J. Agr. Sci. Tech. (2015) Vol. 17: 1161-1170

Serum Biochemical Changes and Acute Stress Responses of the Endangered Iridescent Catfish (*Pangasianodon hypophthalmus*) Supplied with Dietary Nucleotide

M. Yaghobi[1], F. Paykan Heyrati[*1], S. Dorafshan[1], and N. Mahmoudi[2]

ABSTRACT

The effects of dietary nucleotide (NT) were evaluated on some serum biochemical parameters and acute stress responses of the catfish (*Pangasianodon hypophthalmus*). Five experimental diets including 0, 0.25, 0.5, 0.75, and 1% NT were supplied to catfish fry for 10 weeks. At the end of the experiment, fish fed the control and 1% NT diets were subjected to handling and crowding stress. The results showed that the fish supplied with 0.25-0.75% NT had a lower level of lactate dehydrogenase (LDH) ($P<0.05$) while other serum enzymes including alkaline phosphatase (ALP), asparate transaminase (ASP), and alanine transaminase (ALT) were not significantly reduced by NT inclusion in the diet ($P>0.05$). The fish which received the highest doses (0.75 and 1%) of NT exhibited higher levels of triglyceride than the other groups ($P<0.05$) while they showed no significant differences in other biochemical parameters including total protein, albumin, globulin, cholesterol, and glucose ($P>0.05$). To investigate stress responses, cortisol (primary response), serum glucose, and serum ion concentrations including sodium, potassium and calcium (secondary responses) were measured. The results showed significant fluctuations in all the tested components during the sampling intervals for up to 48 h post-stress; the exceptions, however, were glucose in the group on the 1% NT diet as well as serum cortisol and calcium levels in those supplied with the control diet. Based on the results obtained, it may be concluded that dietary NT can improve liver function in iridescent catfish, but it has no obvious positive effects on other serum biochemical parameters and stress responses.

Keywords: Crowding stress, Cortisol, Handling stress, Liver function.

INTRODUCTION

It is well documented that common cultivation practices such as crowding, handling, and transportation can negatively affect popular aquatic health and welfare (Falahatkar and Barton, 2007). The reactions observed in aquatic life under such stress conditions include primary, secondary, and tertiary responses. The primary response starts out in the hypothalamus where stress is perceived. This is followed by stimulation of the pituitary-inter-renal axis that gives rise to the secretion of cortisol and catecholamine as a hormonal response (Nematollahi *et al*., 2013). The action of these hormones triggers metabolic, haematological, and immunological changes, which collectively comprise the secondary response in the organism. If continued, the stress may cause the final fatal stage of the response that is realized as disease or exhaustion, growth retardation, and death (Barton and Iwama, 1991; Barton, 2002). Rising plasma cortisol levels over long periods

[1] Department of Natural Resources, Isfahan University of Technology, Isfahan 84156-83111, Islamic Republic of Iran.
[2] Department of Fisheries, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Islamic Republic of Iran.
* Corresponding author: email address: fheyrati@cc.iut.ac.ir

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

due to such aquaculture-related conditions as overcrowding or handling (Pickering and Pottinger, 1989) lead to unhealthy and retarded growth (Urbinati and Carneiro, 2001; Rowland *et al*., 2006).

To combat or diminish the adverse effects of common stressors in aquacultural environments, a number of measures have been commonly employed. These include aeration, fish density optimization, water quality improvement, water exchange, and inclusion in fish diet of some anti-stress compounds such as nucleotide and medicinal plant extract that counteract the immunosuppressive stress effects (Li and Gatlin III, 2006; Citarasu, 2010).

The recent active research in the field of aquaculture has concentrated on the effects of commercially available nucleotides (NTs) on different biological characteristics in fish (Li and Gatlin III, 2006) and shellfish (Shankar *et al*., 2012). These form a group of low-weight biomolecules with a key role in the physiological and biochemical pathways of a wide variety of animals (Carver, 1994; Carver and Walker, 1995). Reported in the literature is a mass of paradoxical results obtained about the effects of dietary NT on important traits in aquatics such as growth rate (Burrells *et al*., 2001a,b; Barros *et al*., 2013), serum biochemical composition (Yousefi *et al*., 2012; Tahmasebi-Kohyani *et al*., 2012), and stress response (Burrells *et al*., 2001b; Abedian-Kenari *et al*., 2012; Barros *et al*., 2013) in several fish species such as Atlantic salmon (*Salmo salar*), Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), and beluga sturgeon (*Huso huso*). Indeed, there are many scientific gaps in the knowledge about the effects of NT supplementation in diets on different fish species, the dose response, and the time of administration (Li and Gatlin III, 2006). On the other hand, the veritable effects of dietary NT on serum biochemical changes and acute stress responses in fish have not yet been fully understood. Of increasing interest in fish nutrition research nowadays is supplementation in fish diets that supports optimum fish health.

