Journal of the Kalash Science
Volume-10, Number-1, 2022 : 15-22

Website: www.kalashj.com
E-mail: saajjjan@rediffmail.com
ISSN: 2321-7634

Effect of varying protein diets on liver and intestine histology of catla, *Gibelion catla* reared in indoor biofloc system

Sonia Solanki*, S. J. Meshram, H. B. Dhamagaye S. D. Naik, P.E. Shingare and B. M. Yadav
Department of Aquaculture, College of Fisheries (DBSKKV), Shirgaon, Ratnagiri - 415629, Maharashtra, India.
* Corresponding author e-mail: soniyasolanki22693@gmail.com

Accepted : 13 March 2022

Abstract

Gibelion catla is commercially important fish species with other Indian Major Carp (IMC). The planning of the study was done from October 2020 to November 2021. The experiment was conducted for sixty days to investigate the effect of varying dietary protein diets on liver and intestine histology of *G. catla* reared in biofloc system. The experiment consisted of completely randomised design with five treatments *viz.*, Control (T0-30% protein), T1 (20% protein), T2 (25% protein), T3 (30% protein), T4 (35% protein). All the treatments had four replicates. Biofloc was maintained at C:N ratio of 20:1with rice bran as carbon source. Fry of average length (2.65± 0.00cm) and average weight (0.21±0.01g) were stocked @ 30 fry L^{-1}. No adverse effect was observed in liver histology of catla fingerling reared in experimental groups. The result obtained in the study suggests that the different dietary protein diets in biofloc system has effect on fish intestine histology and the fish could be supplied with 30% protein dietin biofloc system.

Keywords: *Gibelion catla*, protein, liver, intestine, histology, indoor biofloc system

Introduction

Histology is the study of cells, tissues and organs as seen through microscope. Histological study correlates morphology with the functioning of structural units of organism and helps in supervising fish health, assessing stress and detect signs of disease not easily recognized on gross examination (Schwaiger *et al.* 1997, Mokhtar 2017). Any alterations or injuries in organs serves as warning signs of damage to animal health, reduced survivaland increase of susceptibility to disease (Velmurugan *et al.* 2009). The protein intake above 25% by fish is remarkably excreted as nitrogenous waste which at higher concentration influences the water quality and leads to mortality, diseases and accumulation of harmful residue in the culture system. In intensive culture system, stress in fish can be initiated by poor water quality, stocking density, feed and feeding technique and infestation by parasites or a disease (Tacon 1992, Ashley 2007).

Biofloc technology (BFT) in aquaculture system focuses on more efficient utilisation of nutrient input with limited or zero water exchange (Widanarni *et al.* 2012). BFT system enhances water quality through addition of extra external carbon sources in accordance with high level of aeration that stimulates the growth of heterotrophic bacteria which in turn convert toxic nitrogenous waste into potentially consumable microbial floc in aquaculture system (Avnimelech 2009, Crab *et al.* 2012, Ahmad *et al.* 2017). Fish obtain their nutritional requirement through the food they consume, and fish larvae show preference to live feed compared to formulated feed (Murugesan *et al.* 2010).

Gibelion catla was selected for the experiment as it is the most preferred farm fish and shows preference to zooplankton dominated food (Natarajan & Jhingran 1961, Jha *et al.* 2015). It is back bone for composite fish culture or polyculture with *Labeo rohita* and *Cirrhinus mrigala* in Indian subcontinent (Dwivedi *et al.* 2004, Tiwari *et al.* 2016) and also shared sizeable proportion in the landing from the Indian rivers (Dwivedi *et al.* 2016, Tripathi *et al.* 2017a, 2017b). In BFT system, *Gibelion catla* fingerlings consume the planktonic contributes in the floc and can easily graze on floating bioflocs (Prajith & Madsoodana 2011, Deb *et al.* 2020).

The liver and intestine are the important organs in digestion and absorption of nutrients from food. Histological analysis of these organs is considered as

a good indicator of the nutritional status of fish (Jasim 2016). Histological changes in liver can reveal the adverse effects of the feed components, inadequate food to the cultured organism and environmental condition that can cause state of pathology in aquaculture production experiments (Tacon 1992, Caballero *et al.* 2004, Fahmi *et al.* 2019). Histopathological changes in the intestine may vary depending on the species and feed used in the experiments (Rašković *et al.* 2011).

