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Effect of Starvation on the Weight and Structure in Some Tissues of Cyprinid Loach, *Misgurnus anguillicaudatus*

In-Seok PARK†

(Korea Maritime and Ocean University)

미꾸리, *Misgurnus anguillicaudatus* 기아시 조직 및 미세구조 변화

박 인 석†

(한국해양대학교)

Abstract

We assessed the effects of 30 days starvation on survival rate, gonadosomatic index (GSI), hepatosomatic index (HSI), intestinosomatic index (ISI) and histological change of kidney, hepatocyte and midgut epithelium of cyprinid loach, *Misgurnus anguillicaudatus*. The average standard length and body weight were greater in order of fed, initial and starved group after 30 days starvation (P<0.05). At the conclusion of this experiment, compared to the 85.0% survival of fed group, starved group showed the decreased survival of 38.7%. GSI, HSI, ISI were greater in fed group than starved group (P<0.05). Hepatocyte nuclear area, Hepatocyte cellular area, nuclear height of midgut epithelium and nuclear height of kidney in fed group was greater than that in the starved group (P<0.05). In starved group, melano-macrophages found in kidney cell increased during 30 days starvation in cyprinid loach. Compared to those of the initial control and fed group, in starved group, the ultrastructure of hepatocytes showed changes. These results from this study appear to be an useful index of the nutritional status in cyprinid loach.

Key words : Cyprinid loach, Histological observation, Starvation, Tissue structure, Tissue weight

Ⅰ. Introduction

Fish starvation studies are significant in determining the fish's nutritional and growth characteristics (Seol et al., 2009). Most fish undergo periods of fasting or starvation due to wintering, spawning, migration or regional decreases in food resources (Hur et al., 2006). Therefore, fish suffered from states of starvation can overcome starvation using biochemical, physiological and behavioral strategies (Seol et al., 2009). Also endogenous energy from basic metabolic accumulations in the body is spent as fish consume their own tissues to remain alive during starvation (Weatherley and Gill, 1987). So histological analysis allows the determination if cause and effect relationships between body structure and starvation (Theilacker, 1986). In addition fish

† Corresponding author : 051-410-4321; ispark@kmou.ac.kr

starvation studies can contribute to our understanding of the nutritional conditions of natural and cultured fish in relation to their growth (Weatherley and Gill, 1987; Park et al., 1998).

The cyprinid loach, *Misgurnus anguillicaudatus* is freshwater fish in the loach family Cobitidae (Nelson, 2006). They are native to East Asia but are also popular as an aquarium fish and introduced elsewhere in Asia and to Europe and North America. The cyprinid loach inhabits mud, ponds and ricefields which are subjected to periodic drying resulting in starvation, and because its domestic market expands rapidly in recent years, cyprinid loach is a commercially important as cultured freshwater species in Korea(Nelson, 2006).

Gonadosomatic index (GSI) is is the calculation of the gonad mass as a proportion of the total body mass. It is a tool for measuring the sexual maturity of fishes in correlation to ovary development and testes development. Hepatosomatic index (HSI) and intestinosomatic index (ISI) are the calculation of the liver and intestinal mass as a proportion of the total body mass respectively. Many studies reported that these indexes are related to starvation of fish(Lee et al., 2000).

During starvation, the essential processes in the fish are maintained at the expense of accumulated energy reserves, resulting in progressive depletion and wastage (degrowth) of body tissues(Weatherley and Gill, 1987). The observed incidence of starvation is essentially the same based on histological criteria (Theilacker, 1986; Park et al., 2001). And the data for the hepatocyte nuclear height in relation to the starvation in fish are generally reported by other researchers. As the results subalimentation caused by starvation of fish result in decrease of hepatocyte nucleus(Alvarez and Cowden, 1966; Storch and Juario, 1983; Strüssmann and Takashima, 1989), it appears that the onset of irreversible starvation is preceded by structural changes in the hepatocyte nuclear height(Park, 2006). Storch and Juario(1983) concluded that this parameter is particularly useful as an indication of the nutritional status in fish. Likewise, within the fish body, the response to starvation is observed in the digestive organs, so intestinal epithelial cell height is useful determining nutritional state in fish(Ehrlich et al., 1976). Meanwhile a reduction in the intestinal epithelial cell height and connective tissues has been observed in common carp, *Cyprinus carpio* and Northern pike, *Esox lucius* larvae(Kostomarova, 1962).

