonic acid (20:4 n6, AA), eicosapentaenoic acid (20:5 n3, EPA) and docosahexaenoic acid (22:6 n3, DHA) are required for a variety of physiological processes in animals (Guler et al., 2007).

Proteins are important biopolymers that can be derived from both animal and plant sources and are recognized as rich sources of nutrients for the body's growth and development. In recent years, their exceptional bioactivities and functions have been explored and their popularity has increased due to their contribution to health promotion and food processing (Rehman et al., 2020). Being considered the richest source of protein among a variety of protein sources, fish plays an active role in human society in terms of nutrition, economy, culture and recreation (Lynch et al., 2016).

Fish protein is known for its content of important amino acids (tryptophan, cystine, lysine, methionine and threonine). It is reported that the protein content varies from species to species and within the same species. Depending on the age, sex and reproductive status of the fish, the biochemical composition of the gonads and muscle may even vary within the same species (Shreni, 1980; Ali et al., 2001; Bhuyan et al., 2003). Both the ovaries and testes increase in size many times over during the maturation phase of the fish, leading to an increase in protein content.

However, evidence of seasonal variation in many biochemical components of the ovary in rainbow trout from Kashmir rivers is lacking. Therefore, the aim of this study was to assess GSI, lipid content and proteins in the female gonads of rainbow trout at different stages of sexual maturity to contribute to the scientific knowledge on proper reproductive management of this species and to understand the nutritional requirements of this commercially important exotic fish in hatcheries.

MATERIALS AND METHODS

Sample collection

Rainbow trout fish used in the present study were collected at regular intervals twice a month per year. The sampled fish were dissected to determine the sex and sexual maturity stage. Ovaries were examined and weighed. The fish were sampled from Verinag (33.55°N and 75.25°E) and Kokernag (33.69°N and 75.22°E) hatcheries in Jammu and Kashmir (India). The water temperature reported in the hatcheries was 11°C-13°C (±0.6°C). The feed ingredient used for fish was composed of fish meal, wheat, soybean, oil, sodium alginate, mineral mix, Vitamin B complex, Vitamin A, B2, D, K3, Vitamin C and Vitamin E. A total of 80 samples of female rainbow trout (n=20/reproductive stage), ranging from 22 to 36.6 cm in length, from 360 to 660 g in weight and from 6.2 to 8.4 cm in width, were analyzed between 2017 and 2019 to determine various assays. All the determinations were made in triplicate. All the fish handling was conducted in the state of anesthesia (MS-222 (300 ppt), Finquel, USA). The biometric indicators such as gonado-somatic index (GSI) values for each month were used according to the following formula: GSI % = gonad weight (g) /body weight (g) X 100.

Biochemical analysis

Protein determination

0.5 g of rainbow trout fish ovarian tissue was mixed with 5 ml of 0.1 M Tris-HCL, pH 8.6, and mechanically homogenized to produce ovarian protein. Ovarian protein samples were centrifuged using GeNei™ centrifuge (India) at 4°C at 6,000 rpm for ten minutes. Pipetted into vials,

the supernatant liquid was held at -20°C before being assayed by Lowry et al. (1951).

Lipid determination

Crude lipids were extracted from the sampled tissue to determine the lipid profile. Total lipid extraction from ovarian samples was performed according to Folch et al. (1957). According to this method, 5 g of ovarian tissue were ground in triplicate at each developmental stage of the fish with 10 g of anhydrous sodium sulfate, and thus the samples were dehydrated. As a result, an anhydrous homogeneous paste was obtained. For total lipid extraction, the paste was shaken in a 1:15 (w:v, weight: volume) methanol-chloroform mixture (1:2 (v:v, volume: volume) for 12 hours. After 12 hours, the agitated mixture was filtered and the organic solvents in the filtrate were evaporated. The oil obtained at the end of the process was used to determine the lipid profile. The glycolipids were carried out following Roughan and Batt (1968), free fatty acid analysis was applied according to Lowry and Tinsley (1976), phospholipid determinations were applied according to Ames (1966) and triglycerides were determined according to the method by Vanhandel and Zilversmit (1957).

Statistical analysis

The results of the analyses were subjected as means ± SEM. Statistical analyses were conducted using SPSS 16.0 software (IBM Inc., Armonk, NY, USA). Statistical significance between different stages was determined by one-way analysis of variance, and Turkey's test was used to detect significant differences between means (P<0.05). The 5% level was taken as the significance level.

