Effect of Starvation Stress on Organ Structure in Mugll Fish *LIZA ABU*

B.M. Jasim
Dep. Fish. Mar. Res., Coll. Agriculture, Univ. Basrah,

Abstract

The present study examined the organ tissue response as well as morphological alterations with relation to stress, induced by food deprivation regimes in *Liza abu*. It is concluded that starvation stress could be observed through organs structure, as is shown in kidney, liver, spleen, gills, skeletal muscles and stomach wall which underwent a damage in different degrees which represents a dysfunction in these organs. The thyroid tissue invaded the neighboring muscles. The ovary showed another response.

Keywords: Starvation, Stress, Food, Nutrition, *L. abu.*

تأثير التجويع على تركيب العضو في سمكة الخشني *Liza abu*

باسم محمد جاسم
قسم الاسماك والثروة البحرية /كلية الزراعة/ جامعة البصرة
البصرة – العراق

الخلاصة

درس تأثير الاجهاد نتيجة التجويع على نسيج العضو والتغيرات المظهرية له في سمكة الخشني *Liza abu* . لوحظ تأثير هذا الاجهاد على تركيب الاعضاء ، كما في الكلية والكبد والطحال والغلاصم والعضلات الهيكلية وجدار المعدة ، حيث اظهرت درجات متفاوته من التلف والذي يمثل نوعا من الخلل الوظيفي في أداء هذه الاعضاء . اما النسيج الدرقي فقد وجد إنه يغزو العضلات المجاورة ، في حين أظهرت المبايض شكلا آخر من الإستجابة.

Introduction

Effects of starvation could be detected by observing a progressive emaciation in fish in the form of body slimness, as it is detected in the changes in organs structure. Histological changes appear in shape or volume of cells, in addition to pigment granules distribution as in kidney and spleen which exhibit variable sizes and types in starved fishes(Oguri,1985).

Nutritional diseases are often a diagnosis of exclusion and, therefore, other explanations for the problem are ruled out (Francis – Floyd, 2002). Rainbow trout was influenced by starvation in its spleen and kidney during three weeks (Agius and Roberts, 1981). On the other hand, neotropical fish *Hoplias malabaricus* starved for more than 35 weeks, as it can survive for about 26 weeks of that period without reduction in metabolism (Rios *et al*., 2002).

Liza abu is one of the most widespread fishes among inland and estuarine Iraqi waters. Yesser *et al*. (1999) investigated the effects of starvation on proximate chemical composition of this species.

The present investigation was carried out to study the effects of prolonged starvation on histological, and morphological structure of some organs in this fish.

Materials and Methods

The experiment was carried out in four circular tanks, each filled with 35L of aged tap water. Twenty specimens of healthy and active *Liza abu* measuring 90- 152 mm in total length were placed in each tank. They were allowed to acclimatize for two weeks and trained to feed regularly on commercial fish diet in proportion of 3% body weight. Then, fishes in two tanks were starved for a period of up to 14 weeks while the fishes in other two tanks were continued to be fed on the regular diet.

Approximately 60- 70% of tank water was changed every 48h. Four specimens were sacrificed initially to provide data on organ structure in control fish. Then fishes were sampled weekly and dissected to obtain samples from kidney, liver, spleen, gills, skeletal muscles, posterior stomach

wall, thyroid tissue and ovary. Each tissue sample was fixed with Bouin's fixative and treated for light microscopic technique. Paraffin sections (5-6µm) were stained with Harris or Ehrlich's hematoxylin and eosin (Bancroft and Stevens,1982). Measurements of glomerulus and its capillary knot were carried out following Hwang and Wu (1988).

Observations were recorded about colour, activity, appearance of organs studied and aggregation of abdomen fluids in the dissected specimens. The whole experiment was repeated twice.

Results

The capture of *L. abu* in tanks resulted in grouping behavior in all trials. At the end of investigation the starved fishes were about to perish, as few of them died actually, in addition to progressive increment in abdominal body fluids.

The effects of starvation on organs are stated below:

kidney

The kidney in the present species is characterized by proximal (I & II), distal and collecting tubules, as well as, glumeruli and veins known in most spiny- rayed fishes.

Three weeks following food deprivation, more necrosis was observed in distal tubules than proximal ones; similarly, central regions showed more necrosis than peripheral. Still, the glumeruli retained their normal diameter (50 µm), as the macrophage centers have a density as that in natural specimens. More macrophages together with nuclei and destroyed membranes of tubules epithelium were spread as infiltration over the section. Hemorrhage in variable degrees was noted, although the cardinal vein was still filled with erythrocytes. Most of degenerated distal tubules are seen with plugged lumen due to dilation in epithelium height, so they may functionally inactive. Some empty spaces can be seen due to necrosis. The brush border inside collecting duct had pale staining in comparison with that in control specimens (Fig. 1).

