Age-Related Variations in the Histochemical Profile of Hepatic Tissues in Common Carp (Cyprinus carpio)

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Abstract

This study investigated the histochemical distribution of total proteins and glycogen in the liver of common carp (Cyprinus carpio) concerning age and sex. Twenty specimens were collected from the Tigris River and categorized into two age groups (3 and 6 months). Liver sections were processed histologically and stained with Mercuric Bromophenol Blue (BPB) for proteins and Periodic Acid-Schiff (PAS) for glycogen. Quantitative analysis was performed using imagej software. Results revealed that adult fish exhibited significantly higher concentrations of both proteins and glycogen compared to juveniles, indicating enhanced metabolic capacity with maturity. Furthermore, females showed markedly greater reserves than males, particularly in the adult group, which is attributed to the energy-intensive process of vitellogenesis. The findings confirm that hepatic metabolic storage in C. Carpio is a dynamic process regulated by developmental stages and reproductive demands.

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Introduction

The determination of total protein content in fish liver is a critical biochemical indication, representing both the animal's nutritional state and its overall hepatic efficiency. In fish, the liver includes a wide range of protein types, from cytoplasmic soluble proteins (enzymes and transcription factors) to structural proteins linked with the cytoskeleton and organelles. These proteins have important roles in metabolism, transport, exocrine activities, and as blood components (Oladimeji et al., 2018; Zhang et al., 2022).

Typically, protein content in liver tissue accounts for 15% to 25% of the fish's weight; however, these levels are extremely dynamic and change depending on species-specific features, health state, environmental stresses, and, most importantly, the fish's age (Foeger et al., 2020).

Hepatic protein concentrations typically peak during periods of vigorous development, while hunger or physiological weariness can cause a considerable drop (Oladimeji et al., 2018). To measure these levels, various standardized biochemical assays—such as the Lowry, Bradford, and BCA techniques—are used to give quick

Furthermore, histochemical techniques based on protein-specific dyes such as Bromophenol Blue (BPB) enable accurate localization and semi-quantitative study of protein distribution inside hepatocytes. This combination of biochemical and histological data reveals differences in protein expression across different liver areas, indicating the metabolic activity of organelles such as the endoplasmic reticulum and mitochondria (Martinez & Chen, 2020; Oliveira et al., 2019).

In addition to protein analysis, hepatic glycogen levels are an important measure of energy stores and metabolic state. Normal glycogen levels in fish liver vary between 2% and 10% of fresh weight, providing a readily available energy source to maintain cellular metabolism and blood glucose levels during dietary absences (Siddique et al., 2019).

The storage and mobilization of glycogen, which is predominantly controlled by hormones such as insulin, glucagon, and adrenaline, is critical for fish to adjust to seasonal food supply (Kumar et al., 2021; Zhang et al., 2020). Histologically, glycogen is stored as intracellular granules within glycosomes, which may be seen and measured by Periodic Acid-Schiff (PAS) staining (Li et al., 2022).

The physiological interaction of protein and glycogen shows the liver's overall response to environmental and biological influences. Low glycogen levels frequently suggest energy deficit or excessive metabolic demand, whereas protein concentration changes may reflect alterations in biosynthetic pathways or cellular stress (Li et al., 2021; Wang et al., 2020).

As a result, a thorough assessment of fish health and hepatic metabolic status necessitates an integrated method that includes the measurement of total protein and glycogen reserves. Such a comprehensive examination, particularly when contrasted across age groups, gives important insights into fish physiological adaptations and metabolic efficiency in their natural environments (Martinez & Lopez, 2020; Patel et al., 2023).

Material and method

Fish Collection and Sampling

Twenty common carp (Cyprinus carpio), ten at three months and ten at six months of age, were collected from a fish farm on the Tigris River in Wasit Governorate. The selection was based on health and sexual maturity. To reduce stress, the fish were transported in Styrofoam containers with original farm water and euthanized humanely upon arrival. Liver samples were then extracted for histological analysis.