Fish melanomacrophages (MMs) are normally located in the stroma of the haematopoietic tissue of the spleen and the kidney, although in amphibians and reptiles, and some fish, they are also found in the liver(Agius and Roberts, 2003). Also, MMs which are similar to human macrophages, metabolize toxic substances and perform various immune functions in the kidney(Agius and Roberts, 2003), and MMs increase in number in response to various pathological and physiological conditions(Palmer et al., 1992), such as starvation, aging(Seol et al., 2009) and vitamin E deficiency(Park, 2006). As the result researchers have recently examined the possibility of using morphological changes in MMs, such as number and melanin content, as a biomarker in aquatic environments(Seol et al., 2009).

The purpose of this study was threefold. The first objective was to acquire physiological basic knowledge about starvation and feeding of cyprinid loach. The second objective was to provide methods, based on histological observation, which can easily be applied in the aquaculture industry to estimate cyprinid loach condition. The third objective was to extend our knowledge of the changes in histological structure that occur in cyprinid loach during

starvation and feeding. Specially, we investigated histological changes in MMs accumulation in the kidney caused by starvation. These data were used to determine nutritional indices for cyprinid loach.

Ⅱ. Materials and methods

Cyprinid loach, *Misgurnus anguillicaudatus* used in this experiment were hatched in June 2012 at a mariculture farm at the Fishery Genetics and Breeding Sciences Laboratory of the Korea Maritime and Ocean University in Busan, Korea. Fed and starved group of experimental groups with initial group were established. Each groups consisted of cyprinid loach whose body weight is around 10 g. All fishes were fed daily with commercial feed (Pellet No. 6, Cheonhajeil feed Corporation, Daejeon, Korea; crude protein: 40.0%; crude fat: 4.0%; crude fiber: 5.0%; ash: 15.0%; calcium:1.0%; phosphorus:1.0%; mineral premix: 1.0%; vitamin premix: 1.0%) at 1-3% of their total body weight for 2 weeks prior to initiation of experiments in October 2012. The starvation and feeding experiment began in August 2012. Initially, fish weight were determined to the nearest 0.01 g using an electronic balance(JW-1, Acom, Pochen, Korea), and their standard lengths were measured to the nearest 0.01 cm using digital vernier calipers(CD-20 CP, Mitytoyo, Kawasaki, Japan).

At the initiation of this experiment, 50 fishes of initial (control) group are fixed in 10% neutral formalin solution in twice. Fish were reared in a recirculating system. Fifty fish were placed in each 1.1-ton fiberglass-reinforced plastic circular tank (118 cm diameter, 100 cm depth); each experimental group consisted of three tanks of fish. Each tank was covered with a net to prevent fish from jumping out. Light was provided by four 40-W (5400 K) fluorescent bulbs controlled by an electric timer, which kept the photoperid at a 12L:12D cycle. No lights were used during the dark period. Water temperature was controlled automatically and held at 25 ± 0.5°C during the experimental period. During the experiment, the fed group was hand-fed two times daily(first feeding occurred between 10:00 and 11:00, the second between 18:00 and 19:00). The fed group received feed ad libitum. The fish in all experiments were kept until the starved group lost vitality rapidly. The fish in all experiments were kept until the starved group ceased activity. At the conclusion of the experiment, to avoid sampling fish with guts that were distended by large quantities of food, fish were starved 24 hours before sampling (Park et al., 1998, 2001).

At the conclusion of the starvation and feeding experiment, fish's standard length was measured to the nearest 0.01 cm using digital vernier calipers and body weight was weighed 0.01 g using an electronic balance after euthanizing with an overdose of lidocaine-HCl (300 ppm lidocaine-HCl / 1,000 ppm $NaHCO_3$) at 25 ± 0.5°C. The survival rate during the starvation and feeding experiment was calculated retroactively, with the dead fish counted every day. The aggregate survival rates of the fed group and starved group during the experimental period were determined for each of the triplicate groups. The starved group was not fed until the end of the starvation and feeding experiment. The fed group was given food continuously.

At the conclusion of the experiment, 50 fishes from each triplicated group were captured. The fish were euthanized with an overdose of lidocaine-HCl (300 ppm lidocaine-HCl / 1,000 ppm $NaHCO_3$) at 25 ± 0.5°C for measuring, according to Park et al. (1988) and Kim et al.(2005) Kidney, liver and gonad

of each fish were dissected respectively. Gonad, liver and kidney weight were measured and GSI, HSI and ISI were calculated with the formula: GSI (%) = (Gonad weight / total tissue weight) × 100, HSI (%) = (Hepatocyte weight / total tissue weight) × 100 and ISI (%) = (Intestine weight / total tissue weight) × 100.