After 13 wks of starvation, a clear destruction was visible in kidney architecture which indicates a failure, to a degree, in its function. Few proximal tubules II and even fewer proximal tubules I were seen; besides, there was scarcity of distal tubules which exhibited plugged lumen and intensively stained epithelium. However, no significant variation was observed in diameter of tubules or vacuolated glumeruli, but additional hemorrhage occurred while the veins appeared practically devoid of erythrocytes.

Obvious increment in the number of macrophages and melano-macrophages accompanied with increased necrosis was observed, but macrophage centres have no such increment (Fig. 2).

The pigments adopted variable patterns; the brown pigments increased in mass, while the yellow pigment increased in size and distribution through kidney tissues.

The capture influenced the erythrocytes count negatively at control specimens as shown in blood vessels at the end of experiment.

Liver

This organ had the usual structure, as described in several fishes; yet in starved specimens it underwent distinct morphological and histological changes which seem to proceed with period progression. In the advanced days of starvation, the liver became deeper in colour and more flabby; hence the microscopic examination revealed structural alterations which indicate dysfunction. The sinusoids appeared dilated in their width and contained more pigment granules, indicating an apparent necrosis, and less defined boundaries among some of hepatocytes were observed; the remainder hepatocytes seemed to be variable in size as most of them appeared binucleated.

The yellow pigment granules increased in number to 5 spots/mm^2 or more, accompanied by increased degeneration, in comparison with four spots or less in control. Some hepatocytes showed slight karyolysis while their nuclei enlarged in diameter from 4 up to 4.5 µm in mean.

