Histopathological changes in the intestine, liver and pancreas of the common carp, *Cyprinus carpio,* during starvation

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ABSTRACT

The present study was conducted to evaluate the effect of starvation on the histological structure of intestine, liver and pancreas of the common carp, *Cyprinus carpio*. The histological changes of fishes were examined after 2, 4, 6, 8 and 10 weeks of starvation. Histological differences between non starved and starved fish after 8 weeks were no longer evident, except for the more abundant goblet cells seen in the anterior and posterior intestine of the non starved fishes. Proteolysis of the intestinal mucosa, especially of enterocytes in apical part of mucosal folds was observed after 10 weeks of starvation. In the liver, progressive reduction of glycogen granules and lipid vacuoles was also observed after 10 weeks. In the pancreas, progressive degeneration of exocrine pancreatic cells, with a decrease in zymogen activity was occurred after 8 and 10 weeks of starvation.

Key words: Histopathological changes, Fish, Intestine, Liver, Pancreas, Starvation, *Cyprinus carpio*

INTRODUCTION

Under natural conditions, several fish species are enduring long periods of starvation, associated with seasonal changes in food availability, spawning migrations and seasonal changes in water temperature (Stepanowska *et al*., 2006). In well-managed aquaculture conditions, food deprivation is not frequent, but farmers may adopt similar conditions for the cultured fishes to avoid risks of overproduction (Krogdhal and Bakke-McKellep, 2005).

Fishes can overcome starvation using biochemical, physiological and behavioral strategies. Endogenous energy from basic metabolic accumulations in the body is spent as fishes consume their own tissues to remain alive during starvation (Wook, 2006). During starvation, essential processes in fishes are maintained on the expense of accumulated (completely endogenous) energy reserves, resulting in the progressive depletion and wastage of body tissues (Hung *et al*., 1997).

Many fish species are subjected to natural starvation periods during the year and have developed the ability to survive without food. Some species can survive starvation for up to four years and no feeding larvae may survive for one month (Ghanshyam and Priyanka, 2003). Starvation (short- or long-term) is an important factor that affects physiological changes in immunity, survival and growth (Wook, 2006).

Histological analysis allows the determination of cause and effect relationships between body structure and starvation, whereas gross morphological measurements provide an index of starvation that is vulnerable to error and bias in calibration and interpretation (Caruso *etal*.,2008). The histopathological changes of digestive structures accompanying such a condition are a good indicator of environmental quality. Histological changes induce by pure starvation should be distinguished from pathological changes induced by ingested material (Guderley *et al*., 2003).

The first objective of the present study was to provide methods, based on histological observations that can easily be applied in the aquaculture industry to estimate the condition of *Cyprinus carpio*. The second objective was to extend knowledge of changes in histological structure that occur in this species during starvation. Specifically, structural changes in digestive structures: intestine, liver and pancreas caused by short-term starvation were investigated, and these data were used to determine the nutritional indices for *C. carpio*.

MATERIALS AND METHODS

The starvation experiment was conducted in the Marine Science Center laboratories, involved 48 common carp (initial average weight 41.51 ± 3.51 gm). The fishes were kept in six (60 litter capacity) constantly aerated glass aquaria equipped with mesh net to prevent them from jumping. Each aquarium was stocked with eight fishes. Prior to experimentation, fishes were acclimatized for two weeks fed *ad libitum* on commercial diet (5.94% moisture, 30.21% protein, 43.73% carbohydrates, 12.56% lipids and 7.56% ash) to minimize the nutritional differences between them. The experiment took 10 weeks during which time the fish were not fed. Fishes were examined after 2, 4, 6, 8 and 10 weeks of starvation.