A histolopgical method was then used to determine the effects of starvation and feeding on the hepatocytes, intestinal epithelium, and kidneys, including MMs over a period of 30 days. First, dissected liver, intestine and kidney of each group were fixed in 10% neutral formalin solution for 24 hours. Fixed samples were refixed in Bouin's fixative for 24 hours, dehydrated through a graded ethanol series, cleared with xylene, infiltrated with paraffin, and embedded for both sagital and frontal sectioning. Serial tissues (6 μm thickness) were sectioned by microtome (HM 315, Microm GmbH, Germany) and stained with hematoxylin and eosin-phloxine B. Finally, the samples were observed under a microscope (Axioskop; Zeiss, Germany) and microphotographed. The nuclear height of intestinal epithelium and kidney were measured with an eyepiece micrometer under a microscope at a ×1,000 magnification. The hepatocyte nucleus and cell area (S) was calculated as $S = \pi ab / 4$, where a and b are the major and the minor axes of the cell and the nucleus, respectively(Seol et al., 2008).

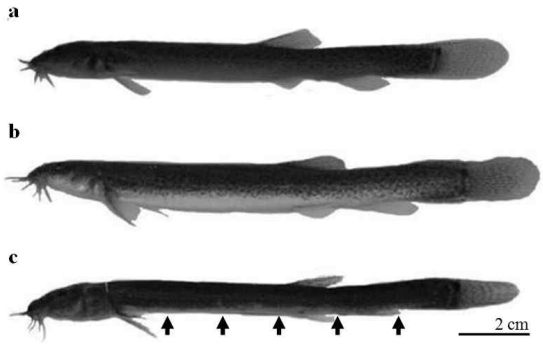
For electron microscopy, small portions of the excised hepatopancreas of fed and starved samples at the conclusion of experiment were pre-fixed in a cold 2.5% glutaraldehyde solution (pH 7.5) for 2 hours. The tissue was pre-fixed for 2 hours at 4°C in 2.5% glutaraldehyde solution buffered by 0.1 M phosphate buffer solution (PBS, pH 7.2). After washing with PBS for 10 min, the samples were post-fixed in 1% osmium tetroxide (OsO_4) for 2 hours at 4°C. Samples were rewashed with PBS, then serially dehydrated with ethanol from 50 to 100%, and embedded in Epon 812.

Sections were cut using an ultramicrotome (LKB, Nova, Sweden) and then stained with toluidine blue to determine the investigation region. The sections were double-stained with uranylacetate and lead citrate solution and examined using a transmission electron microscope (JEM 1200 E-X II, 60-80 kv, JEOL, Tokyo, Japan).

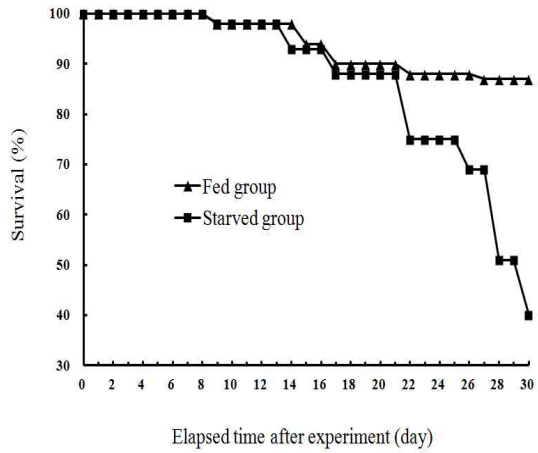
One way analysis of variance (ANOVA) and Duncan's multiple range test were used for the analysis in the SPSS statistical package (SPSS 9.0, SPSS inc., Chicago, IL) to determine whether the values for each set of experimental data were significantly different. The experiments were triplicated.