Biofloc supplementation reduces the requirement of protein in diets. So, the present study was conducted toinvestigate the effect of varying dietary protein diets on liver and intestine histology of *Gibelion catla* reared in biofloc system.

Materials and Methods

A. Experimental design and biofloc preparation

The planning of the study was done from October 2020 to November 2021. The experiment was conducted in HDPE (High Density Polyethylene) drum of 100-L capacity for 60 days in Wet laboratory of Department of Aquaculture, College of Fisheries, Shirgaon, Ratnagiri, Maharashtra, India. The experiment consisted of five treatments with T1, T2, T3 and T4 supplied with different dietary protein level feed at the rate of 20, 25, 30 and 35 % respectively in biofloc system and T0 as control with clear water supplied with 30% protein feed. All the treatments had four replicates randomly assigned as per CRD (Kandathil *et al.* 2020).

The preparation and maintenance of biofloc inoculum were made following the procedure of Avnimelech (1999) and Schryver (2008).

B. Biofloc development in experimental tank, fish stocking and management

A quantity of 5 L of biofloc inoculum and 75 L of freshwater was added to all the experimental tanks at the start of the experiment. The unit was allowed to develop biofloc for subsequent two days and then the fry of average length (2.65± 0.00cm) and average weight (0.21±0.01g) were stocked at the rate of 3no's L^{-1} (i.e. 24 no's per 80L) (Sawant *et al.* 2020).

The addition of rice bran was carried out once in a day based on the calculation described by Schryver (2008) to maintain C:N ratio of 20:1 in the BFT treatment during the experiment.

Table 1. Ingredients and proximate composition of experimental diets.

Ingredients (g kg–1 DM)	**% of ingredients**				
	T1 (20%)	**T2 (25%)**	**T3 (30%)**	**T4 (35%)**	**C (30%)**
Casein fat free[1]	175	235	292	350	292
Gelatin[2]	50	50	50	50	50
Dextrin 160	144	130	115	100	115
Starch	431	385	343	300	343
Cellulose	100	100	100	100	100
Carboxymethyl cellulose	10	10	10	10	10
Sun flower oil + cod liver oil (2:1)	60	60	60	60	60
Vit+Min.[3]	27.5	27.5	27.5	27.5	27.5
Vit.C	2.5	2.5	2.5	2.5	2.5
Proximate composition of diet (% dry weight basis)					
Crude Protein	**21.3**	24	30.8	34.2	30.8
Lipid	**1.9**	1.6	2.1	2.0	2.1
Ash	**12.3**	14.8	16.2	17.3	16.2
Crude fibre	**1.8**	2.1	2.5	3.0	2.5

[1] Casein, Hi- LR: >=92% CP- (Hi Media, India)

[2] Gelatin: 90% CP

[3] Composition of vitamin mineral premix

C. Experimental diet

Four semi- purified diets with different protein levels were prepared viz., Control (T0-30% protein), T1 (20% protein), T2 (25% protein), T3 (30% protein), T4 (35% protein) as shown in Table 1. Feeding trial was conducted for sixty days.

D. Histology

At the end of 60 days feeding trial period, fishes were sacrificed and pooled samples of fish fingerling internal organs such as intestine and liver were collected and fixed in 10% neutral buffered formalin for histological studies. The preserved organs were cut in cross section into a proper size (1-2 mm) and washed under the gentle flow of tap water. The properly washed tissues were dehydrated with a series of paraffin. The paraffin embedded tissues were sectioned longitudinally at 5µ thickness using microtome (MEDIMEAS MRM-RM) and stained with hematoxylin and eosin (H & E). Pathological changes manifested in the tissue sections were examined under light microscope (Zeiss Primo star) and microphotographs were taken with T Capture software (Bullock 1989).

Results

A. Liver

The sections of the liver were examined at the end of the experiment. The results of histological examination of liver are presented in Plate 1 (A-E). No adverse histological changes were observed in the liver of the fish reared in experimented groups. There was no effect of different dietary protein diets on the liver parenchymal morphology in fish reared in biofloc system.

B. Intestine

The sections of the intestine were examined at the end of the experiment (Plate 2: A-E). Increased number of goblet cells and microvilla length were observed in intestine histology of fish with increase in protein in biofloc groups. Intact submucosa, mucosa and serosa were observed in biofloc groups compared to control. Degeneration of serosa layer was noticed in control treatment.