Sections of intestine, liver and pancreas were fixed in Bouin's fluid for 24 hrs. The fixed materials were transferred and processed through ascending grades of alcohol, dried in a wax miscible agent and impregnated in wax. Sectioning was carried out on a rotary microtome at 5 µm. Sections were floated on warm water at 48° C and mounted on chemically clean slides coated with egg albumin. The mounted, unstained sections were dewaxed in three stages of xylene at 1 minute each. The staining was carried out by using haematoxyline and eosin technique (Humason, 1972) and then, histochemically by alcian blue-periodic acid (Pearse, 1985). Stained mounted sections were examined under light microscope.

RESULTS

No mortality was observed throughout the experiment in spite of food deprivation. The starved fishes did not show any significant alteration in their health status in terms of behavior, swimming or body pigmentation.

The intestine of *C. carpio* (Fig. 1&2) consisted of four layers: serosa, muscular, submucosa and the mucosa epithelium. In the anterior intestine, no characteristic pattern of increase or decrease in the height of the mucosal cells was seen between the non starved and starved groups within eight weeks (Fig. 3). The number of goblet cells, however, became higher in non starved group (Fig. 4). In the posterior intestine, an increase in the height of the mucosal cells was remarkable in both non starved and starved samples within eight weeks of starvation. Histological observations had revealed that starved samples had relatively lower mucosal folds compared with the non starved ones. The intestinal mucosal epithelium of *C. carpio* consisted of enterocytes showing basically located large oval nuclei. The apical surface of enterocytes was covered by the brush border. Among the enterocytes, the mucous cells which secreted acidic and neutral mucins were presented.

The structural changes in the intestinal mucosa of the food deprived *C. carpio* appeared already in 10 weeks of starvation and degeneration of enterocytes and the brush border in the apical mucosal fold was observed, accompanied by a shedding of intestinal epithelium (Figs. 5&6). The mucosal lamina propria was considerably enlarged comparing to that observed in the non starved fishes. Its upper region was degenerated, lacked connective tissue, and protruded into the intestinal lumen. The enterocytes were shrunk, and separation of enterocytes was developed between them.

The structure of the liver did not show any anomalies (Fig. 7) and consisted of polygonal hepatocytes with centrally located nuclei. Their cytoplasm stored glycogen and lipids. The hepatocytes were tightly attached to each other, and were separated with narrow sinuses. In the fishes after 10 weeks of starvation, the cytoplasm of hepatocytes was gradually decreased (Figs. 8&9).

The pancreas of non starved *C. carpio* (Figs. 10&11) was dispersed in the intestinal mesentery and around the hepatic portal veins. The Langerhans islets were dispersed within the dispersed pancreas. The exocrine secretory cells showed a pyramidal shape, distinct nuclei and acidophylic zymogen granules situated in the apical region. After 10 weeks of starvation, number of zymogen granules and cell size were considerably reduced in the acinar cells of non starved fishes, and accompanied by pyknosis of the nuclei (Figs 12&13).

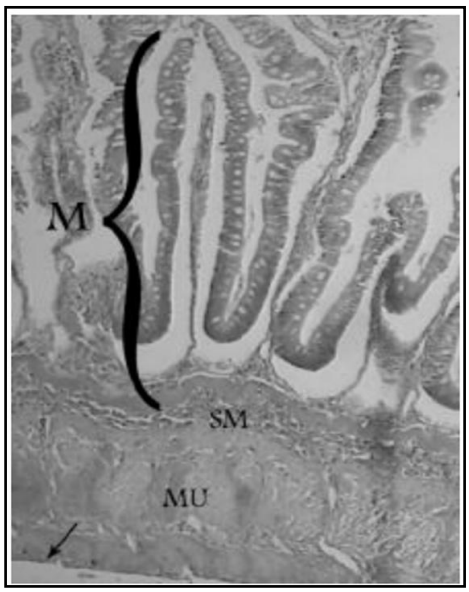

Fig. (1): Mucosa of intestinal wall of *C. carpio* formed by mucosa (M), submucosa (SM), muscular (MU) and serosa (arrow) in non starved fishes. (40X).