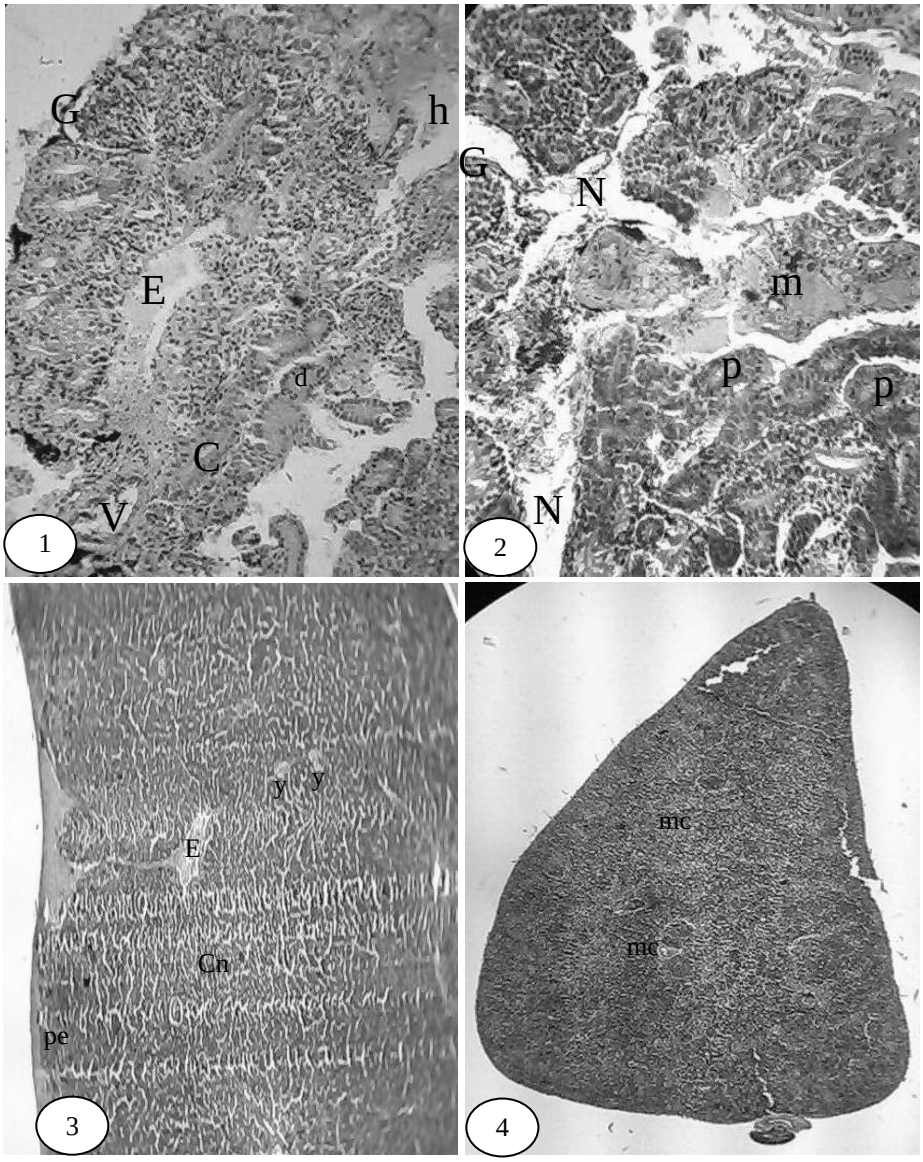

Fig.1. Cross section of kidney after 3-wks of starvation, 400x.
Fig.2. Cross section of kidney after 13-wks of starvation, 400x.
Fig.3. Longitudinal section of liver of stressed specimen, 100x.
Fig.4. Longitudinal section of control spleen with differentiated pulps, 100x.
C, collecting duct; Cn, central zone; d, distal tubule; E, empty spaces; G, glomerulus; h, hemorrhage; m, melano- macrophage; mc, macrophage centre; N, necrosis; p, proximal tubule; pe, periphery; V, cardinal vein; y, yellow pigment granules.

The stress affected central zone of liver more than periphery, where the cytoplasm in alive cells was more acidophilic and the nuclei more faded. The degeneration gave rise to empty spaces together with further macrophages and signs of hemorrhage (Fig. 3). Some hepatocytes have deeply stained strands extending from the nucleus towards cell membrane.
The captivity, in turn, affected the liver structure although the fishes get the diet and show an ordinary activity. Partial necrosis, macrophages increment and relative increment in binucleated hepatocytes were noted; however, no dilation in sinusoids, as the cells still retain the general structure. The yellow pigment granules increased in size although they were found in natural distribution.

Spleen

In control *L. abu* about three macrophage centres per 0.1 mm^2 were shown in histological sections through middle levels of the spleen. Arteries and veins which are filled with blood elements appear with circular and regular walls run adjacent to each other that indicate open type of circulation. Generally, there is scarcity of pigments among the organ tissue and little macrophages in sinusoids. Red pulp is well distinguished from white pulp, while the cortex is stained red with hematoxylin and eosin (Fig. 4).

The spleen in starved specimens showed steady morphological appearance but prominent histological alterations. After 13 weeks of starvation, two enlarged blood vessels were formed opposite to each other in this organ; they exhibited an elliptic shape with a measure up to 162 µm in the aperture as they were highly filled with erythrocytes (Fig. 5). Additional hemorrhage took place along with the starvation period. Besides, considerable necrosis accompanied with increment in macrophages count, where some macrophages lined sinusoids. The macrophage centres raised up to 38/0.1 mm^2, though they adopted a relatively smaller size. The necrotic zones were interposed by randomly distributed nuclei due to cells analysis, which in turn, more abundant in the centre of the spleen than in periphery. The nuclei inside cells adopted deeper stain; still, these cells in both stressed and controlled fishes had different shapes, sizes and staining. The surrounding regions of this organ lost the differentiation between the two pulps. In nutritious fishes the spleen comprised the same figure of macrophage centres but of a larger size after 10 wks of captivity. No degeneration or excess macrophages

