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[Article]

The Effects of Starvation on Physiological Changes and Stress Response in Cultured Cobitid Loach (*Misgurnus anguillicaudatus*) Exposed to Sodium Nitrite

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To investigate effects of starvation on physiological changes, stress response, and survival of cobitid loach (*Misgurnus anguillicaudatus*) exposed to sodium nitrite ($NaNO_2$), a 4-week experiment was conducted. Fewer fish survived in the starved group than those in the fed group during the experiment. Starvation resulted in growth retardation, leading to differences in body length and body depth between fed and starved groups. The fed gorup continued to grow and remained in good condition. Blood chemical analysis (plasma cortisol and glucose) showed significant differences in stress response to nitrite exposure between fed and starved groups ($p < 0.05$). These results suggest that all parameters employed in this study to assess effects of starvation with $NaNO_2$ stress are useful information for researching nutritional status in cobitid loach.

Keywords: Cobitid loach, *Misgurnus anguillicaudatus*, Physiological response, Sodium nitrite, Starvation, Stress response

Introduction

Many species of fish undergo periods of starvation due to various factors, including temperature declines associated with seasonal changes, spawning migration, or regional and seasonal decreases in food supply (Mustafa and Mittal, 1982; Weatherley and Gill, 1987; Lee et al., 1999). To survive periods of starvation, fish utilize biochemical, physiological, and behavioral strategies in addition to endogenous reserves of energy derived from metabolic processes (Mustafa and Mittal, 1982; Weatherley and Gill, 1987; Lee et al., 1999; Park et al., 2002; Hur et al., 2006a, 2006b; Park, 2006). However, the latter can leave little energy for other biological functions such as somatic growth. As a result, growth in body size can slow considerably during starvation.

The efficiency of oxygen consumption in fish is directly related to their metabolic processes through which they can produce physical energy. In turn, this ultimately determines their population density, food supply, and fish yield (Dalla et al., 1998). Mehner and Wieser (1994) have reported the relationship between depletion of energy reserves and changes in oxygen consumption in perch *(Perca fluviatilis)*. Starvation can decrease oxygen uptake efficiency in perch larvae and traira (*Hoplias malabaricus*) subjected to extended periods of starvation (Mehner and Wieser, 1994; Rios et al., 2002).

Stresses can induce the release of catecholamine and cortisol in fish, causing rapid metabolism of high-energy storage compounds (Barton and Iwama, 1991). These catabolic processes have harmful biochemical effects on fish health. They also impair growth (Specker and Schreck, 1980). Fish exhibit primary, secondary, and tertiary responses to stress (Barton and Iwama, 1991). Their primary response involves rapid changes in plasma levels of catecholamine and corticosteroid. When these responses to stressful conditions exceed normal levels, harmful secondary and tertiary responses occur. Therefore, stress can induce changes in energetic metabolic processes, reduce growth rate, interfere with reproduction, and lead to rapid changes in flesh quality following death (Barton and Iwama, 1991).

Blood chemistry can serve as an indicator of an animal's physio-

logical state. Many factors (age, sex, nutrition, season, and circadian rhythm) may affect blood chemistry. Information is available for plasma glucose changes during starvation in Atlantic cod (*Gadus morhua* L.), European eel (*Anguilla Anguilla*), pike (*Esox lucius*), toadfish (*Opsanus tau*), goldfish (*Carassius auratus*), and American eel (*A. rostrata*) (Tashima and Cahill, 1968; Chavin and Young, 1970; Larsson and Lewander, 1973; Ince and Thorpe, 1976; Moon, 1983). Studies of plasma-free fatty acids during starvation stress have also been performed in European eel, American eel, rainbow trout (*Oncorhynchus mykiss*), pike, and toadfish (Tashima and Cahill, 1968; Larsson and Lewander, 1973; Ince and Thorpe, 1976; Moon, 1983; Sumpter et al., 1991). Rios et al. (2002) have noted that erythrocyte senescence and hematological changes are induced by starvation in *Hoplias malabaricus*.