Discussion

Histological study is important for assessing effects of both internal (feed used) and external (aquatic) environmental conditions on the fish cultured (Rajeshkumar & Munuswamy 2011).There is no literature on study of liver and intestine histology of catla fed varying protein diets in biofloc system.

A. Liver

Liver is an important organ in nutrient metabolism and any histological changes in the organ are considered useful indicator for evaluating the nutritional and toxic condition (Najdegerami *et al.* 2016, Bakhshi *et al.* 2018). In the present study, no histological changes were observed in the liver parenchyma cells of fish reared in biofloc system and clear water system. According to Bakhshi *et al*, (2018), due to better nourishment in biofloc system, reducing the feeding rate to 75% of daily feeding had no impact on the liver's histology and enhanced the quantity and qualification of hepatic cells in common carp. In contrast, Romano *et al.* (2018) observed damage in liver due to increase in ammonia-N to dangerously high levels in raw rice bran (RRB) and rice bran incubated for 24 hr with *Bacillus species* under aeration (ResRB) groups of biofloc system which was restored to normal liver with no alterations in liver histopathology through water exchange and stability in water quality during the experiment.

Hence, the different dietary protein feed and environmental condition had no adverse effect on liver of catla indicating better nourishment even at lower protein diet and better water conditions for the fish under biofloc culture.

B. Intestine

Histological change is an important aspect in understanding pathological alteration related to nutritional sources in fish (Shi *et al.* 2017). Intestine epithelium is an important site for the absorption of nutrients, osmotic balance, immunity, recycling of enzymes and macronutrients (Uran *et al.* 2008). Protein nutrients particularly affects the construction of microvilli (Li *et al.* 2017). Increased number of goblet cell was observed with increase in dietary protein fed to the fish in BFT system compared to control. Enterocytes along with goblet cells produce

enzymes essential for proper food digestion (Al Mamun *et al.* 2021). Biofloc shows probiotic properties and like probiotics increase the number of goblet cells in the villi, increasing the absorption of nutrients (Ali *et al.* 2015). Bakhshi *et al.* (2018), reported no detectable changes in gut histoarchitecture of common carp reared in biofloc. Integrity of intestine is presumed to be a key factor for the growth and welfare of farmed fish (Saraiva *et al.* 2015). Degeneration in intestinal mucosa was observed in control group. Damagein intestine may affect the digestion and absorption of nutrients by the fish body which can compromise the healthy growth of fish (Liu *et al.* 2020). Mirzakhani *et al.* (2019), observed enhanced feed efficiency in biofloc group due to increased villus length and diameter of the Nile tilapia fish which resulted in higher nutrient absorption in BFT group. This shows that biofloc has no negative effect on intestine of the catla reared in biofloc indicating the better absorption of the nutrient which might lead to better growth of the fish.

Conclusion

In the present study, the *Gibelion catla* fed varying dietary protein diets in indoor biofloc had no adverse effect on liver and intestine of the fish. The fish can be fed with low protein diets of 30% protein in biofloc culture system as BFT system provides better environmental conditions with reduced physiological changes in the fish. BFT system can enhance better nutrient utilization and absorption of nutrients which can improve growth performance and survival of the fish. There is need to study the enzymatic activity in the intestine and liver for better understanding of the functioning of organ in the biofloc system.

Acknowledgment

Authors are thankful to the authorities of Dr. B.S.K.K.V., Dapoli for gratifying the permission to pursue this study and providing all the necessary facilities at College of Fisheries, Ratnagiri.

References

Ahmad, I., A.M. Babitha Rani, A.K. Verma & M. Maqsood (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture Int.*, **25(3):** 1215-1226.

Al Mamun, M.A., S. Nasren, S.S. Rathore & K. Srinivasiah (2021). Histopathological Studies of Pond Reared Indian Major Carp, *Catla catla* Infested with *Argulus japonicus* and Trial for Argulosis Treatment. *Punjab University J. Zool.*, **36(2):** 131-139.

Ali, S.R., K. Ambasankar, E. Praveena, S. Nandakumar & J. Syamadayal (2015) Effect of dietary mannan oligosaccharide on growth, body composition, haematology and biochemical parameters of Asian seabass (*Lates calcarifer*). *Aqua. Res.*, **46:** 1-10.

Ashley, P.J. (2007). Fish welfare: current issues in aquaculture. *Appl. Anim. Behavi. Sci.*, **104(3-4):** 199-235.