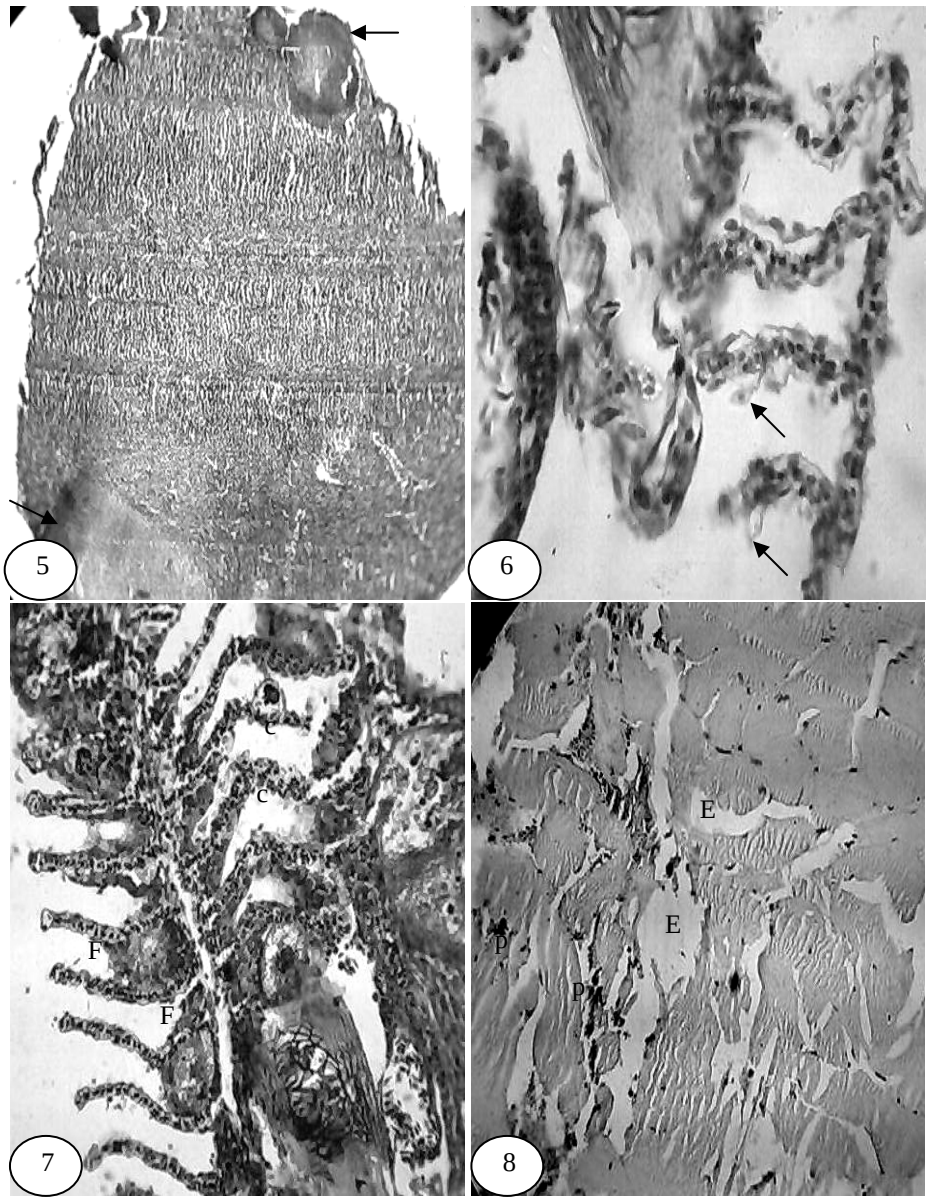

Fig.5. Longitudinal section of spleen with two enlarged blood vessels (arrows), 100x.

Fig.6. Longitudinal section of gills illustrates crooked and hypertrophic lamellae with dropped epidermis (arrows), in starved fish, 1000x.

Fig.7. Longitudinal section of gills illustrates fusion at lamellae bases, 400x.

Fig.8. Cross section illustrates degenerated trunk muscles, 400x.

C, crooked lamella; ca, capillary; E, empty spaces; F, Fusion site; p, pigment aggregation.

Among sinusoids were observed. However, a relatively limited hemorrhage occurred in addition to small fragments of black pigment which were seen on peripheral zones.

Gills

The lamella in its ordinary structure is composed of epithelial cells around capillary which is filled with blood elements. There are some acidophilic cells on the sides of lamellae and mucous cells on their tips and bases. The starvation resulted in marked lesions presented gradually on gills along with period advancement. Some edemas and amebas were observed up and down gill filaments. Besides, there was hyperplasia formation on these filaments. After six wks of starvation a number of lamellae crooked as they contained less erythrocytes inside capillary; also, the acidophilic cells showed a lower count. The epidermis dropped to some extent, whereas hyperplasia started on bases of lamellae, but there was no fusion between lamellae themselves; still, hypertrophy was observed on them (Fig. 6). Finally, the fusion started on lamellae bases, and mucous cells increased. Certain gill arches showed cellular pyknosis and there was an extensive fusion especially at extreme lamellae (Fig. 7). The gills in nourished fishes, in turn, underwent simple damage almost at the end of investigation.

Skeletal Muscles

In the long run of starvation the middle part of the trunk became flexible. The histological sections revealed variable degrees of degeneration in different parts of the body where the bundle arrangement of muscle was lost. Simultaneously, some blank spaces were formed through myocytes. Few nuclei spreaded randomly following myocytes membrane analysis. Macrophages, were also noticed at sites of pigment aggregation (Fig. 8).

In nutritious fishes, small granules of pigment which tended to be spindle in the shape were noted through myocytes at the end of investigation.

Stomach Wall

Prior to rearing, the stomach wall in its posterior portion is characterized by spindle packed muscles with distinct septa and well defined myocytes walls (Fig. 9).

At the termination of starvation period both atrophy and undefined septa could be observed among muscle fibers, whereas the nuclei adopted longitudinal appearance (Fig. 10). Larger masses of pigment granules were set up.