RESULTS

The changes in the GSI during gonad development in a female rainbow trout (Fig. 1) increased rapidly from the immature to the mature stage. GSI was found to be maximum during the mature stage.

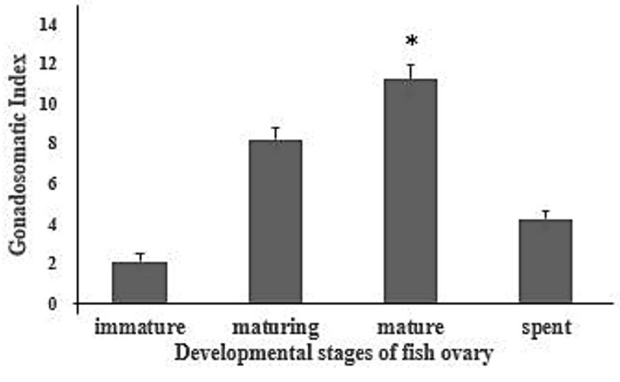

Fig 1. Mean value of gonadosomatic (GSI) indices through breeding cycle of fish. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

During this stage, the female fish was ready to ovulate. The GSI recorded was 11.121. The GSI decreased sharply during the spent stage. The increase was gradual from the spent stage to the maturing stage (Fig. 1). There were significant differences between the mature stage and other breeding stages (Tukey's method of the one-way ANOVA test, $p < 0.05$).

Biochemical changes in rainbow trout ovary

Protein estimation

The protein content differed in fish ovaries at various stages of development. It rose from the immature stage to the mature stage, and the spending phase subsequently showed a small decrease. The protein content was 43.28 ± 2.3 mg/g during the immature period, while the protein content obtained for the maturing stage was 72.4 ± 6.8 mg/g. The protein content was measured as 112.6 ± 8.3 mg/g in the mature stage, while the protein content was decreased to 36.9 ± 1.2 mg/g in the spent phase. There were significant differences between the mature stage and other breeding stages (Tukey's method of the one-way ANOVA test, $p < 0.05$) ($P < 0.05$) (Fig. 2).

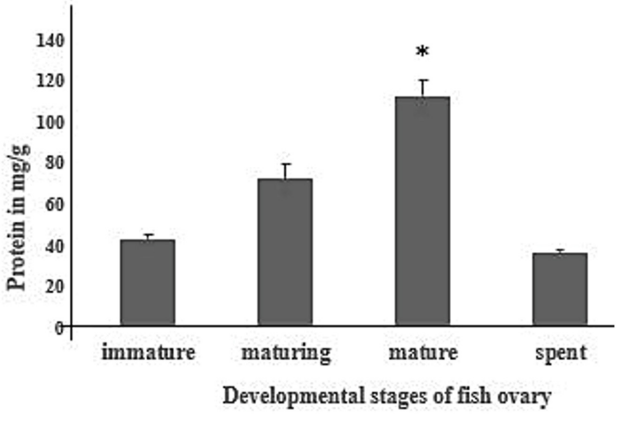

Fig 2. Protein content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

Lipid and their class estimation

The total lipid content in the ovary of rainbow trout fluctuated with maturity to reach a maximum value in the mature ovary (36%), while the minimum value was noticed in the spent stage (9%). There were significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$) (Fig. 3). Cholesterol was also determined during the development of the ovary.

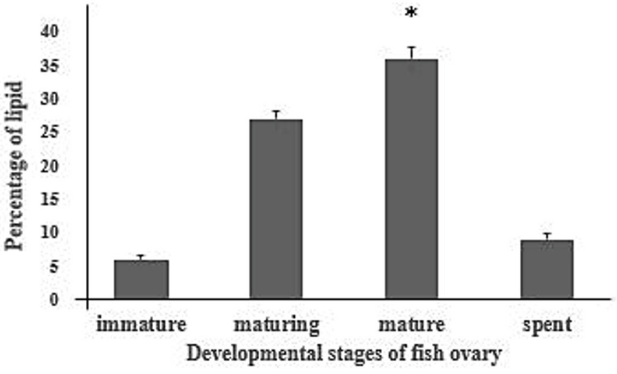

Fig 3. Percentage of lipid in ovary of rainbow trout. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

The results follow the same pattern as that of lipids in the ovary (Fig 4). There were significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$) (Fig. 4).