Sample Collection and Fixation

Immediately following euthanasia, the fish were dissected ventrally to expose the internal organs, at which point liver samples measuring approximately 0.5 \times 0.5 \times 0.5 cm were carefully excised from the lobes using sterile surgical instruments. To preserve the cellular architecture and prevent autolysis, these specimens were immediately immersed in 10% neutral buffered formalin. This fixation process was maintained for 24–48 hours at room temperature using a specific tissue-to-fixative volume ratio of 1:10.

Histological Processing

The fixed liver specimens from both age groups were processed for histological and histochemical analysis following the standardized protocol described by Suvarna et al. (2018). This procedure began with a dehydration series to remove the fixative and cellular water using increasing concentrations of ethanol. The samples passed through six consecutive stations—70%, 80%, 90%, 95%, and two changes of 100%—for 45 minutes each to ensure complete water replacement while preserving tissue integrity. Following dehydration, the samples were cleared of alcohol using two changes of xylene, the first for 45 minutes and the second for one hour. The tissues were then infiltrated with molten paraffin wax in an oven maintained at 58–60°C for two hours to replace the clearing agent. Once infiltrated, the specimens were oriented and embedded into paraffin wax blocks using molds, which were then allowed to solidify at room temperature for 24 hours to provide the necessary support for thin sectioning.

For the final preparation, a rotary microtome was utilized to cut the paraffin blocks into thin sections with a thickness of 6 µm. These sections were floated in a warm water bath maintained at 5–10°C below the wax melting point to remove wrinkles before being carefully mounted onto clean glass slides coated with a thin layer of egg albumin-glycerin (1:1) mixture. To prevent fungal growth, a small amount of thymol crystals was added

to the adhesive. Finally, the slides were dried in a thermostatic oven at 40°C for 24 hours to ensure proper tissue adhesion prior to the staining process.

Histochemical Staining Protocols

The assessment of metabolic and structural components within the liver tissue was conducted using two specialized histochemical staining techniques. To assess the total protein content, liver sections were subjected to the Mercuric Bromophenol Blue (BPB) technique according to Chapman (1975). This involved immersing deparaffinized sections for two hours in a solution of 1 g mercuric chloride and 0.05 g sodium bromophenol blue dissolved in 100 ml of 2% aqueous acetic acid. After incubation, differentiation was achieved with 0.5% acetic acid for five minutes, followed by a 15-minute distilled water wash. The process concluded with dehydration, clearing in xylene, and mounting in D.P.X. medium.

Parallel to this, the detection of glycogen and neutral mucopolysaccharides was performed using the Periodic Acid-Schiff (PAS) technique as described by Suvarna et al. (2018). After deparaffinization and rehydration through a descending ethanol series, the slides were treated with periodic acid for 10–15 minutes to oxidize carbohydrate hydroxyl groups. Following a distilled water rinse, Schiff's reagent was applied for 15 minutes until a characteristic magenta color developed, which was then intensified under running tap water for 10 minutes. Hematoxylin was employed as a counterstain for 10 minutes to visualize the nuclei, and the slides were finally dehydrated, cleared, and stabilized with D.P.X. medium for microscopic examination.

Photomicrography and Image Acquisition

Tissue slices were inspected and photographed with a Canon EOS 650D digital camera connected to a Meiji microscope via a 1/2X adaptor. To document the liver's histological and histochemical properties, photomicrographs were taken at three different magnifications (10x, 40x, and 100x). To maintain uniformity for future digital analysis, all photographs were taken in standardized lighting settings and recorded in high-resolution format.

Statistical Analysis

The quantitative data from imagej was arranged in Microsoft Excel and evaluated using IBM SPSS Statistics (Version 26). Descriptive data were given as Mean ± SD. The significance of differences between the two age groups (3 months vs. 6 months) was determined using an Independent Samples T-test. P-values < 0.05 were deemed statistically significant.

Results

Protein Distribution

The histochemical analysis using Mercuric Bromophenol Blue (BPB) revealed that total proteins were primarily localized within the hepatocyte cytoplasm as dense, dark blue areas. A clear distinction was observed between the two age groups, as adult fish exhibited significantly more intense staining compared to juveniles. Adult livers were characterized by larger and more continuous dark blue patches, whereas juvenile fish showed smaller, more scattered protein granules. This increased accumulation of protein in adults compared to juveniles is quantitatively detailed in Table 1, illustrated in Chart 1, and visually represented in Figure 1.