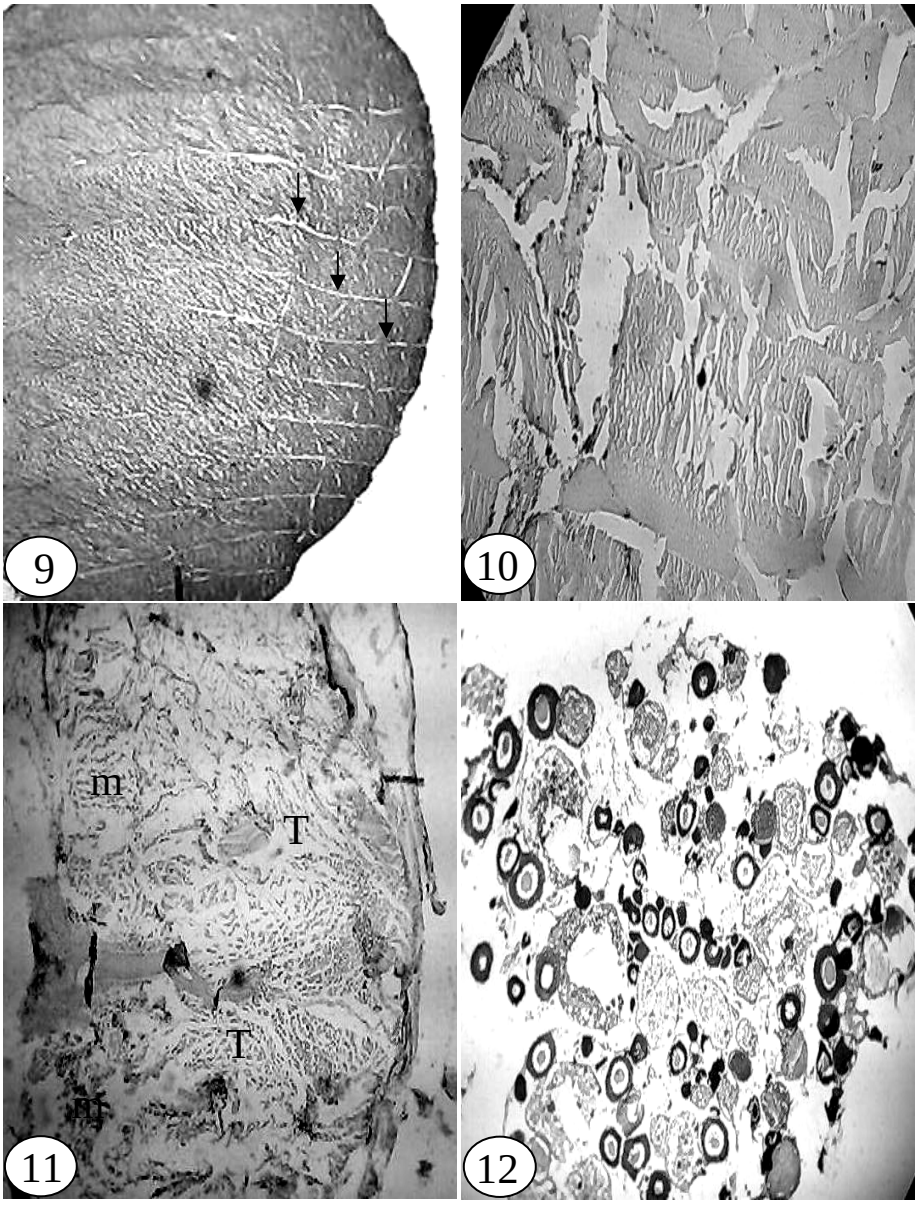

Fig.9. Cross section illustrates the distinct septa (arrows) in stomach wall muscles, 100x.

Fig.10. Cross section illustrates degenerated muscles of starved stomach wall, 100x.

Fig.11. Cross section of thyroid tissue invades the adjacent muscles, 100x.

Fig.12. Cross section illustrates the primary vitellogenesis oocytes stage, 100x.

m, degenerated muscles; T, thyroid tissue.

The main influences of captivity on this organ were slight disarrangement of septa, minor expansion in blood vessels which contained less blood and extension of myocytes nuclei which were darker in stain. However, the gross muscles structure remained almost perfect.

Thyroid Tissue

No morphological or outer alterations, which pertain this portion, could be distinguished in all specimens under investigation. In nourished fishes the thyroid tissue is confined one that is distinguished from the muscular tissue closed to it.

The starvation gave rise to hyperplasia in this tissue and dilation in the cells of it. After 13 wks the thyroid tissue in starved fishes invaded the neighboring muscles and occupied their areola spaces as concluded by the C- cells diffused from the tissue. As a result, there was an obvious degeneration in muscles facing the thyroid (Fig. 11).

Ovary

In the outset of investigation the ovary in all trials was falling under primary growth phase, when there were chromatic nuclear oocytes and perinuclear oocytes. In the end of investigation the ovaries were converted in some fishes to primary vitellogenesis oocytes (Fig. 12). The results revealed the persistence of ovary development, at least for this stage, disregarding of starvation stress.