Ⅲ. Results

After 30 days, the starved group of cyprinid loach, *Misgurnus anguillicaudatus* had rapidly lost vitality, so the experiment was finished. The average standard length and weight of initial group were 10.4 ± 0.39 cm and 6.9 ± 0.66 g, respectively[Fig. 1]. And the average standard length and weight of fed group were 12.7 ± 1.49 cm and 10.8 ± 2.24 g, respectively [Fig. 1]. Those of starved group were 9.7 ± 1.14 cm and 5.7 ± 1.20 g, respectively [Fig. 1]. [Fig. 2] shows survival rate of fed and starved group during the 30 days starvation and feeding experiment. At the beginning of the experiment, survival rate of fed and starved group was 100%. In the fed group, the survival rate tended to declined to 87.5 ± 1.36% during 22 days but it was almost constant to the end of the experiment. The survival rate of fed group after 30 days was 85.0 ± 1.25%. The survival rate of



[Fig. 1] Typical external morphology of the cyprinid loach, *Misgurnus anguillicaudatus* at the conclusion of the experiment. Initial group (a), starved group (b) and fed group (c) for 30 days starvation and feeding experimental period. Note the lateral ventral region is thin in starved group (Arrows in Fig. 1-c).



[Fig. 2] Mean survival of fed and starved cyprinid loach, *Misgurnus anguillicaudatus* during 30 days starvation and feeding experimental period.

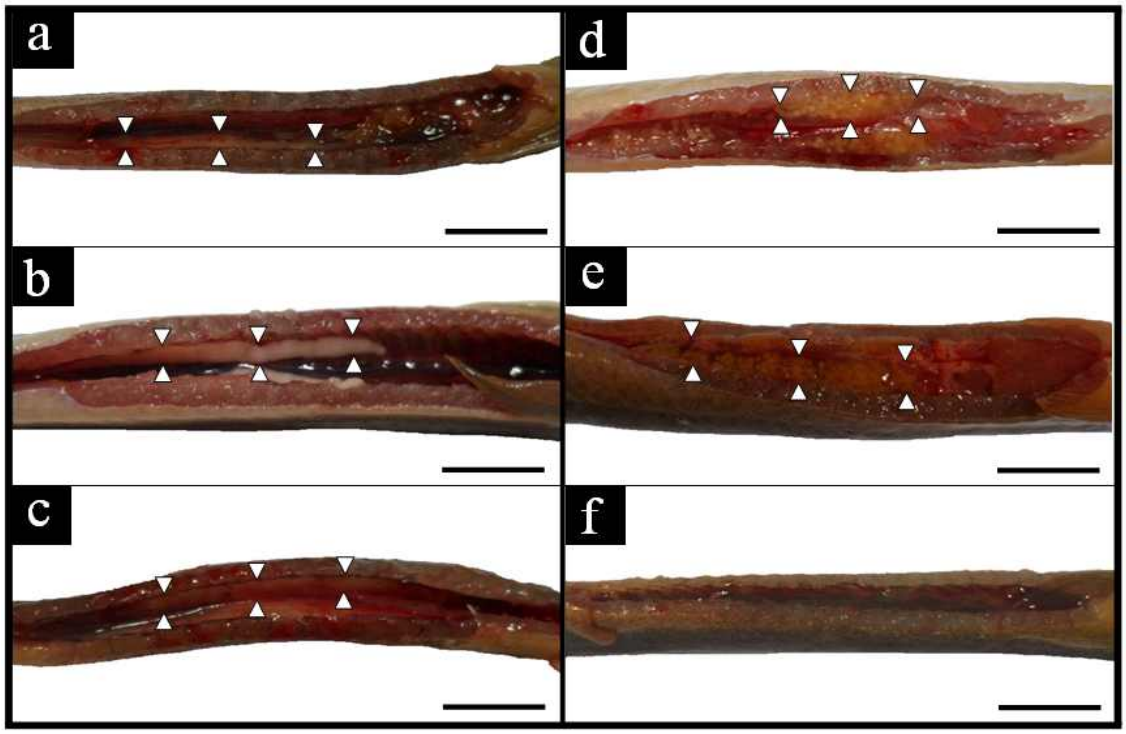
starved group was 97.4 ± 6.41% during 12 days, after that the parameter declined gradually till the end of experiment, so the survival rate showed 38.7 ± 1.05% at 30 days after the experiment. Rapid decline was occurred at 27 days after experiment.

<Table 1> shows the change of GSI, HSI and ISI among initial, fed and starved group. External morphology of gonad is shown in [Fig. 3]. [Figures 3a, 3b and 3c] are testis and [Figs. 3d, 3e and 3f] are ovary in initial, fed and starved group, respectively (ovary of starved group in [Fig. 3f] was invisible to the naked eye). As shown in <Table 1> female GSI of initial group (4.2 ± 2.20) was not different from that of fed group (4.6 ± 2.61) (P>0.05).