The iridescent or striped catfish, *Pangasianodon hypophthalmous*, is one of the most important native fresh water fish species of the Southeast Asia which is heavily fished from natural river basins as a food source. It is considered to be suitable for cultivation as a food fish as well as a common species in the fish keeping hobby. More recently, this valuable species has been identified as an endangered species (Vidthayanon and Hogan, 2011).

Based on the above observations, this experiment was conducted to examine the effects of dietary nucleotides on serum biochemical parameters and stress responses of iridescent catfish exposed to acute stress.

MATERIALS AND METHODS

Experimental Animals and Design

Eight hundred striped catfish fry (0.7 ± 0.05 g) were obtained from a commercial supplier (Sepanta Mahianab Aria Pars, Isfahan, Iran). The fish were acclimatized to lab conditions for two weeks prior to the experiment before they were randomly weighed (1.52 ± 0.11 g) at the beginning of the experiment and stocked into 15 aquaria of 150-L (50 fish per aquarium) in triplicates for each dietary treatment. The water level in the aquaria was kept stable and about 50% of the water volume was changed every two days to maintain constant water quality parameters. Among these parameters, water temperature (30 ± 2.0 °C), pH (7.5 ± 0.2) and dissolved oxygen (6 mg L^{-1}) were monitored on a daily basis at 18:00 hrs. Fish were fed about 5% of their body weight three times a day at 10:00, 13:00, and 16:00 hrs for 70 days.

Experimental Diets

A diet containing 39% crude protein, 14% fat, 21.7% carbohydrate, 3% fibre, and less than 10% moisture (National Research Council (NRC), 1973) was formulated to

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

make a practically iso-caleric experimental diet (14.25 kJg^{-1} diet) with fish meal, soybean oil, soybean meal, corn, corn gluten, and fish oil. The diet was supplemented with the commercial nucleotide mixture "Optimun" (Chemoforma, Augst, Switzerland) to give 0, 2.5, 5, 7.5, and 10 g of mixed NT/Kg diets. Optimun contained inosine monophosphate (IMP), adenosine monophosphate (AMP), guanosine monophosphate (GMP), and ribonucleic acid (RNA).

Stress Challenge

The stress challenge was imposed according to Tahmasebi-Kohyani *et al*. (2012) with some modification. Briefly, at the end of the feeding trial, 120 fish from both the control and the experimental group receiving 1% NT (the highest dietary NT dose which showed the highest growth performance, data not shown) were subjected to the stress challenge. For this purpose, the fish remained on the experimental diets and were acclimatized for 1 week as described above. After the acclimation period, 9 fish per tank were removed for sampling (time 0) prior to subjecting the remaining fish in the tank to acute stress. The stress consisted of netting the remaining fish from the tank and holding them out of water for 5 min before being crowded at an approximate density of 100 gL^{-1} in a plastic mesh bucket placed in their original tank for 3 h. The experimental fish were sampled after 1 h of crowding and later as they were released from the crowding stress (3 h) at 8, 24, and 48 h.

Blood Sampling and Biochemical Analyses

At the end of the experiment, at least 9 fish from each replicate were anaesthetized with clove powder (100 ppm) and blood sampling (0.5-1 mL) was performed individually from caudal puncture (G.18 needle). The blood was centrifuged at 3000 rpm for 10 min. The serum was then collected and kept frozen at -80 °C until analysis for enzymatic activity of lactate dehydrogenase (LDH), aspartate transaminase (AST), alkaline phosphatises (ALP), and alanine transaminase (ALT) activities (Peyghan and Azary Takamy, 2002) as well as biochemical characteristics including the level of total protein (Tietz, 1986), albumin (Doumas *et al*., 1977), globulin (total protein-albumin; gdL^{-1}), and albumin:globulin (A:G) ratio (Kumar *et al*., 2005). The manual presented by Davidson and Nelson (1977) was used to determine the levels of triglycerides and cholesterol.

To evaluate the effects of NT on response to handling and crowding stresses in the catfish, the fish (9 individuals from each treatment at each sampling interval) were captured with minimal disturbance to the other fish. Blood sampling as well as serum preparation and storage were performed as described above. Cortisol and glucose levels were determined using the radioimmunoassay method (Immunotech, France) and the colorimetric glucose oxidize procedure (Benfey and Biron, 2000). The levels of ions including sodium, potassium, and calcium were assayed according to Braun *et al*. (2010). All the measurements were made in duplicates.