The cobitid loach (*Misgurnus anguillicaudatus*) is a freshwater species in the loach family of Cobitidae (Nelson, 2006). This species is native to east Asia. As a popular aquarium fish, it has been introduced to other places in Asia, Europe, and North America. The cobitid loach inhabits mud, ponds, and rice fields that are subjected to periodic drying, resulting in starvation. As the domestic market has expanded rapidly in recent years, cobitid loach has become a commercially important freshwater species in Korea. The objective of this study was to investigate effects of starvation on physiological and metabolic profiles of cobitid loach.

Materials and Methods

The specimens of cobitid loach (*Misgurnus anguillicaudatus*) (mean body weight ± SD: 7.4 ± 0.62 g; mean total length ± SD: 11.2 ± 0.78 cm; n = 100) used in this experiment were hatched in October 2017 at the aquaculture facility at the Fishery Genetics and Breeding Sciences Laboratory of the Korea Maritime and Ocean University (Busan, Korea). At the beginning of the experiment two experimental groups were established: a fed and a starved group. For two weeks prior to commencement of the experiment all the fish were fed daily with a commercial feed (containing 40.0% crude protein, 4.0% crude fat, 5.0% crude fiber, 15.0 ash, 1.0% calcium, 1.0% phosphorus, and 1.0% minerals; Table 1) at a rate of 1~3% of their total body weight. The fed group was hand fed three times daily at 4-h intervals. The first feeding occurred between 08.00 h and 12.00 h, the second between 12.00 h and 16.00 h, and the third between 16.00 h and 20.00 h (but always with 4 h between successive feeds). The commercial feed used prior to the start of the experiment was also used for the fed group during the experiment. The fed group received food *ad libitum*, whereas the starved group was fasted throughout the experiment.

Table 1. Composition of the experimental diets used in this starvation experiment[*1]

Nutrition	Content (%)
Crude protein	40.0
Crude fat	5.0
Crude fiber	5.0
Ash	14.0
Calcium	1.0
Phosphorus	1.0
Mineral premix[*2]	1.0
Vitamin premix[*3]	1.0

[*1] Cheonhajeil Feed Coporation (Daejeon, Korea)
[*2] Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1} mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003
[*3] Mineral premix contained the following ingredients (g kg^{-1} premix): NaCl, 43.3; $MgSO_4 \cdot 7H_2O$, 136.5; $NaH_2PO_4 \cdot 2H_2O$, 86.9; KH_2PO_4, 239.0; $CaH_4(PO_4) \cdot 2H_2O$, 135.3; ferric citrate, 29.6; $ZnSO_4 \cdot 7H_2O$, 21.9; Ca-lactate, 304.0; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0

The fish were raised in a recirculating system. They were placed in 1.1 ton fiberglass-reinforced circular tanks (118 cm diameter × 100 cm depth). Twenty fish were placed in each tank. During the experimental period the water temperature was controlled automatically at 25±0.5℃, and artificial lighting, controlled by an electric timer, was used to maintain the photoperiod on a 12:12 h light/dark cycle. Survival was recorded throughout the experiment. The starved group was not fed until the end of the starvation experiment, while the fed group was provided food continuously. The fish in all experimental treatments were kept until the starved group rapidly lost vitality. The survival rate during the starvation experiment was calculated retrospectively from daily counts of any dead fish. The aggregate survival rates of the fed and starved groups during the experimental period were determined for each of the triplicate groups.