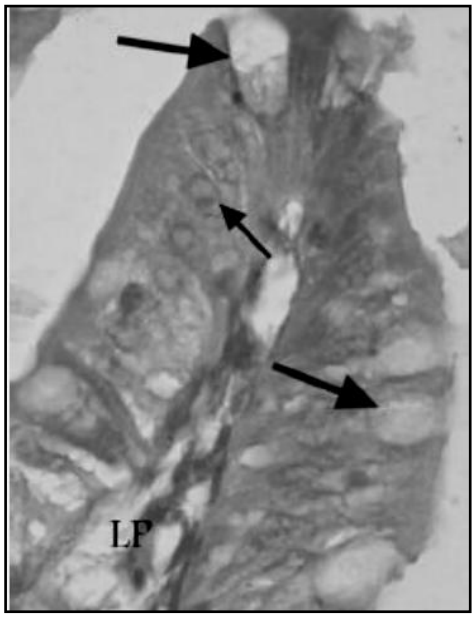

Fig. (2): Upper portion of a intestinal villus of *C. carpio*: Basally located nuclei of the enterocytes (small arrow), goblet cell (large arrows), lamina propia (LP) and brush border (BB) in non starved fishes.

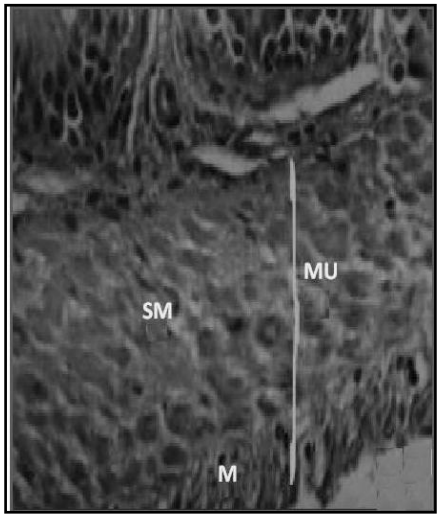

Fig. (3): Section of the anterior intestine of *C. carpio* showing the muscularis layer (MU). The height of mucosa (M) and the thickness of both submucosa (SM) and muscularis (MU) are greater in non starved fishes. (1000X).

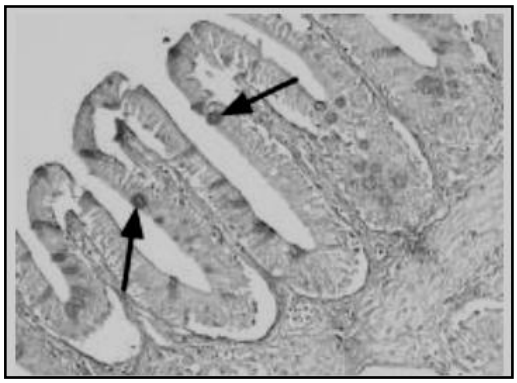

Fig. (4): The mucosa of intestine of *C. carpio* stained with periodic-acid, the goblet cell are positive (arrows) in non starved fishes. (100X).

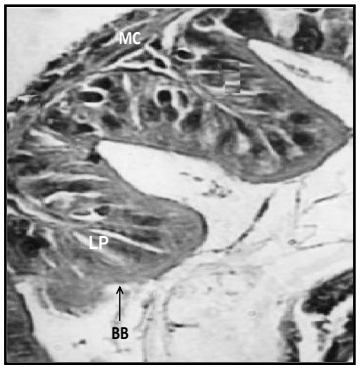

Fig. (5): Longitudinal section of intestinal epithelium of *C. carpio* after 10 weeks of starvation, arrow indicates desquamation of apical part of mucosal folds, lamina propria (LP), brush border (BB), mucus cells (MC). (200X).