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, **176:** 227-235.

Avnimelech, Y. (2009). Biofloc technology: A practical guide book. World Aquaculture Society. Baton Rouge, 182.

Bakhshi, F.H., E. Najdegerami, R. Manaffar, A. Tokmechi, K. Rahmani Farah & A. ShalizarJalali (2018). Growth performance, haematology, antioxidant status, immune response and histology of common carp (*Cyprinus carpio* L.) fed biofloc grown on different carbon sources. *Aqua. Res.*, **49(1):** 393-403.

Bullock, A.M. (1989). Laboratory methods. In: R.J. Roberts (ed). Fish Pathology, 2nd ed. Bailliere Tindal, London, 374-405.

Caballero, M.J., M.S. Izquierdo, E. Kjørsvik, A.J. Fernandez & G. Rosenlund (2004). Histological alterations in the liver of sea bream, *Sparus aurata*L., caused by short-or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *J. Fish Diseases*, **27(9):** 531-541.

Crab, R., T. Defoirdt, P. Bossier & W. Verstraete (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*, **356:** 351-356.

Deb, S., M.T. Noori & P.S. Rao (2020). Application of biofloc technology for Indian major carp culture (polyculture) along with water quality management. *Aqua. Engin.*, **91:** 102106.

Dwivedi, A.C., A. S. Mishra, P. Mayank & A. Tiwari (2016). Persistence and structure of the fish assemblage from the Ganga river (Kanpur to Varanasi section), India. *Journal of Geography and Natural Disasters*, **6:** 159. **Doi:** 10.4172/2167-0587.1000159.

Dwivedi, A.C., N. P. Tewari & K. R. Singh (2004). Present structure of capture and culture fishery of the Faizabad District (U.P.). *Bioved*, **15(1, 2):** 95-98.

Dyková, I., J. Žák, R. Blažek, M. Reichard, K. Součková & O. Slabý (2022). Histology of major organ systems of *Nothobranchius* fishes: short-lived model species. *J. Vertebrate Biol.,* **71(21074):** 21074-1.

Fahmi, U., I. Andriani, S. Salmah, T.H. Hatta, S.B.A. Omar & D.K. Sari (2019). Histopathology of liver and intestine of pangkilan bare fish (*Oryziasmatanensis*) Polluted by nickel and iron in Lake Matano, South Sulawesi. In IOP Conference Series: Earth and Environmental Science, **370(1):** 012078.

Jasim, B.M. (2016). Effects of using fish biosilage as fish meal replacer on feeding, growth and gut histology in common carp *Cyprinus carpio* L. fingerlings. *Basrah J. Vet. Res.*, **15(1):** 35-47.

Jha, D.N., K. D. Joshi, A. C. Dwivedi, P. Mayank, M. Kumar & A. Tiwari (2015). Assessment of fish production potential of Chitrakoot district, Uttar Pradesh. *J. Kalash Sci.,* **3(3, Special Volume):** 7-10.

Kandathil, D. Radhakrishnan, K. Velayudhannair & B.V. Schmidt (2020). Effects of bio-flocculated algae on the growth, digestive enzyme activity and microflora of freshwater fish *Catla catla* (Hamilton 1922). *Aqua. Res.,* **51(11):** 4533-4540.

Li, S., H. Ji, B. Zhang, J. Zhou & H. Yu (2017)> Defatted black soldier fly (*Hermetiaillucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. *Aquaculture*, **477:** 62-70.

Liu, H., X. Dong, B. Tan, T. Du, S. Zhang, Y. Yang, S. Chi, Q. Yang & H. Liu (2020). Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama* Forsskál)(1775). *Aqua. Nut.,* **26(5):** 1724-1735.

Mirzakhani, N., E. Ebrahimi, S.A.H. Jalali & J. Ekasari (2019) Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc systems with different carbon sources and input C:N ratios. *Aquaculture*, **512:** 734235.

Mokhtar, D.M. (2017). Fish histology: from cells to organs. Apple Academic Press.

Murugesan, S., V. Sivasubramanian & K. Altaff (2010). Nutritional evaluation and culture of freshwater live food organisms on *Catla catla*. *J. Algal Biomass Utln*, **1(3):** 82-103.

Najdegerami, E.H., F. Bakhshi & F.B. Lakani 2(016). Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. *Fish physiol. Biochem.*, **42(2):** 457-465.