To avoid sampling fish with guts that were distended by large quantities of food, at the conclusion of the experiment the fish in

each group were starved 48 h prior to sampling (Park et al., 2001; Hur et al., 2006a). The oxygen consumption rate was measured according to the method of Jo and Kim (1999). The respirometer chamber was based on a simple circulating system, whereby the water was pumped from a reservoir (30 ℓ) to a header tank, from where it flowed through the respirometer chamber and back to the reservoir. The header tank was equipped with a temperature controller, and the water was filtered (10 μm and 3 μm cartridge filters) to exclude particles. A flow-through UV lamp was used to reduce oxygen consumption by microbes. Water from the respirometer chamber flowed into an oxygen measurement chamber. During the experiment the average water flow was 59.6 ± 0.5 l/h. The respirometer chamber comprised an acrylic resin box 10 cm width × 25 cm length × 10 cm height (wall thickness 8 mm). A rubber pad was used to cover the respirometer chamber to prevent the inflow of air; a hole in the cover contained a small valve for the removal of air from the chamber. Water flowing into the respirometer chamber was diffused through a 10 mm pipe that had a cap at the end in which there were several holes. Water flowed from the chamber into the dissolved oxygen measurement chamber (10 cm width × 10 cm length × 6 cm height). The respirometer system could operate three chambers at the same time, each connected to a separate dissolved oxygen measurement chamber.

Each dissolved oxygen measurement chamber was equipped with an oxygen probe, and air in this chamber was removed using the same method as for the respirometer chamber. Dissolved oxygen was measured using an oxygen measurement electrode and a multidata logger system (Oxyguard, Denmark). The dissolved oxygen in the inflow and outflow of the respirometer chamber was measured at 5- minute intervals over 24 h using μLog VL 100 Software. Measurements of the oxygen concentration and the oxygen consumption rate at each temperature were recorded using the multidata logger.

From the commencement of the experiment measurements were made at 6-h intervals over 48 h. Prior to each dissolved oxygen measurement the pH, and ammonium (NH_4^+) and carbon dioxide (CO_2) concentrations were measured, and the respiratory frequency (gill cover movement) was measured using a counter and a digital timer. The NH_4^+ and CO_2 concentrations were measured spectrophotometrically (DR2800, HACH, Loveland, Colorado, USA). The dissolved oxygen and pH were measured using an oxygen measurement electrode and a multidata logger system (Oxyguard, Denmark). Measurements of oxygen concentrations and oxygen consumption rates for each experimental group were recorded using the multidata logger, as described by Cech (1990). Data were analyzed by one- and two-way ANOVA using the SPSS statistical package (SPSS 9.0, SPSS Inc., USA). Means were compared using Duncan's multiple range test, and were considered significantly different at $p < 0.05$. The experiment was performed in triplicate.

Table 2. Acute toxicity test condition of sodium nitrite for cobitid loach, *Misgurnus anguillicaudatus*

Test parameter*	Conditions
Temperature (℃)	26 ± 0.5
pH	7.1 ± 0.65
DO (dissolved oxygen; mg/l; Saturated concentration in 26℃)	7.6
Salinity (ppt)	0.3 ± 0.07
Ammonia (ppm)	0.01
Nitric acid (ppm)	1.8 ± 0.14
Nitrous acid (ppm)	0.01
Conductivity (μs/cm)	238

*Temperature, pH, dissolved oxygen and salinity were measured using an oxygen measurement electrode and a multi-data logger system (Oxyguard, Denmark). Ammonia, nitric acid, nitrous acid and conductivity were measured using spectrophotometer (DR-2800, HACH, Loveland, Colorado, USA). The values are means of triplicate groups

Tests of acute and sublethal nitrite toxicity were conducted over a 96-h period. For each replicate in each treatment (nitrite concentration) 20 individuals (83.3 ± 7.26 g, 16.5 ± 1.03 cm) were placed in a 150-ℓ fiberglass-reinforced plastic tank containing 150 ℓ of fresh water (Table 2). Nitrite (sodium nitrite; Sigma, St. Louis, MO, USA) was added to the tanks to create nitrite concentrations of 40, 80, 120, 160, and 200 mg/l; a nitrite-free treatment served as a control. Three replicates were included for each test concentration and the control. Determining the median lethal concentration (LC_{50}), and statistical analysis of the data were based on the method described by Peltier (1978).