Significant sex-related differences in protein deposition were also observed across both age groups, as detailed in the comparative analysis in Table 2 and visually represented in Chart 2. Female fish consistently exhibited superior protein accumulation compared to their male counterparts. In adult specimens, females displayed the most intense reactivity (Figure 2: a), particularly concentrated within the sinusoidal regions, which far exceeded the staining intensity observed in adult males (Figure 2: b). A similar trend was noted in the juvenile stage, where juvenile females maintained higher protein deposition (Figure 2: c) than juvenile males. Notably, juvenile males exhibited the lowest staining intensity recorded across all experimental groups (Figure 2: d).

Table 1. Mean values and standard deviation of total protein in the liver of adult and juvenile fish.

Group	Mean ± SD
Adult Cyprinus carpio	4.852 ± 0.416
Juvenile Cyprinus carpio	2.167 ± 0.0926
P-value	0.001

Table 2. Mean values and standard deviation of total protein in the liver of adult and juvenile fish male and female.

Group	Male (Mean ± SD)	Female (Mean ± SD)
Adult Cyprinus carpio	3.465 ± 0.254	4.679 ± 0.147
Juvenile Cyprinus carpio	1.551 ± 0.200	2.084 ± 0.034

P-value	0.001	0.001

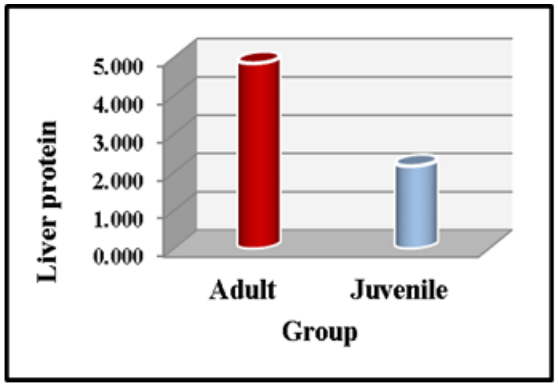


Chart 1. The proportion of total protein content in the liver of both ages of fish.

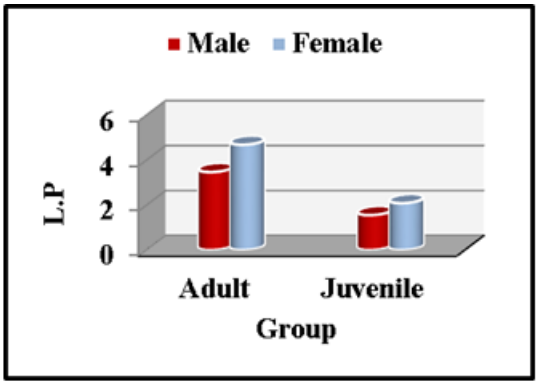


Chart 2. The proportion of total protein content in the liver of both ages of fish male and female.

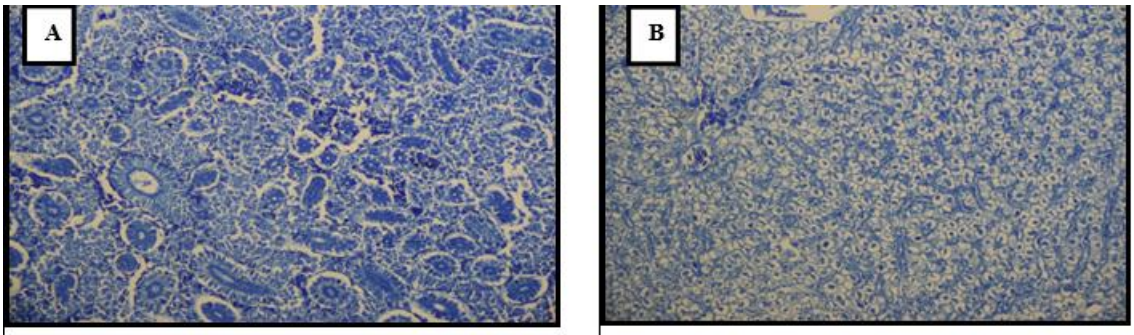


Fig. 1. Photomicrograph of cross section in liver A: Adult fish sections showed the total protein contents appeared as darkly stained granules, B: Juvenile fish showed the total protein contents appeared as darkly stained granules Bromophenol blue stain, 200 X.