<Table 1> The change of gonadosomatic index (GSI), hepatosmatic index (HSI), and intestinosomatic index (ISI) in initial, fed, and starved groups for this 30 days starvation and feeding experiment in cyprinid loach, *Misgurnus anguillicaudatus*[*1]

Group	GSI[*2]		HSI[*3]	ISI[*4]
	Female	Male		
Initial	4.2 ± 2.20[b]	0.8 ± 0.14a	1.5 ± 1.05[b]	0.6 ± 0.16[b]
Fed	4.6 ± 2.61[b]	1.1 ± 0.92[b]	1.3 ± 0.81[b]	0.7 ±0.10[b]
Starved	3.3 ± 3.15[a]	0.6 ± 0.16[a]	0.4 ± 0.30[a]	0.5 ± 0.30[a]

[*1]The value are means±SD (n=50) of triplicated groups. Means in columns having same superscript letter are not significantly different among experimental grops (P>0.05).
[*2](Gonad weight / total tissue weight) × 100.
[*3](Hepatocyte weight / total tissue weight) × 100.
[*4](Intestine weight / total tissue weight) × 100.



[Fig. 3]. Typical external morphology of gonad at the conclusion of this 30 days starvation and feeding experiment in the cyprinid loach, *Misgurnus anguillicaudatus* (Arrowed with white arrow heads). Testis: (a) initial group, (b) fed group and (c) starved group; ovary: (d) initial group, (e) fed group and (f) starved group. Ovary of starved group (f) was so thin invisible to the naked eye. Bars indicate 1 cm.

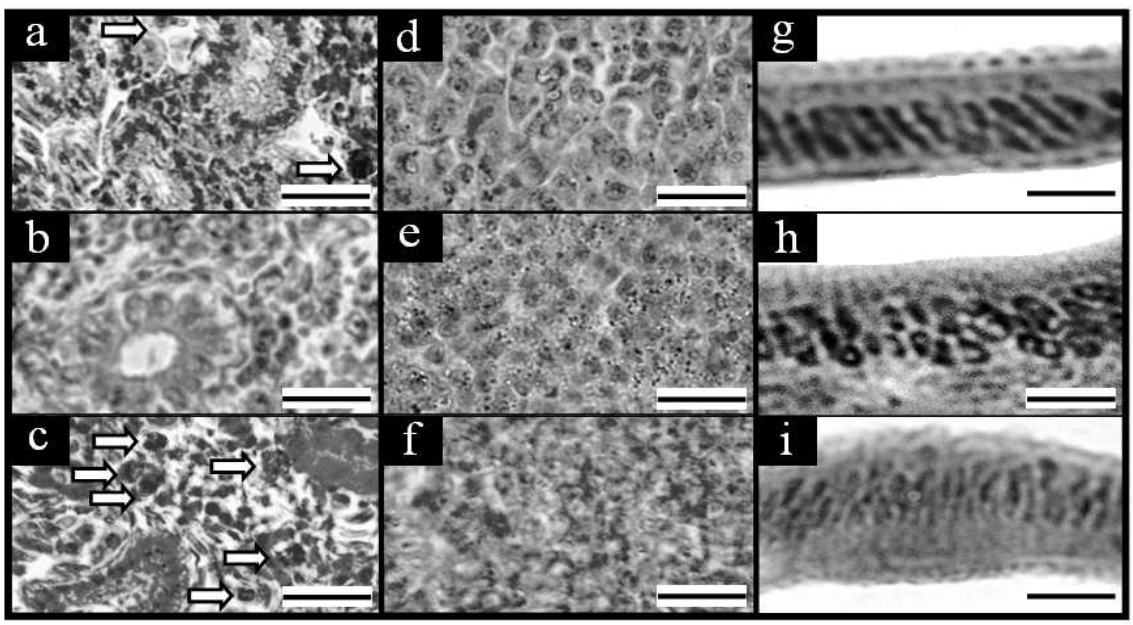
But in starved group, female GSI (3.3 ± 3.15) showed lower value compared with initial and fed group (P<0.05). Male GSI of fed group (1.1 ± 0.92) showed the highest value of three groups (P<0.05), there wasn't significant difference between initial (0.8 ± 0.14) and starved group (0.6 ± 0.16) (P>0.05). The values of HSI in initial group (1.5 ± 1.05) and fed group (1.3 ± 0.81) had no significant difference (P>0.05), but greater than values of HSI in starved group (0.4 ± 0.30) (P<0.05). In the value of ISI, there was no significant difference between initial (0.6 ± 0.16) and fed group (0.7 ± 0.10) (P>0.05). But the ISI in starved group (0.5 ± 0.30) showed the smallest value of all groups (P<0.05).