Statistical Analysis

Statistical analysis was performed by one way ANOVA at 5% significance level. A multiple comparison test (Tukey's studentized range tests, TMRT) was conducted to compare the significant differences among the groups using SPSS V.19. Values were presented as means ± standard error of mean. The *T*-test was used to evaluate the differences between stress-related parameters analyzed at the same time between the two experimental groups.

RESULTS

Biochemical Analysis

At the end of the experiment, no significant changes were observed in the

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

serum levels of ALT and ALP (P>0.05; Table 1) while the levels of the two other enzymes, AST and LDH, were significantly affected by NT inclusion in the diet (P<0.05; Table 1). The lowest levels of LDH were observed in the groups of fish on medium NT doses (0.25-0.75%). Feeding iridescent catfish with diets containing different levels of commercial NT had effects neither on their total protein nor on their albumin levels (P>0.05; Table 2). The globulin levels were in the range of 1.95-2.26 gdL^{-1} without any significant differences among the groups (P>0.05; Table 2). Similar results were observed for Alb:Glb ratio, which was not affected by NT levels (P>0.05; Table 2). The cholesterol and glucose levels ranged over 227.6-255.16 and 129.77-149 $mgdL^{-1}$, respectively (Table 2). The results indicated that 10 weeks of administering diets enriched with different levels of NT had failed to cause any significant changes in the serum levels of these components in the iridescent catfish (P>0.05; Table 2).

The serum triglyceride (TG) levels showed significant changes with increasing NT doses (P<0.05; Table 2). NT inclusion in the diet at doses of 0.75% or higher caused significant increases in TG levels compared to other groups receiving lower doses of NT (P<0.05; Table 2).

Stress Challenge

At the beginning of handling and crowding stress, cortisol levels measured as low as 7.26 ± 1.68 and 4.73 ± 0.86 ng mL^{-1} in the control and the 1% NT treatments, respectively, but there were no significant differences between the two groups at that time (P>0.05; Figure 1a). In the group of fish supplied with 1% NT, serum cortisol level increased significantly to 14.26 ± 2.76 at 3h post-stress, while the fish on the control diet showed a very limited and

Table 1. Serum enzymes (U L^{-1}) in iridescent catfish fed different levels of dietary nucleotide (NT) for 10 weeks.[a]

NT dose (%)	0 (Control)	0.25	0.5	0.75	1
AST[b]	261±19.85[ab]	326.66±12.14[a]	230.33±19.93[b]	228±17[b]	219±22.27[b]
ALT[c]	9±0.57[a]	9.33±0.88[a]	7.33±0.88[a]	9.01±2.03[a]	7.02±1.01[a]
ALP[d]	141.66±20.3[a]	185.66±12.1[a]	191.66±23.78[a]	191.5±23.5[a]	132.33±3.84[a]
LDH[e]	2805.6±398.97[a]	1283±391.04[b]	1291.33±381.03[b]	1432±357.5[b]	1670±382.52[ab]

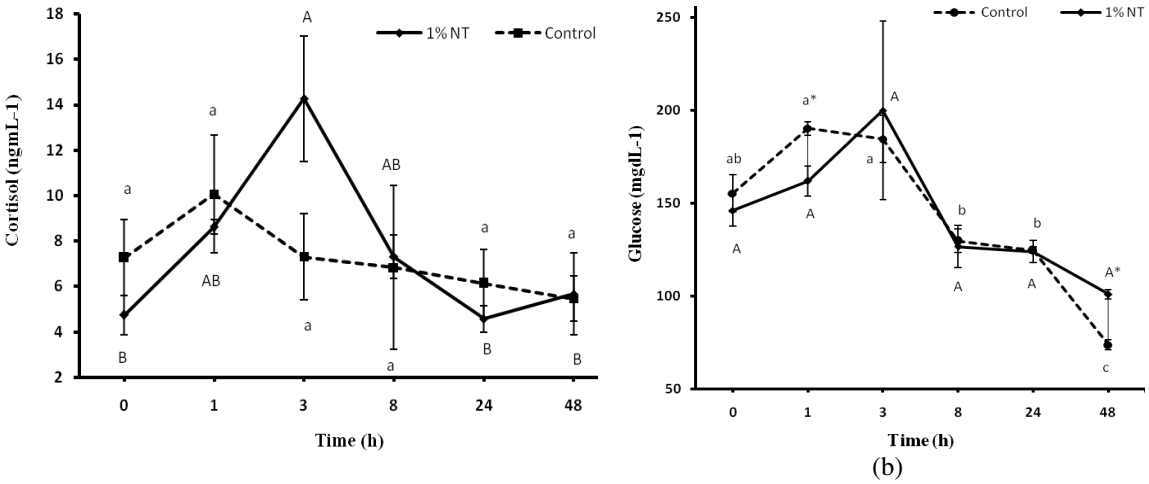
[a] Mean values with at least one identical superscript are not significantly different (P> 0.05). Values are Means±SEM. [b] Alkaline phosphatase; [c] Asparate transaminase; [d] Lactate dehydrogenase, [e] Alanine transaminase.