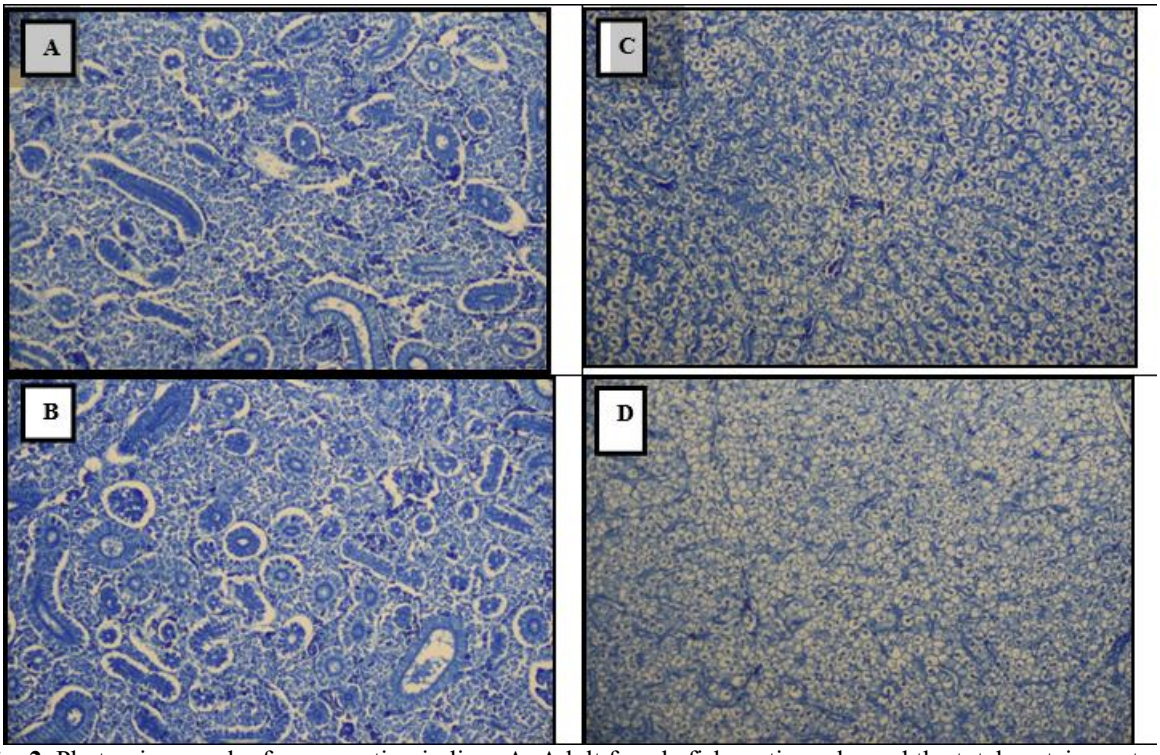


Fig. 2. Photomicrograph of cross section in liver A: Adult female fish sections showed the total protein contents appeared as darkly stained granules, B: Adult male fish showed the total protein contents appeared as darkly stained granules, C: Juvenile female fish sections showed the total protein contents appeared as darkly stained

granules, D: Juvenile male fish sections showed the total protein contents appeared as darkly stained granules Bromophenol blue stain, 200 X.

Glycogen Distribution

The Periodic Acid-Schiff (PAS) reaction showed extensive magenta staining within the hepatocyte cytoplasm, indicating significant glycogen presence. Adult fish displayed a more robust and widespread staining pattern compared to juveniles, with adult livers characterized by dense glycogen clusters while juveniles showed a more diffuse distribution. This increased accumulation of glycogen in adults relative to juveniles is quantitatively detailed in Table 3, illustrated in Chart 3, and visually confirmed in Figure 3.

The analysis of glycogen distribution revealed distinct sex-related variations within each age category, as summarized in Table 4 and illustrated in Chart 4. Female fish generally exhibited higher glycogen storage levels than males, reflecting differences in metabolic demands between the sexes.