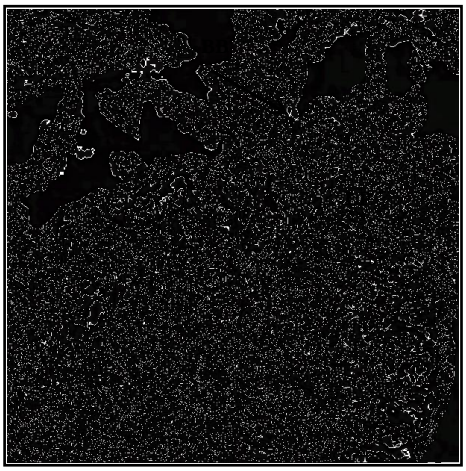

Fig. (6): Longitudinal section of intestinal epithelium of *C. carpio* after 10 weeks of starvation, arrow indicates desquamation of apical part of mucosal folds, LP-lamina propria, BB- brush border. (400X).

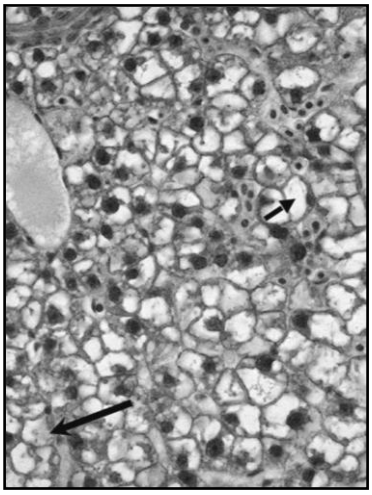

Fig. (7): Liver section containing vacuolated hepatocytes, lipid vacuoles (short arrow) and polygonal hepatocytes containing glycogen (long arrow) of non starved *C. carpio*. (400X).

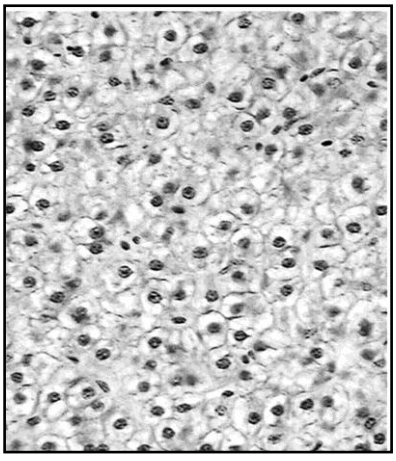

Fig. (8): Ten weeks starved *C. carpio* liver showing reduction in cytoplasm area, glycogen and lipids vacuoles. (400X).

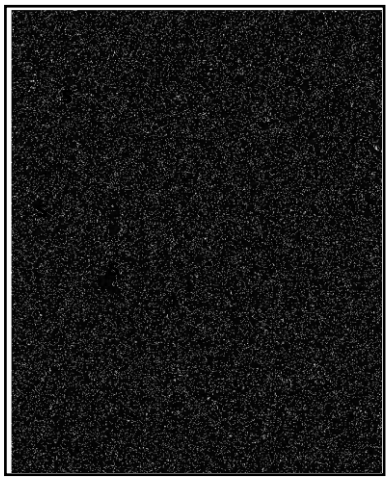

Fig. (9): Ten weeks starved *C. carpio* liver showing large intercellular space, collapsed cytoplasm and enlarged sinusoids (s). (400X).

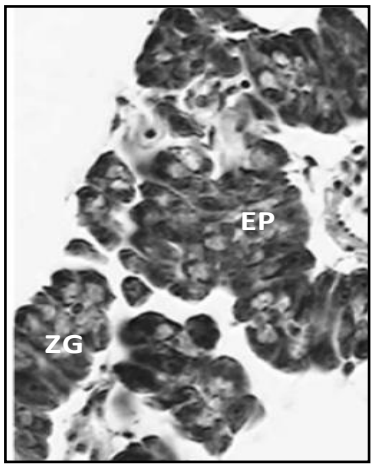

Fig. (10): Pancreas section of non starved *C. carpio* showing langerhans islets (EP), exocrine pancreas (ZG), acidophylic zymogen granules. (1000X).