Discussion

The deposits of pigment granules in kidney were classified as the second type (mixed of yellow and dark brown pigments) according to Oguri (1985) who studied them in 20 marine teleosts.

It seems that melano-macrophage centres observed in *L. abu* contributed to tissue catabolism which were raised from prolonged starvation. Agius and Roberts (1981) observed that melano-macrophage centres in the kidney and spleen of starved plaice increased from 10 to 35 centres/mm^2 after 16 wks of starvation, whereas other species had different patterns of formation or increment of such centres.

The necrotic appearance in the kidney of nutritious fishes might be due to vitamin D deficiency in artificial diet, whereas the hemorrhage be due to vitamin A deficiency (Snieszko, 1972). Necrosis and apoptosis are two distinct forms of cell death (Mitchell and Cotran, 2003). It is evident from the present results that upon starvation, *L. abu* exhibits adaptive response, including cytometric changes in nephrons which indicated a profound changes in kidney functions.

Agius and Roberts (1981) stated that the tissue of kidney and spleen in fishes can relatively degenerate during starvation but melano- macrophages density increase at a ratio higher than tissue degeneration. This relationship seemed not to be found in accordance with *L. abu* which showed obvious degeneration but less melano- macrophage increment in both organs.

Starvation stress showed no effect upon liver structure, at least according to light microscopy examination in *L. abu* during 3-4 wks. Nakagawa *et al.* (1984) observed that hepatosomatic index was not significantly distinctive between starved ayu *P. altivelis* and those of the control group after 21 days of starvation.

Some individuals of *L. abu* perished after about three months of food deprivation, so it seems to be influenced faster than other species like *C. carpio* which characterized by reduction of cellular volume and then the disappearance of cellular organization in the liver during eight months of above stress (Love, 1980). The neotropical fish *Hoplias malabaricus* starved more than 35 wks, and it could survive for about 26 wks of that period without reductions in metabolism (Rios *et al.*, 2002).

It is evident that liver in *L. abu* could be employed as a parameter for the nutritive status. Storch *et al*. (1983) concluded that hepatocytes might be used as an indicator of quantity and quality of food in teleosts, they observed that hepatocytes in *C. chanos* frys decreased in area by 25% from 7-d to 9-d of starvation, which was accompanied by moderate increase in the amount of lysosomal bodies. The present observations showed less defined boundaries among infected hepatocytes; the damage which may lead to liver dysfunction such as reduction of xenobiotics metabolism, for

example rendering lipophilic compounds water soluble (Hinton *et al*., 2004).

Fishes with nutritional deficiencies or fishes in poor health tend to have more or larger macrophage aggregates (Blazer and Dethloff, 2000). This phenomenon was distinct at kidney, liver and spleen in the present study. Lesions noticed on nourished specimens liver might be attributed to feeding. Bac (1987) observed alterations in liver of seabass induced by artificial feeding (dry pellets).

Although there was remarkable digital increment in macrophage centres among spleen in starved fishes, they were not big in size as that observed in plaic (Agius and Roberts, 1981). Increased number of macrophages seemed to be a natural mechanism to remove damaged blood cells, or so- called increased erythrophagia. On the other hand, captivity and handling must be considered, for they have a significant role in erythrocyte content within spleen (Yamamoto *et. al., 1983;* Kita and Itazawa, 1989). Spleen is the storage organ for erythrocytes supply into the circulating blood. Degeneration of gills may cause hypoxia which induces spleen to supply more erythrocytes as a physiological response.

The starved animals show usually a reduction in lymphocytes and spleen weight (Grunfeld, 2002). Moreover, the spleen is a hematopoietic organ in fishes, so dysfunction can affect the whole - organism level and immune system (Blazer and Dethloff , 2000).

Epithelial hyperplasia in gills could be a result of malnutrition as suggested by Snieszko(1972), or due to continuous exposure to low concentrations of NH_4OH for some weeks (Eller, 1975; Smart,1976), and this agent results in erythrocytes decrease along with nutritional gill diseases as noted in the present study. Rios *et al*.(2002) observed that haematocrite and the count of erythrocytes at gills and skin dermis of neotropical fish decreased after 150 and 240 days of starvation respectively. Damage in gills limits ammonia excretion, where 80% of it is excreted through gills. In addition, the putrefaction of proteins from desquamated gut epithelium during fasting can act as a source of ammonia (Smutna *et al.* 2002).