In adult specimens, females displayed the most intense accumulation of glycogen granules (Figure 4: a), particularly in the periportal areas, whereas adult males showed a less intense distribution (Figure 4: b). A similar trend was observed in the juvenile stage, where juvenile females showed moderate glycogen reactivity (Figure 4: c) that remained higher than that of their male counterparts. Juvenile males consistently displayed the weakest glycogen staining among all experimental groups (Figure 4: d).

Table 3. Mean values and standard deviation of glycogen in the liver of adult and juvenile fish.

Group	Mean ± SD
Adult Cyprinus carpio	2.948± 0.398
Juvenile Cyprinus carpio	1.317± 0.0443
P-value	0.001

Table 4. Mean values and standard deviation of glycogen in the liver of adult and juvenile fish male and female.

Group	Male (Mean ± SD)	Female (Mean ± SD)
Adult Cyprinus carpio	1.278 ± 0.0412	3.510 ± 0.188
Juvenile Cyprinus carpio	1.086 ± 0.0374	1.112 ± 0.0429
P-value	0.001	0.001

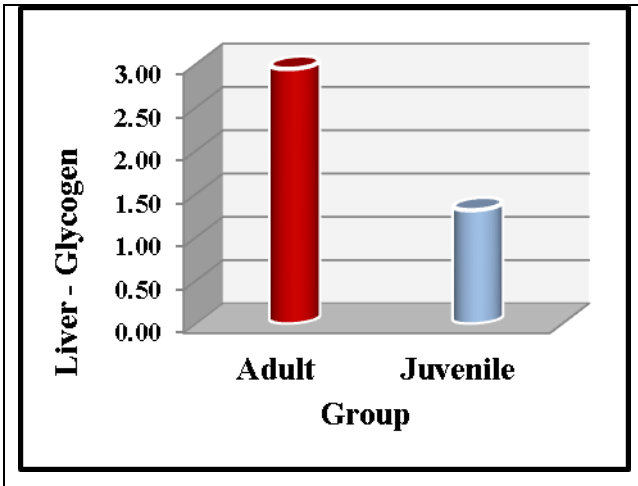


Chart 3. The proportion of glycogen content in the liver of both ages of fish.

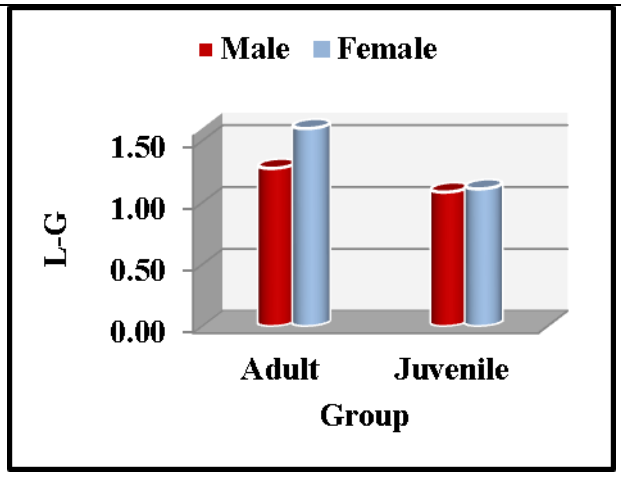
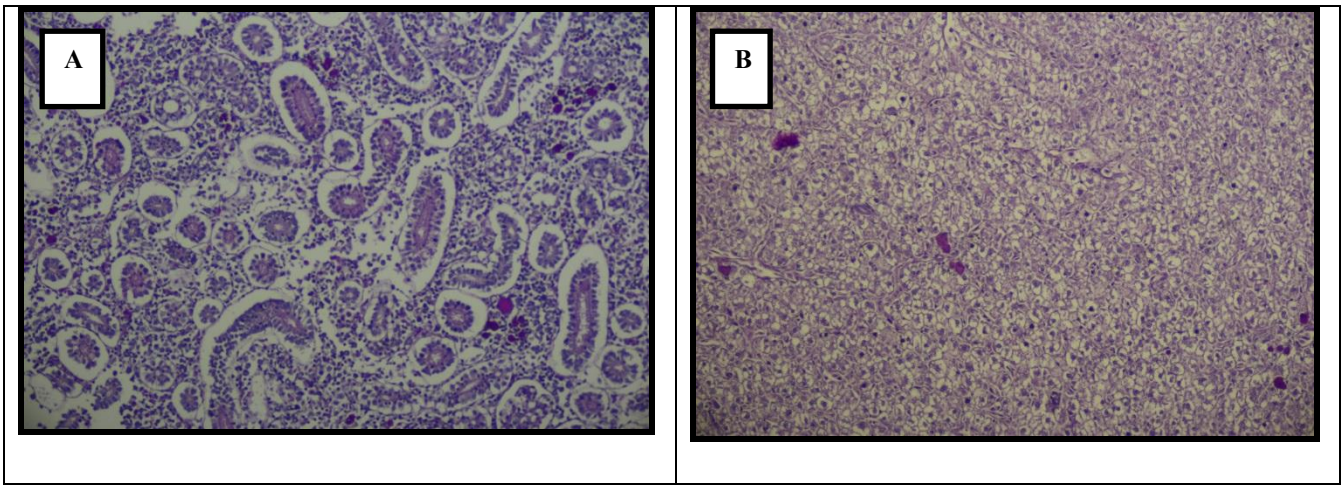