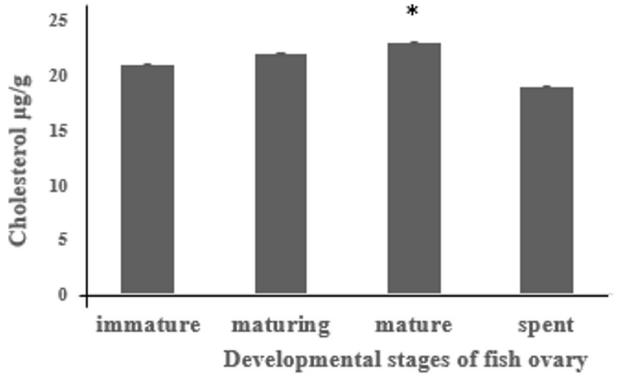

Fig 4. Cholesterol content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

In ovaries, glycolipid concentrations showed a clear accumulative pattern through maturation. Glycolipid increased significantly during the mature stage to reach the maximum levels of 29 ± 0.12 µg/g, then glycolipid decreased drastically with a value of 16 ± 0.04 µg/g 20 ± 0.09 µg/g. The increase was gradual from the immature to the mature stage. There were significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$) (Fig. 5).

The free fatty acid content also increased from the immature stage to the mature stage. After the breeding stage, there was a decrease in the free fatty acid content (Fig. 6). There were significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$) (Fig. 6).

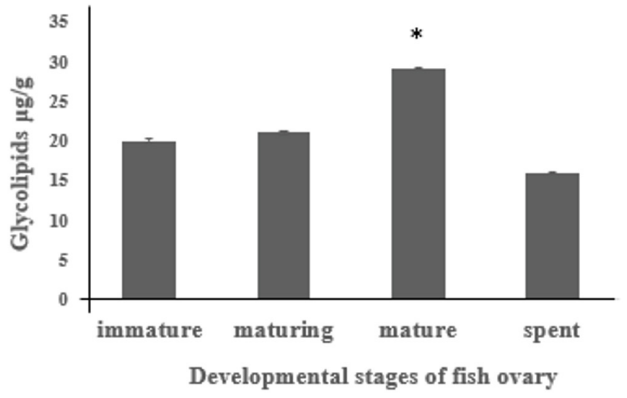

Fig 5. Glycolipid content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

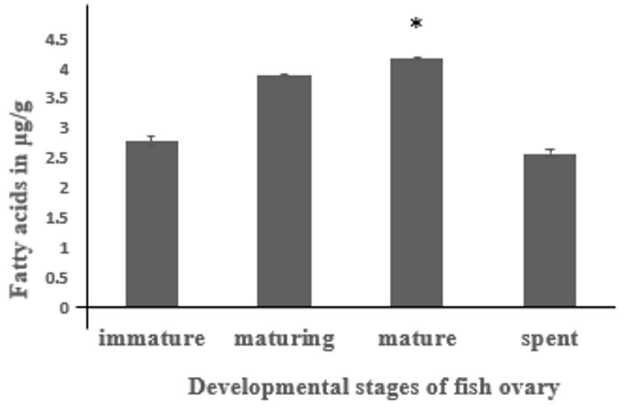

Fig 6. Free fatty acids content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

Phospholipids also showed an accumulative pattern in the ovary of rainbow trout. There was a slight increase in the phospholipids from the immature to the maturing stage 2.54 ± 0.07 µg/g. There were significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$) (Fig. 7).

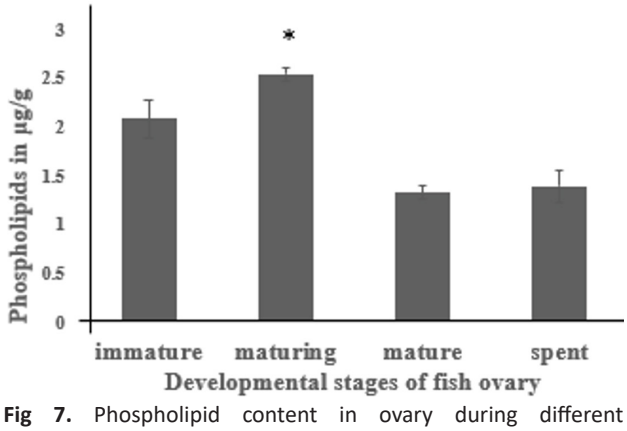

Fig 7. Phospholipid content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

In mature rainbow trout females, the total ovarian content of triglycerides showed significant differences between the mature stage and GSI (Tukey's method of the one-way ANOVA test, $p < 0.05$). In the immature stage, the triglyceride content was 15 ± 0.07 µg/g. It steadily began to increase in the maturing stage with a value of 17 ± 0.014 µg/g (Fig. 8).