We observed the change of kidney <Table 2, Figs. 4a, 4b and 4c], hepatocyte <Table 2, Figs. 4d, 4e and 4f] and intestinal epithelium <Table 2, Figs. 4g, 4h and 4i] in the cyprinid loach during 30 days starvation and feeding experiment. The value of height of kidney nucleus was higher in order of fed (4.1 ± 0.62 ㎛), initial (3.4 ± 0.50 ㎛), and starved group (2.5 ± 0.33 ㎛) (P<0.05) <Table 2>. Kidney nucleus in fed group was larger than that in other two groups, the parameter in starved group was small and dented [Figs. 4a, 4b and 4c]. Kidney cells in MMs were not shown in the initial and fed group, or the number was small [Figs. 4a, 4b and 4c].

<Table 2> Changes in nuclear area and cellular area of hepatocyte, and nuclear height of midgut epithelium and kidney in the initial, fed and starved groups for this 30 days starvation and feeding experiment in cyprinid loach, *Misgurnus anguillicaudatus*[*1]

	Initial group	Fed group	Starved group
Hepatocyte area (μm^2)			
Nucieus	0.7 ± 0.27[b]	1.2 ± 0.56[c]	0.3 ± 0.20[a]
Cell	9.9 ± 2.67[a]	16.5 ± 3.62[b]	9.9 ± 3.06[a]
Nuciear height (μm)			
Midgut epithelium	5.3 ± 0.48[b]	6.7 ± 0.73[c]	4.5 ± 0.55[a]
Kidney	3.4 ± 0.50[c]	4.1 ± 0.62[c]	2.4 ± 0.33[a]

[*1]The value are means±SD (n = 50) of triplicated groups. Means in columns having same superscript letter are significantly different among experimental grops (P>0.05).

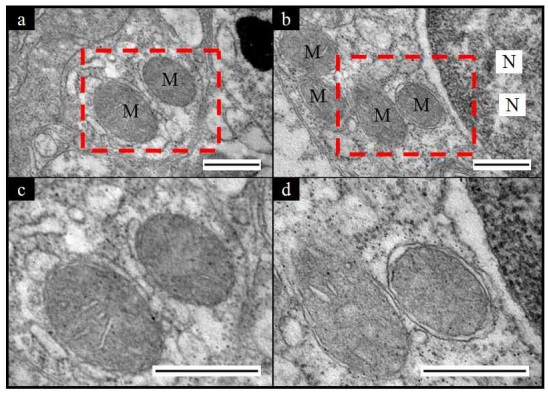


[Fig. 4]. Histological appearance of the kidney, liver and midgut epithelium under starved after the 30 days of this starvation and feeding experiment in cyprinid loach, *Misgurnus anguillicaudatus*. Histological observations of melano-macrophage (MM) centres on the kidneys of (a) the initial group, (b) the fed group and (c) the starved group. Note the white arrows in Fig. 4-c indicate the exact increase in the degree of MM deposition during starvation. Livers of (d) the initial group, (e) the fed group and (f) the starved group. Note the reduction in the size of the hepatocyte nuclei in the starved group (Fig. 4-f). Midgut epithelium of (g) the initial group, (h) the fed group and (i) the starved group. Note the reduction in the nuclear height of the midgut epithelium in the starved group (Fig. 4-i). Hematoxylin and eosin staining. Bars indicate 20 μm .

On the other hand, MMs could be observed that its number increased [Figs. 4a, 4b and 4c]. The area of the hepatocyte nucleus in the fed group (1.2 ± 0.56 μm^2) was greatest (P<0.05), in starved group (0.3 ± 0.20 μm^2) was the smallest (P<0.05) <Table 2>.

The area of hepatocyte cell in the fed group (16.5 ± 3.62 μm^2) was also greatest while the parameter in the initial (9.9 ± 2.67 μm^2) (P<0.05) and starved group (9.9 ± 3.06 μm^2) had no significantly difference each other (P>0.05) <Table 2>. As shown [Figs. 4d, 4e and 4f], in the fed group cell and nucleus were large and infrequent while irregular, small and frequent in the starved group. Similarly kidney nucleus, the value of intestinal epithelial nucleus height was higher in order of fed (6.7 ± 0.73 μm), initial (5.3 ± 0.48 μm), and starved group (4.5 ± 0.55 μm) (P<0.05) <Table 2>. Intestinal epithelial nucleus in the fed group was large and regular, whereas that in starved group had a long and irregular shape [Figs. 4g, 4h and 4i].