Results

The starved group of cobitid loach (*Misgurnus anguillicaudatus*) rapidly lost vitality. Therefore, the experiment was terminated. The cumulative survival was 87 ± 1.2% in the fed group and 40 ± 2.4% in the starved group in each of triplicate tanks (Fig. 1). Star-

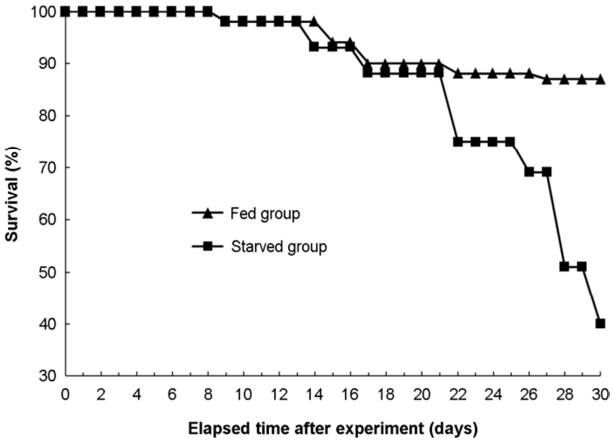

Fig. 1. Survival rates of fed and starved cobitid loach, *Misgurnus anguillicaudatus* for 30 days experimental period. The values are means of triplicate experiments.

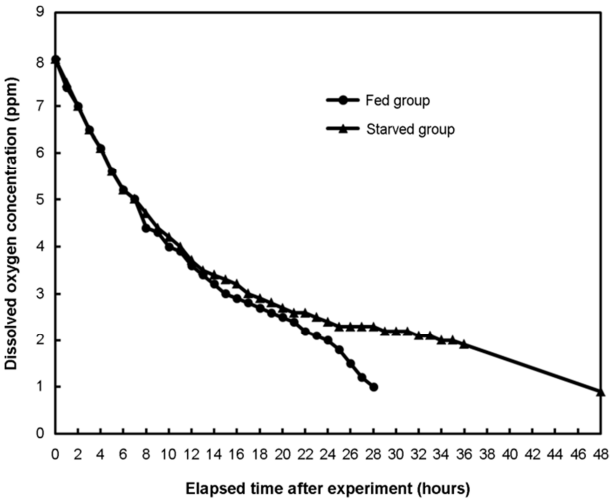

Fig. 2. Dissolved oxygen concentration between fed group and starved group on cobitid loach, *Misgurnus anguillicaudatus*. The values are means of triplicate experiments.

vation resulted in growth retardation which was evident based on differences in body length and depth between the fed and starved groups, although they continued to grow and remained in good condition. Results of two-way ANOVA and measurements of the oxygen consumption rate between the fed and starved groups are shown in Tables 3, 4 and Fig. 2. For the starved group, the dissolved oxygen concentration decreased to 1 ppm over 48 h. In the fed group, it decreased to 1 ppm over 28 h (Fig. 2). Table 3 shows pH values and respiratory frequency (gill cover movement)

Table 3. Respiratory frequency (gill cover movement) and pH values between fed group and starved group of cobitid loach, *Misgurnus anguillicaudatus*[*]

Time (hours)	Respiratory frequency (min^{-1})		pH	
	Fed group	Starved group	Fed group	Starved group
Pre-experiment	95^a	114^b	7.6^a	7.6^b
6	104^a	118^b	6.4^a	7.2^b
12	92^a	127^b	4.6^a	4.9^b
18	65^a	114^b	4.1^a	4.6^b
24	64^a	84^b	3.9^a	4.3^b
30	63^a	82^b	3.6^a	3.9^b
36	62^a	81^b	3.4^a	3.7^b
42	60^a	76^b	3.2^a	3.5^b
48	59^a	70^b	3.1^a	3.4^b

[*]Respiratory frequency was measured while 1 min. Difference between fed group and starved group is significant at this level ($p < 0.05$). The values are means of triplicate experiments

Table 4. Carbon dioxide (CO_2) and ammonium (NH_4^+) concentration between fed group and starved group of cobitid loach, *Misgurnus anguillicaudatus*[*]