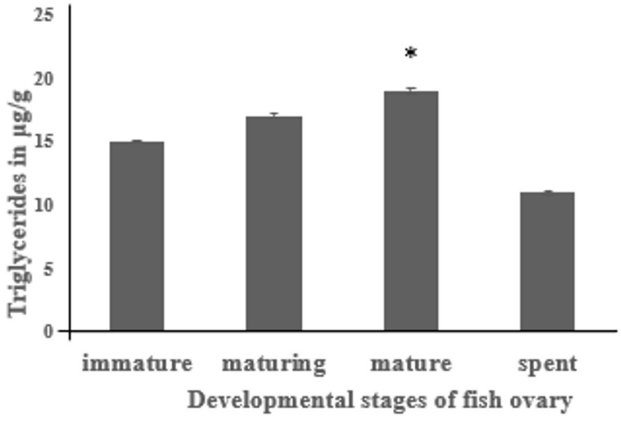

Fig 8. Triglyceride content in ovary during different developmental stages. Values are presented as means ± SEMs (n=20/reproductive stage), differences considered statistically significant when $p < 0.05$ and are denoted with asterisks

DISCUSSION

Rainbow trout is a fish with a synchronized annual reproductive cycle. The GSI not only reflects the timing of ovarian maturation and spawning in rainbow trout but can also be used to identify the reproductive phase or peak of the fish. The present study focuses on reproductive biological factors and ovarian maturation studies involving GSI, proteins and total lipids, including its various classes. In female rainbow trout, the highest GSI was found in the mature phase and the lowest in the spent phase. Similar results were obtained in previous studies on fish such as trout and *Dentex dentex* (Wallaert and Babin, 1994; Chatzifotis et al., 2004).

According to the present results, the biochemical profile of the ovary of rainbow trout changed during the breeding cycle. Biochemical analyses such as protein and lipid analysis, including their different classes, were significantly higher in the mature stage of the ovary, indicating their function in maturation. The protein content was also higher at the mature stage, indicating that the ovaries have a higher demand for proteins at this stage.

In terms of protein, it has been reported that protein concentrations (% wet gonad weight) rose with gonad maturation. El-Halfawy and Amadan (2015) reported that protein exhibited a positive relationship with the maturation of gonads in *Nemipterus japonicus*. A similar type of positive correlation to gonadal maturation was reported in the present study. Similar observations in other teleosts were also made for protein content by Nuriyal and Singh (1985) in *Puntius chilinoides*, and Sivakami et al. (1986) has reported the maximum crude protein content in the ripe fish and the minimum in spent and early maturation stages in *Cyprinus carpio*. However, Kurbah and Bhuyan (2018) reported higher content of protein during pre-breeding stages and lower in the spent stages of *Monopterus cuchia*.

The present results show that the proportion of lipids changes at each stage of ovarian development in fish. During the development of the ovaries from immaturity to maturity, the lipid content increased from immaturity to maturity and then decreased at the maturity stage. In a study conducted on *Heteropneustes fossils*, it was observed that during the mature phase of the ovaries, its lipid content tends to increase (Singh and Singh, 1979; Shreni, 1980). A different species of fish *Labeo dyocheilus* has been used in research that obtained similar results (Verma, 2013).

Lipids are important structural and energetic elements that aid gonadal development. Lipoprotein lipase (Lpl) hydrolyzed lipids carried by plasma very low density of lipoprotein (VLDL), releasing dietary fatty acids that are used as an immediate energy source by surrounding tissues or re-esterified for preservation (Tochler, 2003). Various lipid classes such as cholesterol, glycolipids, phospholipids, free fatty acids and triglycerides were analyzed in rainbow trout ovaries during the present study. The results of the present research showed that the content of cholesterol was highest in the mature period and minimum in the spent phase. In *Halobatrachus didactylus*, related observations have also been documented by Munoz-Cueto et al. (1996). During the incubation period, the cholesterol content in the ovary of Notopterus notopterus increased and the highest cholesterol content was found during this phase (Shankar and Kulkarni, 2007). The increase in cholesterol volume is attributed to the need for ovarian development and vitellogenesis to synthesise cortisol.