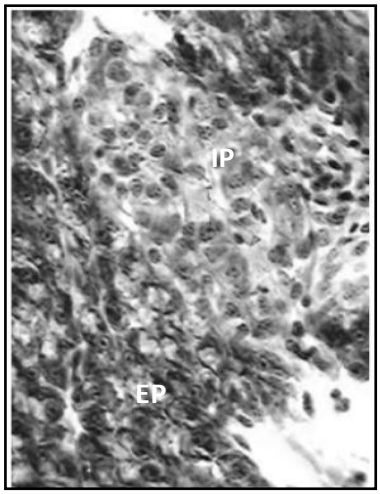

Fig. (11): Pancreas section of non starved *C. carpio* showing exocrine pancreas (EP), langerhans islets (IP). (1000X).

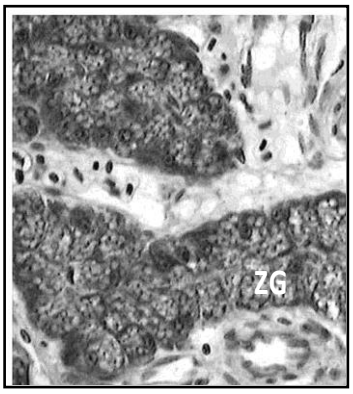

Fig. (12): Ten weeks starved *C. carpio* pancreas showing acidophilic zymogen granules (ZG). (1000X).

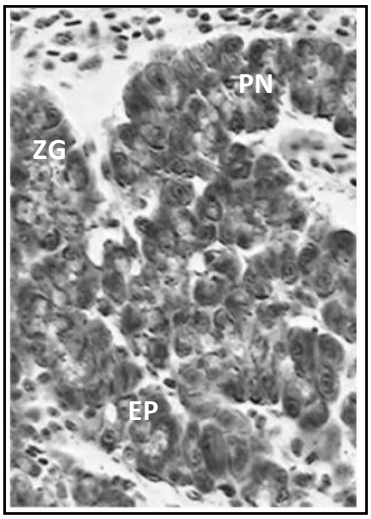

Fig. (13): Ten weeks starved *C. carpio* pancreas showing acidophylic zymogen granules (ZG), exocrine pancreas (EP) and pyknotic nuclei (PN). (1000X).

DISCUSSION

A long time of starvation may induce considerable changes in fish digestive structures that make digestion difficult or even impossible (Guderley *et al*., 2003). The starvation related changes disturb metabolism and selective nutrient utilization, which may result in tissue degeneration (Xie *et al*., 2001). The histological observations of the digestive system of *C .carpio* of the present study indicated that even a short-term food deprivation induces histopathological changes in the intestinal epithelium, liver and pancreas structure.

The height of enterocytes is considered as a good indicator of starvation or malnutrition (Green and McCormick, 1999). Reduction of the enterocytes height may be related to the smaller size of food-deprived fish (Xie *et al*., 2001), or proteolysis of the epithelium observed even during a short-term starvation (Green and McCormick, 1999). Such a proteolysis of enterocytes of the apical region of intestinal folds observed in the starved fishes of the present study would prevent

intracellular digestion and absorption of nutrients if the fish were fed again, until regeneration of destroyed epithelium. Therefore, the real time of starvation would be extended by a time of digestive inability (Dou *et al*., 2002).

In the non starved samples, more goblet cells were found. The mucous layer coating the anterior and posterior intestine is secreted primarily by these cells, which were interspersed among the enterocytes covering the mucosal folds. Some of the functions of the mucus of the goblet cells, common to all the regions of the digestive tract, include trapping of sloughed cells and undigested particles and facilitating their clearance, and holding IgA that binds to and inactivates microorganisms (Krogdhal and Bakke-McKellep, 2005). The abundant mucous granules seen in the non starved fishes may not be due to a higher activity in terms of functions just mentioned. Instead, it may be attributed to the abundance of raw materials present for its formation. The basic structure and path of synthesis and secretion of all mucins are similar. Mucins are large glycoproteins that contain greater than 50% by weight carbohydrates arranged as chains of oligosaccharides linked to peptide backbones. Mucin polymers are continually packed into secretory granules on the trans surface of the Golgi apparatus and transported to the apical region of the cell (Krogdhal and Bakke-McKellep, 2005). Within seconds after exocytosis, the released mucins are hydrated, resulting in a 600-fold expansion in volume. This addition of water, along with the association of polymers with each other, creates an unstirred layer of mucous gel covering the cell surface (Dou *et al.*, 2002). This efficient process was seen in non starved fishes where the continuous ingestion of food was not delayed or disrupted at any time of development.