Time (hours)	CO_2 concentration (mg/l)		Ammonium (NH_4^+)	
	Fed group	Starved group	Fed group	Starved group
Pre-experiment	5.1^a	5.1^b	0	0
6	13.6^a	13.4^b	0.11^a	0.10^b
12	15.2^a	14.8^b	0.14^a	0.12^b
18	17.6^a	16.8^b	0.17^a	0.15^b
24	19.2^a	19.8^b	0.18^a	0.17^b
30	19.8^a	20.2^b	0.20^a	0.20^b
36	20.4^a	20.6^b	0.22^a	0.22^b
42	21.0^a	20.8^b	0.24^a	0.23^b
48	21.4^a	21.0^b	0.25^a	0.24^b

[*]Difference between fed group and starved group is significant at this level ($p < 0.05$). The values are means of triplicate experiments

Table 5. The 96 hr LC_{50} values of cobitid loach, *Misgurnus anguillicaudatus* exposed to sodium nitrite*

Experimental group	96 hr LC_{50} (ppm) [95% confidence ranges]	Experimental conditions	
		DO (mg/l)	pH
Fed group	132.45 [112.93~167.77]	7.6 ± 0.42	7.1 ± 0.47
Starved group	93.89 [73.26~119.41]	7.6 ± 0.38	7.1 ± 0.51

*Fed group (345±20.1 mm: mean body length ± SD; 562±52.8 g: mean body weight ± SD), starved group (243±11.6 mm: mean body length ± SD; 246±45.8 g: mean body weight ± SD). The values are means of triplicate experiments

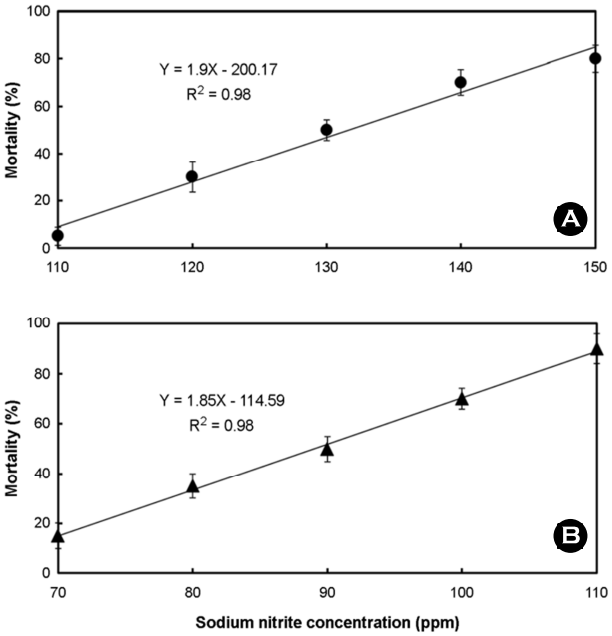

Fig. 3. Dosage-mortality response of cobitid loach, *Misgurnus anguillicaudatus* exposed to sodium nitrite for 96 hours. (A): fed group, (B): starved group. Error bars represent the standard error of triplicate experiments ($p < 0.05$).

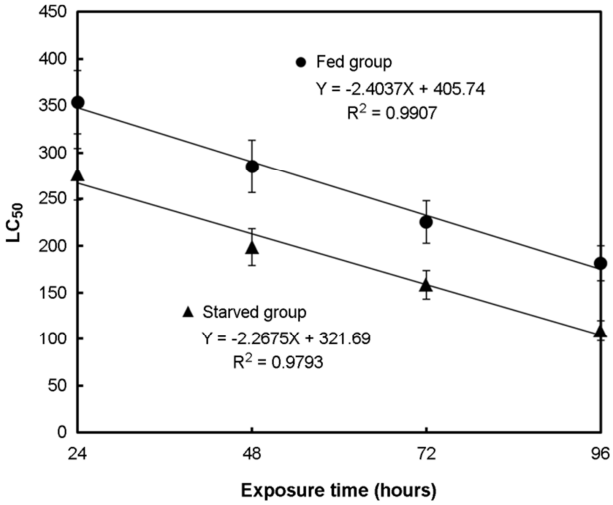

Fig. 4. Effect of starvation on LC_{50} changes of cobitid loach, *Misgurnus anguillicaudatus* exposed to sodium nitrite at each exposure time. Error bars represent the standard error of triplicate experiments ($p < 0.05$).