Chart 4. The proportion of glycogen content in the liver of both ages of fish male and female.

Fig. 3. Photomicrograph of cross section in liver A: Adult fish sections showed the glycogen contents appeared as darkly stained granules, B: Juvenile fish showed the glycogen contents appeared as darkly stained granules



PAS stain, 200X.

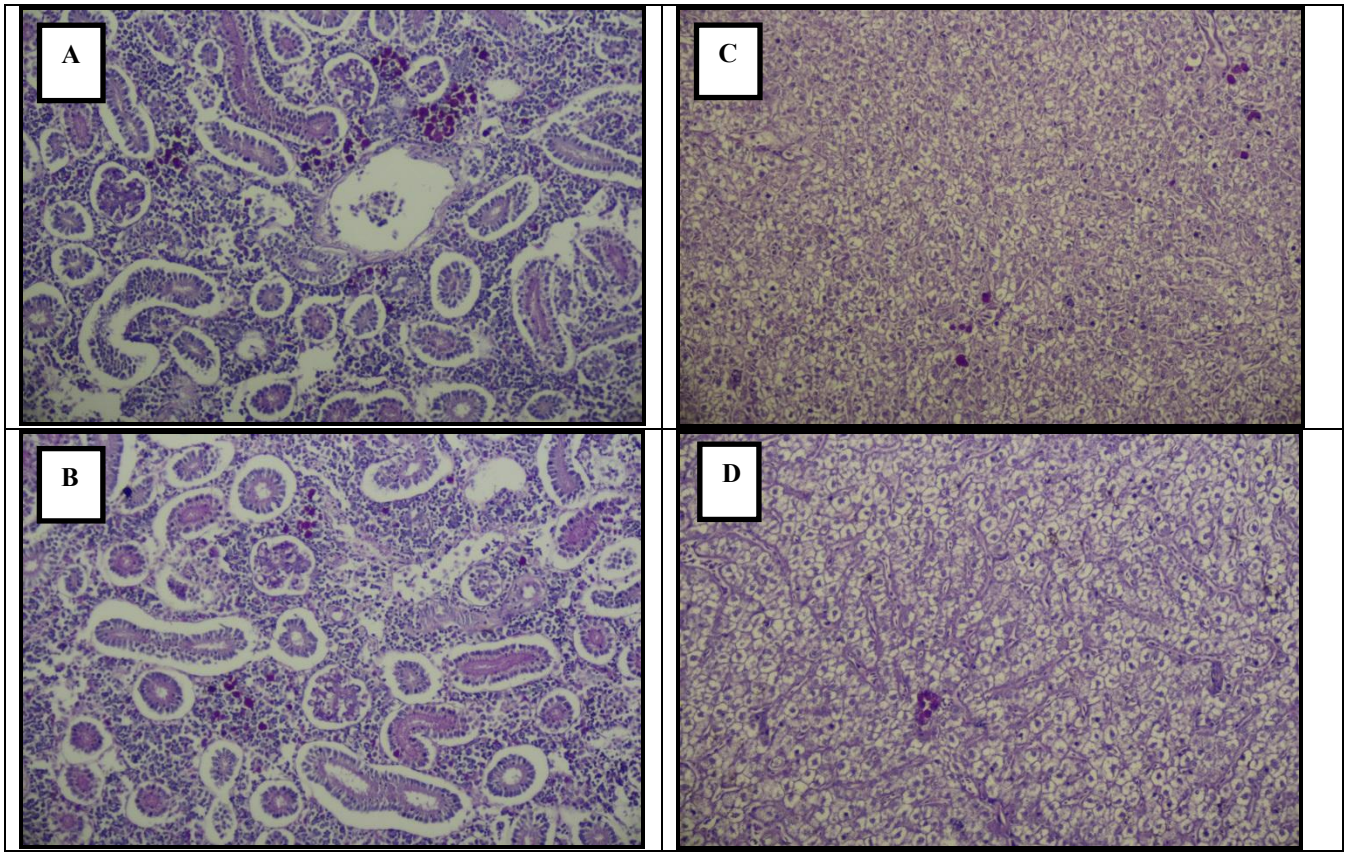


Fig. 4. Photomicrograph of cross section in liver A: Adult female fish sections showed the glycogen contents appeared as darkly stained granules, B: Adult male fish showed the glycogen contents appeared as darkly stained granules, C: Juvenile female fish sections showed the total protein contents appeared as darkly stained granules, D: Juvenile male fish sections showed the total protein contents appeared as darkly stained granules PAS stain, 200X.

Discussion

Adult C. Carpio exhibit greater protein accumulation, indicating a physiological shift toward improved metabolic and synthetic capacities as the fish matures. This observation aligns with the findings of Martínez-Álvarez et al. (2020) and Reading et al. (2017), who demonstrated that this developmental growth is primarily fueled by the multiplication of cellular organelles, such as ribosomes and rough endoplasmic reticulum. These researchers established that such cellular expansion enables the high protein synthesis rates necessary for somatic maintenance and energy consumption.

The biological process of vitellogenesis is principally responsible for the significantly greater protein levels observed in females, especially throughout maturity. As concluded by Tyler and Sumpter (1996) and Mommsen et al. (1999), the liver serves as the primary site for vitellogenin manufacturing during the reproductive season. Furthermore, Guderley and Seebacher (2018) identified that this specific metabolic requirement intensifies ac-

tivity within female hepatocytes, directly resulting in the extensive protein deposition found in the sinusoidal areas.