During starvation, glycogen and lipids stored in fish hepatocytes are the primary energy sources (Figueroa *et al*., 2000). During prolonged starvation, amino acids may become as important source of energy (Gisbert and Doroshov, 2003). The gradual reduction of hepatocyte size, and very fast disappearance of stored nutrients from the liver of starved fishes confirm these observations. Similar liver degeneration, hepatocyte size reduction, and sinus enlargement were reported for starving fishes (Green and McCormick, 1999), and the larvae of several fish species (Gisbert and Doroshov, 2003). The changes observed in liver of starving fishes might have adversely affected metabolic processes such as glucose catabolism, gluconeogenesis, and lipid metabolism, which was observed by Green and McCormick (1999) in *Amphiprion melanops*.

The secretion of pancreatic enzymes is related to the level of feeding, diet composition or starvation (Zambonino and Cahu, 2001; Wold *et al*., 2009). Histological observations of pancreas and proteolysis of intestinal epithelium showed that in the starved fishes, zymogen was produced in the acinar cells, and secreted into the intestinal lumen. The presence of zymogen granules in the acinar cells, and gradual degeneration of pancreas in the starved fish was reported also by Gisbert and Doroshov (2003). On the contrary, Segner *et al*. (1993) and Gwak *et al*. (1999), observed a decrease in the enzymatic activity of some starved fishes.

The physiological response to starvation (epithelium shedding, hepatocytes size and changes in the exocrine pancreas) observed in the present study was reported also in other fish species (Cook *et al*., 2000), in the larvae (Green and McCormick, 1999) and in the adults (Gisbert and Doroshov, 2003). Even a short-term food deprivation, is generally considered as a safe index for histopathological changes in the intestinal epithelium, liver, and pancreas of *C. carpio*. The results of the present study suggest the need to investigate the histological changes during a long-term starvation, particularly at the moment when food digestion is no more

REFERENCES

Caruso, G.; Maricchiolo G.; Micale V., Genovese, L.; Caruso, R. and Denaro, M. (2008). Physiological responses to starvation in the European eel (*Anguilla anguilla*): effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol. Biochem., 31: 12-22.

Cook, J. T.; Sutterlin, A. M. and McNiven, M. A. (2000). Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). Aquaculture, 188: 47–63.

Dou, S., R. Masuda, M. Tanaka, and K. Tsukamoto. (2002). "Feeding resumption, morphological changes and mortality during starvation in japanese flounder larvae."J. Fish Biol., 60: 1363-1380.

Guderley, H.; Lapointe, D.; Be´dard, M; and Dutil, J. D. (2003). Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, Gadus morrhua L. Comp. Biochem. Physiol., 135: 347–356.

Figueroa, R. I.; Rodriguez-Sabaris, R. M.; Aldegunde, and Soengas, J. L. (2000). Effects of food deprivation on 24h- changes in brain and liver carbohydrate and ketone body metabolism of rainbow trout. J. Fish Biol., 57: 631-646.

Ghanshyam, T. and Priyanka V. (2003). Starvation-induced impairment of metabolism in a freshwater Catfish. Z. Naturforsch., 58: 446-451.

Gisbert, E. and Doroshov, S.1. (2003). Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Aeipenser medirostris*). Aquat. Living Resour, 16: 7789 – 7798.

Green, B.S. and McCormick, M.1. (1999). Influence of larval feeding history on the body condition of *Amphiprion melanops*. J. Fish BioI., 55: 1273-1289.