for fed and starved groups. Respiratory frequency also gradually decreased over 48 h (Table 3). The pH decreased markedly over 12 h. It then decreased gradually up to 48 h (Table 3). The respiratory frequency of the starved group was significantly higher than in the fed group. It decreased with time during the experiment ($p < 0.05$). Oxygen uptake per unit of respiratory movement was less for starved group than that for the fed group. CO_2 and NH_4^+ concentrations increased markedly for 6 h. They then increased gradually up to 48 h (Table 4). Their concentrations in the fed group were significantly higher than those in the starved group. They increased during the experiment ($p < 0.05$).

Dose-related mortality occurred in the fed fish exposed to 120 mg/l nitrite and in the starved fish exposed to 80 mg/l nitrite (Fig. 3). Most fish survived 96 h of exposure to 0 and 70 mg/l of nitrite whereas 75% of fish survived 96 h of exposure to 110 and 120 mg/l of nitrite in the fed group or 70 and 80 mg/l of nitrite in the starved group. LC_{50}, 95% confidence ranges, and experimental conditions for various exposure groups are summarized in Table 5 and Fig. 4. LC_{50} value at 96 h was 132.45 mg/l for the fed group and 93.89 mg/l for the starved group. The LC_{50} value at 24 h was 351.32 mg/l for the fed group and 274.91 mg/l for the starved group. The LC_{50} for each group increased with shorter exposure time ($p < 0.05$).

Figures 5~7 show variations in stress response during 48 h of exposure to sublethal nitrite concentrations (110 ppm for the fed group and 70 ppm for the starved group). In the fed group,

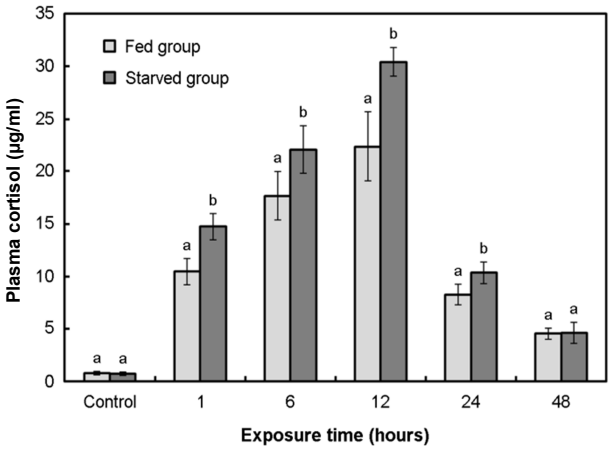

Fig. 5 Variations of the plasma cortisol concentrations exposed sublethal concentration of nitrite during 48 hours on the blood plasma of the cobitid loach, *Misgurnus anguillicaudatus*. Sublethal concentrations of nitrite were 110 ppm on fed group, 70 ppm on starved group, respectively. Values are means ± SE (n=30). Actually n=5 for each experiment because the means and SE were calculated separately for each group.