Regarding energy reserves, adult fish possess greater glycogen stores than juveniles, indicating a higher potential for storage. This trend supports the assertions made by Mommsen and Walsh (1992) and Evans et al. (2013), who found that as fish age, their metabolic demands for somatic maintenance and reproductive preparation increase, thereby necessitating larger glycogen reserves.

The research conducted by Evans et al. (2013) and Guderley and Seebacher (2018) further implies that sex-based differences in glycogen levels allow females to maintain larger energy reserves to power the energy-intensive stages of oogenesis. Siddique et al. (2019) proved that glycogen acts as a crucial, readily accessible energy source for vitellogenin production. Their study suggests that the concentration of these granules around the major veins in adult females indicates a purposeful regionalization of metabolic activity within the liver parenchyma, a mechanism evolved to promote reproductive success.

Conclusion

This study shows that the histochemical profile of the liver in common carp (Cyprinus carpio) is dynamically affected by both developmental age and biological sex. The data show a positive link between aging and hepatic protein and glycogen accumulation, indicating that as fish mature, their metabolic capability and energy storage efficiency improve. Furthermore, a considerable sexual dimorphism was discovered, with females—particularly adults—exhibiting higher levels of metabolic reserves. This rise is biologically related to the high energy requirements of vitellogenesis and ovarian growth during the reproductive season.

Furthermore, the use of imagej software proved to be an accurate technique for converting qualitative microscopic observations into rigorous quantitative data. Finally, the zonal distribution of these metabolites is an important bio-indicator of the fish's physiological health, hormonal control, and reproductive readiness in the Tigris River's aquatic ecosystem.

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