Guderley, H.; Lapointe, D.; Be´dard, M. and Dutil, J. D. (2003). Metabolic priorities during starvation:

enzyme sparing in liver and white muscle of Atlantic cod, *Gadus morrhua* L. Comp. Biochem. Physiol., 135: 347–356.

Gwak, W. S.; Seikai, T. and Tanaka, M. (1999). Evaluation of starvation status of laboratory reared Japanese flounder *Paraliehthys olivaeeus* larvae and juveniles based on morphological and histological characteristics. Fish. Sci., 65: 339-346.

Humason, G. L. (1972). Animal tissue techniques. 3rd ed., W. H. Freeman Co., an Francesco: 661 pp.

Hung, SSO; Liu, W.; Storebakken, L. H. and Cui, Y. (1997). Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. Aquaculture, 151: 357 – 363.

Krogdhal A., and Bakke-McKellep A. M. (2005). Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol., 141: 450–460.

Pearse, AG.E. (1985). Histochemistry. Theoretical and Applied. Vol. 2. Analytic Technology. Churchill Livingstone, New York, 1055 pp.

Segner, H.; Rosch R., Verreth, J. and Witt, U. (1993). Larval nutritional physiology: studies with *Clarias gariepinus*, *Coregonus lavaretus* and *Seophthalmus maximus*. J. World Aquacult. Soc., 24: 121-134.

Stepanowska, K.; Nedzarek, A. and Rakusa-Suszczewki, S. (2006). Effects of starvation on the biochemical composition of blood and body tissue in the Antarctic fish *Notothenia coriiceps* (Richardson, 1844) and excreted metabolic products. Polar Biosci., 20: 46 –54.

Xie, S.; Zhu, X.; Cui, Y.; Wotton, R. I.; Lei, W. and Yang, Y. (2001). Compensatory growth of the gibe carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. J. Fish BioI., 58: 999-1009.

Wold, P.A.; Hoehne-Reitan, K.; Cahu, C.L.; Infante., J. Z.; Rainuzzo, J. and Kjørsvik, E. (2009). Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morha* L.) larvae. Aquacult. Nutr., 15: 73- 84.

Wook H.J.; Jo J.H. and Park In-Seok (2006). Effects of long-term starvation on hepatocyte ultrastructure of olive flounder *Paralichthys olivaceus*. Ichthyol. Res., 53: 306–310.

Zambonino, I.L and Cahu, E. (2001). Ontogeny of the gastrointestinal tract in marine fish larvae. Compo. Biochem. Physiol., 130: 477-487.

التأثيرات المرضية النسجية في أمعاء وكبد وبنكرياس أسماك الكارب الشائع *Cyprinus carpio* L. خلال التجويع

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أجريت هذه الدراسة لتقييم تأثير التجويع على انسجة الأمعاء والكبد والبنكرياس في أسماك الكارب الشائع *Cyprinus carpio*. تم فحص التغيرات النسيجية للأسماك بعد 2 و4 و6 و8 و10 أسابيع من التجويع. التغييرات النسجية بين الأسماك المجوعة وغير المجوعة بعد 8 أسابيع لم تكن واضحة، باستثناء الخلايا الكأسية goblet cells حيث كانت أكثر وفرة في الأمعاء الأمامية والخلفية للأسماك غير المجوعة. لوحظ التحلل البروتيني في مخاطية الأمعاء، وخاصة في الجزء الداخلي القمي من طيات المخاطية بعد 10 أسابيع من التجويع. ظهر اختزال تدريجي في حبيبات الكلايكوجين والدهون في انسجة الكبد والاختزال في الفراغات بين الخلايا الكبدية بعد 10 أسابيع من التجويع أيضاً، كما أن التجويع لـ 8 و10 أسابيع سبب في انحطاط تدريجي لخلايا البنكرياس مع انخفاض في فعالية نشاط الزايموجين zymogen.