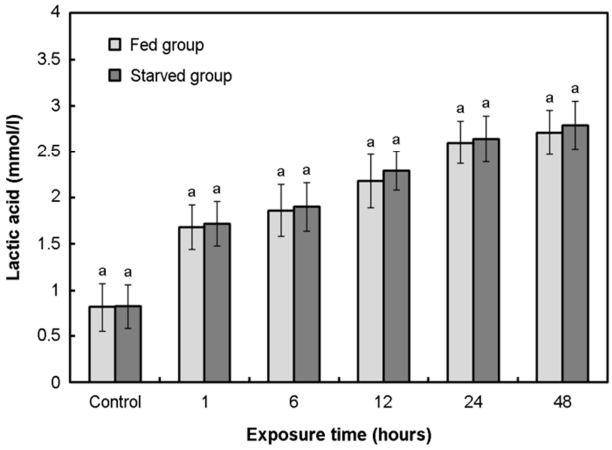

Fig. 7. Variations of the lactic acid concentrations exposed sublethal concentration of nitrite during 48 hours on the blood plasma of the cobitid loach, *Misgurnus anguillicaudatus*. Sublethal concentrations of nitrite were 110 ppm on fed group, 70 ppm on starved group, respectively. Values are means ± SE (n=30). Actually n=5 for each experiment because the means and SE were calculated separately for each group.

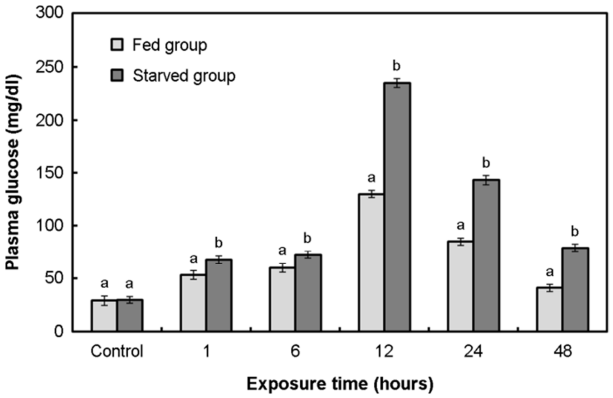

Fig. 6. Variations of the plasma glucose concentrations exposed sublethal concentration of nitrite during 48 hours on the blood plasma of the cobitid loach, *Misgurnus anguillicaudatus*. Sublethal concentrations of nitrite were 110 ppm on fed group, 70 ppm on starved group, respectively. Values are means ± SE (n=30). Actually n=5 for each experiment because the means and SE were calculated separately for each group.

plasma cortisol concentration increased significantly (p < 0.05) from 1.7 ± 0.2 μg/dl to 22.9 ± 3.4 μg/dl over 12 h, but decreased significantly (p < 0.05) to 8.9 ± 2.9 μg/dl at 24 h and 4.7 ± 0.4 μg/dl at 48 h (Fig. 5). In the starved group, plasma cortisol concentration increased significantly (p < 0.05) over 12 h from 1.7 ± 0.2 μg/dl to 30.5 ± 1.5 μg/dl, but decreased significantly (p < 0.05) at 24 h (10.6 ± 1.1 μg/dl) and 48 h (4.9 ± 1.0 μg/dl) (Fig. 5). Plasma cortisol levels in fed or starved groups differed significantly between 1 and 24 h (p < 0.05). Plasma glucose concentrations in each group increased significantly (p < 0.05) over 12 h, from 29.7 ± 0.7 mg/dl to 132 ± 4.4 (in the fed group) or 240 ± 3.9 mg/dl (in the starved group) (Fig. 6). Plasma glucose concentrations decreased significantly from 12 to 48 h. Plasma glucose levels in fed or starved group differed significantly between 1 and 48 h (p < 0.05). At 12 h, plasma cortisol and plasma glucose levels in fed and starved groups differed greatly (p < 0.05). Lactic acid content of both groups increased over 48 h (p < 0.05). For this parameter, there was no significant difference between the two groups (p > 0.05; Fig. 7).

Discussion

Larsson and Lewander (1973) have noted that many fishes undergo natural periods of starvation during the year. They consequently gain ability to withstand prolonged food shortages. Such periods may amount to weeks, months, or even years. They may cause extensive loss of energy stores in the body as fish consumes its own tissues to remain alive (Weatherley and Gill, 1987). In this study, cobitid loach (*Misgurnus anguillicaudatus*) were starved

over 30 days. This demonstrates that this species can tolerate long-term food shortages.