In the hepatocyte ultrastucture of initial control, fed and starved groups showed that the infoldings of inner mitochondrial membrane were usually lamellar [Fig. 5]. In the starved group after a starvation period of 4 weeks, the hepatocytes showed changes, in comparison to those of the initial control and fed group. The mitochondria were separated from the shell and were destroyed by starvation [Fig. 5].



[Fig. 5]. Transmission electron microscopy of hepatocyte in fed group (a and c) and starved group (b and d) of cyprinid loach, *Misgurnus anguillicaudatus*. Figs. 5-c and 5-d: high power view of red dotted line box in Figs. 5-a and 5-b. M: mitochondria; N: nucleus. Bars indicate 0.1 μm.

Ⅳ. Discussion

During starvation, fish maintain survival using the accumulation of energy consumption (Weatherly and Gill, 1987). So this survival strategy leaves little energy for other biological functions, including somatic growth. As a result, growth in body size slows considerably during starvation (Weatherley and Gill, 1987). In this study, standard length and body weight of cyprinid loach, *Misgurnus anguillicaudatus* were decreased in the starved group and increased in the fed group during the 30 days experiments. Specially, the body weight was increased by about two times. As a result of 12 weeks of starvation and feeding experiments in olive flounder, *Paralichthys olivaceus* while standard length and body weight in fed group increased by 136.2% and 310.0% compared with initial group, those in starved group decreased by 92.1% and 56.0% (Park, 2006). Rainbow trout, *Salmo gairdneri* also showed that body weight decreased by 14.5% and 32.5% respectively during the 3 and 13 weeks starvation (Weatherley and Gill, 1981). Given these results, the change due to feeding and starvation is considered to be greater in body weight than in standard length. Likewise this study, Sumpter et al. (1991) reported that decrease of weight was greater than length in a

year rainbow trout during 6 weeks starvation.

Many 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 4 years(Hur et al., 2006), and nonfeeding larvae may survive for 1 month(Hur et al., 2006). In this study, after 30 days, while fed group maintained 85.0% survival rate, starved group showed 38.9% survival rate (about 45% of survival rate in fed group). Similar tendency with this study observed in previous study of Park(2006), as the result of 12 weeks starvation and feeding experiment in olive flounder, fed group maintain 90.0% survival rate but starved group maintain 77.5% survival rate. Seen as cyprinid loach shows much lower survival rate than olive flounder, cyprinid loach is suggested to be more affected by starvation than olive flounder.

Generally, feeding or starvation affects weight of many tissues and the change of HSI in teleost fish is related to development of gonad (Lee et al., 2000). Lee et al.(2000) reported that HSI in chub mackerel, *Scomber japonicas*, greenling, *Hexagrammos otakii* and sockeye salmon, *Oncorhychus nerka* was directly proportional to development of gonad, whereas that in ayu, *Plecoglossus altivelis* and eel, *Anguillia japonica* was inversely proportional to. In this study, female GSI, HSI and ISI was not significantly different between initial and fed group, but was lowest in the starved group. On the other hand male GSI was highest in the fed group of this study, Additionally, female GSI is higher than male GSI in every group. It demonstrated that ovarium was heavier than testis and starvation was more influential than feeding to kidney and hepatocyte.

Under the optical microscope, MMs appear as small-to-large round or oval structure which are easily distinguished from surrounding lymphatic tissue, whereas under the electron microscope they appear as groups of macrophages(Seol et al., 2009). MMs are observed in normal fish, but are more numerous in physiologically abnormal states, i.e., because of disease or stress. In our study, kidney nuclear height increased in fed group and decreased in starved group compared with initial group. In addition, the shape of kidney nucleus in starved group was dented. We found MMs in the kidney cell, they were increased during 30 days starvation. MMs deposition increased significantly in the starved group similar to results reported for plaice, *Cleisthenes pinetorum*, rainbow trout, swordtail, *Xiphophorus hellerii*, tilapia, *Tilapia mossambica* (Agius and Roberts, 1981), blue tilapia, *Oreochromis aureus* (Seol et al., 2009), sea bream, *Diplodus annularis*(Micale and Perdichizzi 1990), masu salmon, *O. masou*(Mizuno et al., 2002) and olive flounder(Hur et al., 2006). On the other hands, MMs showed no significant difference between fed and initial group. This result is found in Hur et al. (2006). MMs deposition ranged from 0.21% to 0.29% in the control group and 0.25% to 0.28% in the fed group during the 12 weeks experiment(Hur et al., 2006). This result suggests that there is no MMs difference by the food amount.