Hyperplatic clubbing of gill filaments, occasionally accompanied by partial fusion, were observed in fingerlings S*almo giardneri* as a result of natural infection (Kudo and Kimura, 1983 a) or artificial infection by bacterial gill disease (Kudo and Kimura, 1983 b). Also, it might be associated with pantothenic acid deficiency (Moeller, 2004), or with vitamin C deficiency as noticed in catfish (Adham *et al*, 2000). Pantothenic acid deficiency may induce distal fusion of lamellae (Snieszko, 1972; Francis - Floyd, 2002) or gill lesions(Eller, 1975).

The partial damage in gills of nutritious fishes might attribute to chemical irritant from excretory products of the fishes themselves or it might be due to dusty dry diet (Eller, 1975).

The trunk bending appeared as a result of backbone morbid changes due to calcium lack which alters various physiological processes (Ashley, 1972). Skeletal muscles response in *L. abu* to fasting had a shorter period in comparison with *C. carpio.* Love (1980) stated that cross section through the muscles of carp starved for six months had essentially the same appearance as that of the unstarved one; However, there was little increase in the extracellular spaces, but the fish starved for 11 months showed extensive cellular shrinkage and an increase in extracellular fluid. Yesser *et al.*(1999) concluded that contribution to metabolized energy in *L. abu* was higher in muscles. It seemed that muscles of posterior stomach wall were influenced less than the skeletal muscles. Degeneration in skeletal or stomach muscles of starved specimens might be attributed to vitamin C deficiency as suggested by Moeller (2004). The obvious destruction of muscle structure could explain the feebleness in movement and response during the last days of investigation.

The hyperplasia in thyroid tissue is related to iodine deficiency (Moeller, 2004). Thyroid tumer is more probable in captive fishes where the environment is devoid of iodine (Snieszko,1972). It was reported that starvation reduces the concentration of thyroxine circulating in the blood of *Salvelinus fontinalis* (Love,1980).

Females employed in the present study (90-152 mm in total length) are mature individuals as suggested by Yousif (1983). It was concluded here that ovary development from primary growth phase to primary vitellogenesis oocytes was not influenced by food deprivation for over 14 wks. It was noted that the ovaries were insulated from the depletion that occurred in carcase and liver of stickleback, but the ovaries maintained or increased their lipid and glycogen content (Wootton *et al.*, 1978). However, the final stages of maturation need abundance of nutrition because the gonads require specific fatty acids for their development (Ballantyne *et al*., 1996).

It was detected that *L. abu* was susceptive to organs damage caused by malnutrition in patterns of the so-called necrosis or apoptosis. The destruction was firstly noticed at kidney then at gills and then at liver and spleen, where the stomach wall and thyroid tissue started to be destructed after prolonged starvation.

The progressive increment of abdomen fluids may reveal falling of body lipids due to malnutrition. Salam *et al*. (2000) observed an increase in total water content in starved *C. catla* which indicated a reverse relationship between the body lipid and water content.

The changes in different organs might result from the manner of cultivation. The captivity may result in various endocrine and metabolic changes (Billard *et al.*, 1981).

References

ADHAM, HASHEM, ABU-SHABANA AND KAMEL, 2000. Vitamin C deficiency in the catfish *Clarias gariepinus. Aquac. Nutrition.*, **6**:129-139(Abstract).

AGIUS,C. AND ROBERTS, R.J.,1981. Effects of starvation on the melano-macrophage centers of fish. *J. Fish Biol.*, **19**:161-169.

ASHLEY, L.M., 1972. Nutritional pathology. **In** *Fish nutrition* (ed. J. E. Halver), pp. 439- 537. New York.

BAC,N., 1987. Hepatic disturbances induced by an artificial feed in the seabass (*Dincentrarchus labrax*) during the first year of life. *Aquaculture*, **67**:93-99.

BALLANTYNE, J. S., MERCURE, F., GERRITS, M. F., VAN DER KRAAK, G., McKINLEY, S., MARTENS, D.W., HINCH, S. G. AND DIEWERT, R.E., 1996. Plasma nonesterified fatty acid profiles in male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration. *Can. J. Fish. Aquat. Sci.*, **53**:1418-1426.

BRANCFORT, J.D. AND STEVENS, A. (EDS.). 1982. Theory and practice of histological techniques. 2nd ed. Churchill Livingstone. Edinburgh. 662 pp.

BILLARD,R.,BRY,C.AND GILLET, C., 1981. *Stress, environment and reproduction in teleost fish.* **In** Stress and fish (ed. A.D. Pickering), pp. 185-208. London.

BLAZER,V.S. AND DETHLOFF, G. M., 2000. Immune system indicators. **In** *Biomonitoring of environmental status and trends (BEST) program:* selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems. U.S.G.S. (ed. C. J. Schmitt and G.M. Dethloff), pp. 25-30. USGS/BRD.

ELLER, L.L., 1975. Gill lesions in freshwater teleosts. **In** *The pathology of fishes* (ed. W. E. Ribelin and G. Migaki), pp. 305-330. Wisconsin.