Natarajan, A.V. & A.G. Jhingran (1961). Index of preponderance - a method of grading the food elements in the stomach analysis of fishes. *Indian J Fish.*, **8(1):** 54-59.

Prajith, K.K. & K.B. Madhusoodana (2011). Application of Biofloc Technology (BFT) in the nursery rearing and farming of giant freshwater prawn, *Macrobrachium rosenbergii (deMan)* (Doctoral dissertation, Cochin University of Science and Technology).

RajeshKkumar, S. & N. Munuswamy (2011). Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, South East Coast, India. *Chemosphere*, **83(4):** 415-421.

Rašković, B., M. Stanković, Z. Marković & V. Poleksić (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Ag. Sci. (Belgrade), **56(1):** 87-100.

Romano, N, A.B. Dauda, N. Ikhsan, M. Karim & M.S. Kamarudin (2018) Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles. *Aqua. Res.*, **49(12):** 3691-3701.

Saraiva, A., J. Costa, J. Serrão, C. Cruz & J.C. Eiras (2015). A histology-based fish health assessment of farmed seabass (*Dicentrarchuslabrax* L.). *Aquaculture*, **448:** 375-381.

Sawant, K.S., S.J. Meshram, H.B. Dhamagaye, B.R. Chavan, R.M. Tibile & V.R. Vartak (2020). Growth and survival of *Labeo rohita* (Hamilton, 1822) fry in biofloc system using various dietary protein levels. *J. Exp. Zool., India*, **23 (Suppl. 1):** 765-769.

Schryver, P.D., R. Crab, T. Defoirdt, N. Boon & W. Verstraete (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, **277:** 125-137.

Schwaiger, J., R. Wanke, S. Adam, M. Pawert, W. Honnen & R. Triebskorn (1997). The use of histopathological indicators to evaluate contaminant-related stress in fish. *J. Aquatic Ecosyst. Stress Recov.,* **6(1):** 75-86.

Shi, X., Z. Luo, F. Chen, C.C. Wei, K. Wu, X.M. Zhu & X. Liu (2017). Effect of fish meal replacement by Chlorella meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for Crucian carp *Carassius auratus*. *Aqua. Res.*, **48(6):** 3244-3256.

Tacon, A.G. (1992). Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish **(Vol. 85, No. 22)**. Food & Agriculture Organisation.

Tiwari, A., A. C. Dwivedi & P. Mayank (2016). Time scale changes in the water quality of the Ganga River, India and estimation of suitability for exotic and hardy fishes. *Hydrology Current Research,* **7(3):** 254. doi:10.4172/2157-7587.1000254.

Tripathi, S., A. Gopesh & A. C. Dwivedi (2017a). Framework and sustainable audit for the assessing of the Ganga river ecosystem health at Allahabad, India. *Asian J. Environ. Sci.*, **12(1):** 37-42. DOI: 10.15740/HAS/AJES/12.1/37-42.

Tripathi, S., A. Gopesh & A. C. Dwivedi (2017b). Fish and fisheries in the Ganga river: current assessment of the fish community, threats and restoration. *J. Exp. Zool., India,* **20(2):** 907-912.

Urán, P.A., J.W. Schrama, J.H.W.M. Rombout, A. Obach, L. Jensen, W. Koppe & J.A.J. Verreth (2008). Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. *Aqua. Nut.*, **14(4):** 324-330.

Velmurugan, B., M. Selvanayagam, E.L. Cengiz & E. Unlu (2009) Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to Dichlophos. *Int. J. Braz. Archives Biol. Tech.,* **52(5):** 1291-1296.

Widanarni, E., J. kasari & S. Maryam (2012). Evaluation of biofloc technology application on water quality and production performance of Red Tilapia *Oreochromis sp*. cultured at different stocking densities. *Hayati J. Biosci.,* **19:** 73-80.

Plate 1. Histological analysis of liver sections of catla (*Gibelion catla*) fed different dietary protein diets in the biofloc system. **A.** Control (T0) (30% CP), and biofloc system; **B.** T1 (20% CP), **C.**T2 (25% CP), **D.**T3 (30% CP), **E.**T4 (35% CP). **H**- Hepatocytes, **S**- Sinusoid, **V**- vacuolation. Stain: H & E (40X).