The content of phospholipids improved from the stage of immature to the stage of maturing. The phospholipid value consequently declined in the mature and spent stages. In *Halobatrachus didactylus*, related observations have also been previously documented (Munoz-Cueto et al., 1996). It has been reported that phospholipids contributed significantly to total lipids in *Thunnus albacares* gonads (Tochler, 2003) since phospholipids play an important role in cellular membranes and tissue formation, thus these results are in agreement with the present study. Another study on *Salmo salar* has reported significant differences in fatty acid and phospholipids between immature and mature ovaries (Bogevik et al., 2020). These previous results are in the support of the present study. In the present study, it was observed that the highest volume of glycolipids was in the reproductive stage and the lowest was in the non-reproductive stage. Triglycerides are an essential lipid class and have exhibited fluctuations at

various stages of ovarian development. The highest level of triglycerides was observed in the present study during the mature phase, while the lowest amount was found during the spent phase. These observations are supported by the findings of *Halobatrachus didactylus* by Munoz-Cueto et al. (1996). Excessive dietary energy is stored in the form of triglycerides deposited in adipose and gonads. These are an important fat deposits in salmonids, including rainbow trout during maturation (Kiessling et al., 1991).

CONCLUSION

Understanding the biochemical makeup of fish helps clarify their nutritional, physiological and environmental status. The ratio between the weight of the gonads and the body weight of the fish is called the gonadosomatic index (GSI). Since the size of the ovaries of gravid females increases rapidly during the spawning season, the GSI is particularly useful in determining the spawning season. The knowledge gained can contribute to better management and avoidance of fish catches during the breeding season in order to maintain fish diversity. The data can be used as indicators of the dietary requirements of the broodstock, although it also encourages further research on the seasonal variation in the reproductive biology of rainbow trout from Kashmir hatcheries.

AUTHOR CONTRIBUTIONS

All authors were involved in the conception and design of the study. The article was written by Rayees Ahmad Bhat, Osman Sabri Kesbiç and Francesco Fazio. Material preparation, data collection and analysis were carried out by Rayees Ahmad Bhat, Francesca Arfuso and Concetta Saoca. The data were analysed by Enrico D'Alessandro and Alessandro Zumbo. All authors commented on earlier versions of the manuscript. All authors read and approved the final manuscript.

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SADRŽAJ LIPIDA I PROTEINA KOD KALIFORNIJSKE PASTRVE U ODNOSU NA RAST GONADA

SAŽETAK

Svrha ovog istraživanja bila je istražiti različite tipove lipida kao i sadržaj proteina u jajnicima kalifornijske pastrve. Ukupno 80 uzoraka *Oncorhynchus mykiss* (Walbaum 1792) prikupljeno je iz mrijestilišta Kokernag i Verinag (Jammu i Kashmir, Indija) između 2017. i 2019. godine. Zreli stadij gonada kod ženki imao je najviši gonadosomatski indeks (GSI) i minimalni GSI u potrošenom stadiju. Veći sadržaj lipida u jajniku zabilježen je tijekom zrele faze (36%) u usporedbi s drugim fazama prije parenja. Slično, klase lipida također su pokazale fluktuacije tijekom razvoja jajnika. Klase lipida, tj. glikolipidi, slobodne masne kiseline, kolesterol i trigliceridi, imale su veći sadržaj u zrelom stadiju, dok je sadržaj fosfolipida bio veći u zrelom stadiju. Sadržaj proteina također je bio veći u zrelom jajniku, nego u drugim razvojnim fazama. Studija daje referentne vrijednosti za različite biokemijske parametre kalifornijske pastrve koji bi mogli biti korisni za programe praćenja populacije i za razvoj prehrane i metoda upravljanja u kontroliranim uvjetima.

Ključne riječi: kalifornijska pastrva, lipidi, riblji jajnici, glikolipidi, sezona parenja, bjelančevine

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