In most fish the size and shape of the hepatocyte nucleus is constant if adequate nutrition is supplied. In the absence of adequate nutrition the contents of the hepatocyte nucleus is converted to non-chromosomal protein, resulting in a change to the size and shape of the nucleus(Alvarez and Cowden, 1966; Storch and Juario, 1983). In this study, hepatocyte nuclear area was increased in fed group and decreased in the starved group, hepatocyte cell area was large in fed group. Additionally hepatocyte nuclear was regular and rare in fed group but irregular and numerous in the starved group. Similarly the results of this study, the immature

rainbow trout showed similar regressive changes in the liver tissue after starvation for three months at a water temperature of 13°C (Robertson et al., 1963). Because observation on hepatocyte nuclear size is useful to determine nutritional state, the study of starvation in pejerry, *Odontesthes bonariensis* (Strüssmann and Takashima, 1989), larvae of red spotted grouper, *Epinephelus akaara* (Lee et al., 1998), larval rockfish, *Sebastes schegeli* (Park et al., 1998) and chum salmon, *O. keta* fry has been reported.

The digestive organs of the jack mackerel, *Trachurus symmetricus* including the intestinal epithelium, were the first affected by starvation (Theilacker, 1978). In our 30 days starvation and feeding experiments in cyprinid loach, intestinal nuclear height was highest in fed group and lowest in starved group. The shape of intestinal nucleus was plump and regular in fed group but dented and irregular in starved group. Park(2006) reported that average nuclear height of midgut epithelium reduced or increased respectively during 12 weeks starvation and feeding experiment in olive flounder. Likewise this result, atrophy of the stomach tissue accompanied by a reduced ability to exercise during starvation was reported by many researchers (Park, 2006).

Liver structure and ultrastructure have recently been identified as relevant parameters used for detecting and assessing the toxicity of several xenobiotics in fish, but their use in fish nutrition is much rarer, except in nutritional studies(Segner and Braunbeck, 1988; Braunbeck et al., 1990; Abi-Ayad and Kestemont, 1994; Arnold et al., 1996).

Microvesicular hepatocellular degeneration was reported in hybrid striped bass fed experimental and practical diets(Brown et al., 1993). In a percid, *Perca fluviatilis,* hepatocyte ultrastructure was noticeably affected by dietary treatments(Kestemont et al., 1996). The progressive increase in glycogen storage and lipid droplets, both in size and abundance, and the concomitant reduction in essential organelles involved in the oxidation processes and protein synthesis, such as RER and mitochondria, can be considered preliminary signs of impaired liver function. Lipid storage, however, appeared to be limited to large droplets into the cytoplasm itself, without the formation of liposomes, i.e., lipid droplets that are formed within the cisternae of the ER, or intranuclear lipid inclusions of microvesicular and macrovesicular nature(Baglio and Farber, 1965).

Our results show that cyprinid loach has a carbohydrate-oriented liver. The carbohydrates are stored as alpha-glycogen, a predominant feature of hepatocytes in the liver of well-fed fish. We also observed that this species undergo marked changes that proceed at an accelerated pace as starvation time is prolonged. After 4 weeks of starvation, the hepatocytes of cyprinid loach underwent a part of ultrastructural changes that have previously been described for other species. Widened intercellular spaces, reduction in size, hypertrophy of the lysosomes, reduction in the diameter of the cell nucleus, condensation of the chromatin material, depletion of ribosome-studded ER and glycogen, and mitochondrial enlargement and destruction all appear to be the characteristic features of hepatocytes in starved teleosts(Renaud and Moon, 1980; Moon and Johnston, 1981; Storch and Juario, 1983).

We determined the effects of 30-days starvation and feeding on the survival rate, various indexes, and histological changes in cyprinid loach. During the starvation, essential processes in fish are maintained at the expense of accumulated energy

reserves, resulting in the progressive depletion and wastage of body tissues. Therefore, studies of fish nutrition in relation to growth and condition can enhance understanding of the responses of wild and cultured fish to feeding conditions(Weatherley and Gill, 1987; Park et al., 1998, 2001). Analysis of the various results investigated in this study has been shown to provide an accurate indication of the nutritional conditions of other fish.

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