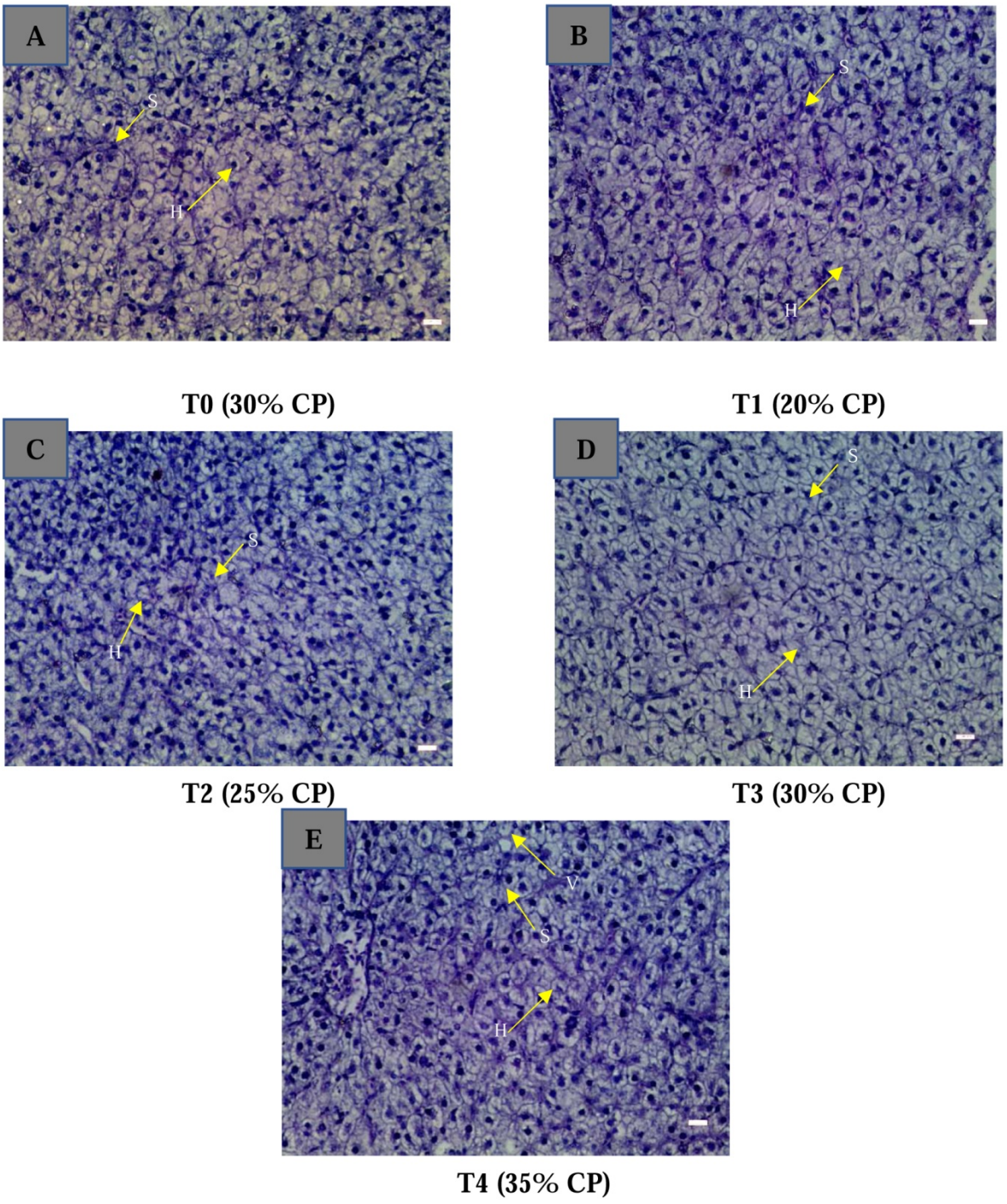

Plate 2. Histological analysis of intestine (L. S.) of catla (*Gibelion catla*) fed different dietary protein diets in the biofloc system. **A.** Control (T0) (30% CP), and biofloc system; **B.** T1 (20% CP), **C.** T2 (25% CP), **D.** T3 (30% CP), **E.** T4 (35% CP). **M**- Mucosa, **Bm**-Basement membrane, **G**- Goblet cells, **Sm**- Submucosa, **Ms**-Muscularis, **S** -Serosa, **D**- Degeneration of serosa layer, **V**- Villi. Stain: H & E (40X).