When a fish is exposed to chronic stress, its metabolic reactions are altered by changes in the hypothalamic-pituitary-adrenocortical axis (HPA axis), with hypothalamic secretion of corticotropin-releasing hormone (CRH) occurring through the limbic system and the reticular formation. CRH can stimulate the release of adrenocorticotropic hormone (ACTH) from the pituitary gland which then stimulates the release of cortisol from the target organ (the interrenal gland), promoting a metabolic stress reaction. Thus, cortisol is an important index of stress reaction (Specker and Schreck, 1980). Severe starvation can result in coma and catabolic disease of the gastrointestinal system. Basic metabolic reactions maintain energy levels and body tissues (Jung et al., 2003). Guyton (1991) has reported three stages of physiological change during starvation. In the first stage, glycogen stored for immediate use is hydrolyzed, releasing glucose. In the second stage, acetyl-CoA is oversupplied relative to oxaloacetate (because of lipid use), leading to acidosis. In the third stage, fish are compromised by protein exhaustion. In the process of starvation, sugars, lipids, proteins, and other essential nutritional elements decrease rapidly, ultimately leading to the collapse of immune, circulatory, and endocrine systems that result in death (Guyton, 1991).

In our study, dissolved oxygen concentration decreased over 48 h in the starved group and over 28 h in the fed group. The respiratory frequency also gradually decreased over 48 h, indicating that respiratory function was decreased in all experimental groups during the experimental period. The pH and concentrations of CO_2 and NH_4^+ showed different trends to those of dissolved oxygen concentration. However, decreasing rates of pH and concentrations of CO_2 and NH_4^+ were similar to the decreasing rate of oxygen consumption. That is, the metabolic rate of fish in all experimental groups decreased during the experimental period.

Toxicity and effects of nitrite vary among fish species. They depend on test conditions, including fish size, water ionic composition, and temperature (Doblander and Lackner, 1997). Chloride and other anions in water can provide protection against nitrite during active branchial uptake (Williams and Eddy, 1986). Thus, small amount of Cl^- (e.g. 1 mM) is likely to afford protection against high nitrite levels (Eddy et al., 1983). The effect of nitrite on fish is greater in Cl^--poor water. Thus, nitrite is more toxic to freshwater organisms than to organisms living in seawater (Grosell and Jensen, 1999).

Nitrite accumulation in the plasma probably causes methemoglobinemia and malfunction of hemopoietic activity, both of are effects of nitrite intoxication (Costa et al., 2004). Although methemoglobinemia is not directly related to high mortality in fish exposed to nitrite (Costa et al., 2004), the passage of nitrite into the blood stream may increase blood cell lysis (Knudsen and Jensen, 1997), changes in plasma electrolyte balance (Huertas et al., 2002), and efflux of K^+ from red blood cells (RBCs; Martinez and Souza, 2002) which is evident in an increase in the number of shrunken RBCs. Dysfunctional erythrocytes may be removed from blood circulation because of oxygen shortage, causing a reduction in total erythrocyte count (Park et al., 2007). Stress response may explain the significant difference between fed and starved groups in this study.

Plasma cortisol and glucose levels are useful indicators of stress in fish (Park et al., 2008). Plasma levels of cortisol and glucose are elevated in red drum (*Sciaenops ocellatus*) simultaneously exposed to stressor (Massee et al., 1995). Barton and Iwama (1991) stated that "Usually, the phenomenon that plasma cortisol concentration of fishes rises by stress is the first order reaction and the phenomenon that plasma glucose concentration rises is the result of second-order reaction by hormone rise reaction caused by stress". A similar trend has been reported in gray mullet (*Mugil cephalus*) and kelp grouper (*Epinephelus bruneus*) (Park et al., 2008). In the present study, starvation in cobitid loach caused greater stress response to nitrite. Our results may be useful as a guide in the regulation and scheduling of feeding as an indicator of sodium nitrite stress and in developing an index to determine nutritional status of cobitid loach.

Acknowledgement

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