FEIST, S.W., BIGNELL,J.P., BATEMAN, K., GREEN, M., RILEY,C. AND STENTIFORD, G.D., 2003 Development in the application of hepatocellular pathology biomarkers of flatfish in environmental monitoring. **In** 11th *international conference, " Diseases of fish and shellfish"*.EAFP.

FRANCIS- FLOYD,R., 2002. Fish nutrition. Extension, institute of food and agricultural sciences. VW114:1- 4.

GRUNFELD,C., 2002. Editorial: Leptin and immunosuppression of malnutrition. *J.Clin. Endocrinol. Metab*. **87**: 3038-3039.

HINTON, D.E., WAKAMATSU, Y., OZATO, K. AND KASHIWADA,S., 2004. Imaging liver development / Remodeling in the see - through medaka fish. *Comp. Hepat*. **3**: 1-5

HWANG,P.P. AND Wu,S.M., 1988. Salinity effects on cytometrical parameters of the kidney in the euryhaline teleost *Oreochromis mossambicus* Peters .*J.Fish Biol*., **33**: 89-95.

KITA, J. AND ITAZAWA, Y., 1989. Release of erythrocytes from the spleen during exercise and splenic constriction by adrenaline infusion in the rainbow trout. *Japan. J .Ichthyol*., **36**: 48-52.

KUDO, S. AND KIMURA, N., 1983a. The recovery from hyperplasia in a natural infection. B*ull. Japan. Soc. Sci. Fish*., **49**:1627-1633.

_____1983b. The recovery from hyperplasia in an artificial infection. *Ibd.*, **49**:1635-1641.

LOVE, R.M.,1980.The chemical biology of fishes, vol.2.London.

MITCHELL, R. N. AND COTRAN, R.S., 2003. Cell injury, adaptation, and death. **In** *Robbins basic pathology* (ed.V. Kumar, *et al.*), pp. 3-31. Philadelphia.

MOELLER, R. B., 2004. Nutritional deficiencies. http:// vetpath 1. afip. mil/ Vet Services/VS.html.

NAKAGAWA,H., KASAHARA, S., TSUJIMUR, A. AND AKIRA, K.,1984. Changes of body composition during starvation in *Chlorella-extract* fed ayu. *Bull. Japan. Soc. Sci. Fish.,* **50**:665-671.

OGURI, M., 1985.Pigment granules in the renal interstitial tissue of marine teleosts. *Bull. Japan.Soc. Sci. Fish.,* **51**:1447-1449.

RIOS, F.S., KALININ, A.L. AND RANTIN, F.T., 2002.The effects of long-term food deprivation on respiration and haematology of the neotpropical fish *Hoplias malabaricus. J. Fish Biol.*, **61**:85-95.

SALAM, A., ALI, M. AND MASUD, S., 2000. Effect of various food deprivation regimes on body composition dynamics of thaila, *Catla catla*. *J. res. Sci*.,**11**: 26- 32.

SMART, G., 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). *J. Fish Biol*., **8**: 471- 475.

SMUTNA, M., VORLOVA, L. AND SVOBODOVA, Z., 2002. Pathobiochemistry of ammonia in the internal environment of fish (Review)., *Acta Vet. Brno*, **71**: 169- 181.

SNIESZKO, S.F., 1972. Nutritional fish diseases. **In** *Fish nutrition* (ed. J. E. Halver), pp. 403- 437. New York.

STORCH, V., STAHLIN, W. AND JUARIO, J. V., 1983. Effect of different diets on the ultrastructure of hepatocytes of *Chanos chanos* fry (Chanidae: Teleostei): an electron microscopic and morphometric analysis. *Mar. Biol*., **74**: 101- 104.

WOOTTON, R. J., EVANS, G. W. AND MILLS, L., 1978. Annual cycle in female three- spined sticklebacks (*Gasterosteus aculeatus L.*) from an upland and lowland population. *J. Fish Biol.*, **12**: 331- 343.

YAMAMOTO, K., ITAZAWA, Y. AND KOBAYASHI, H., 1983. Erythrocyte supply from the spleen and hemoconcentration in hypoxic yellowtail. *Mar. Bio*.,**73**: 221- 226.

YESSER, A. K. T., HINDE, M. J. AND AHMED, H. A., 1999. Effects of starvation on proximate chemical composition of *Liza abu* (Heckel, 1843). *Mar. Mesopotamica*, **14**: 11- 17.

YOUSIF, U. H., 1983. Ecological study of *Carasobarbus luteus* (Heckel) and *Liza abu* (Heckel) from Mehaijeran canal, south of Basrah, M. Sc. thesis, Univ. Basrah.