Table 2. Some biochemical parameters of iridescent catfish fed different levels of dietary nucleotide (NT) for 10 weeks.[a]

NT dose (%)	0 (Control)	0.25	0.5	0.75	1
TP[b]	4.12±0.13[a]	4.08±0.11[a]	4.3±0.64[a]	3.83±0.13[a]	4.1±0.28[a]
Alb[c]	1.86±0.06[a]	1.92±0.08[a]	1.62±0.19[a]	1.66±0.47[a]	1.81±0.12[a]
Glb[d]	2.26±0.07[a]	2.16±0.03[a]	1.95±0.12[a]	2.17±0.09[a]	2.12±0.05[a]
Alb:Glb	0.82±0.01[a]	0.88±0.02[a]	0.9±0.16[a]	0.76±0.02[a]	0.79±0.03[a]
Chol[e]	227.6±11.76[a]	231.12±11.62[a]	255.16±41.43[a]	235.75±19.55[a]	250.12±21.71[a]
Glu[f]	139.5±4.09[a]	141.14±4.75[a]	149±30.73[a]	140.62±3.75[a]	129.77±9.84[a]
TG[g]	408.8±24.52[a]	373.75±28.53[a]	358.4±59.97[a]	584.14±52.53[b]	630.42±65.89[b]

[a] Mean values with at least one similar superscript are not significantly different (P> 0.05). Values are Mean±SEM. [b] Total protein; [c] Albumin(g dL^{-1}); [d] Globulin (g dL^{-1}); [e] While cholesterol; [f] Glucose (mg dL^{-1}), [g] Triglyceride (mg dL^{-1}).

[DOR: 20.1001.1.16807073.2015.17.5.9.8]



(a) (b)

Figure1. (a) Serum cortisol (ng mL^{-1}) (b) Serum glucose (mg dL^{-1}) levels in iridescent catfish on both the control and 1% NT diets subjected to acute handling followed by 3 h of crowding stress. Data are presented as means ± standard error as error bars. Significant differences between different times of blood sampling in the same group are indicated by unlike letters (P< 0.05; Tukey's test) while significant differences between two groups (0 and 1% of NT) at the same time (P< 0.05; t-test) are shown by the asterisk (*).

insignificant rise in their cortisol compared to time 0. However, cortisol returned to its pre-stress levels within 8 h in both groups and remained in the plateau status until the end of the experiment, i.e., 48 h (Figure 1a). There were no significant differences between the two groups in cortisol levels at different sampling intervals (P>0.05; Figure 1a).

Serum glucose levels showed an elevating trend in fish subjected to stress in both groups (Figure1b). Glucose reached its maximum concentrations at 1 and 3 h post-stress in the control and the 1% NT groups, respectively, although the elevations were not significant compared to time zero (P>0.05; Figure1b). Serum glucose returned to its pre-stress levels within 8 h (Figure1b). The lowest glucose concentrations (73.66 ± 2.66 and 101 ± 2.51 $mgdL^{-1}$) were measured at 48 h post-stress in the control and the 1% NT groups, respectively (Figure1b).

The results of ion regulation investigations showed that at time 0, the serum sodium levels were 139.73 ± 4.34 and 127.66 ± 3.09 $mmolL^{-1}$ in the control and the 1% NT groups, respectively (P>0.05; Figure 2a). In both groups, sodium concentrations decreased after stress challenges and reached their minimum values at 3 h post-stress (Figure 2a), beyond which they gradually returned to their pre-stress values until 48 h (Figure 2a). During all the sampling intervals, the levels of serum sodium were lower in fish supplied with the 1% NT diet than in the control although our statistical analysis only showed significant differences at 8 h (P<0.05; Figure 2a). An opposite trend was observed in potassium levels in that the fish fed the 1% NT diet exhibited higher levels of K^+ than the control (P<0.05; Figure 2b) in most of the sampling times. In both groups, the highest levels of potassium were recorded at 8 h post-stress which was significantly higher than those reported at time 0, beyond which potassium concentration decreased sharply and returned to its pre-stress values (in the control) or even less (in the 1% NT treatment) (P<0.05; Figure 2b). In the control group, calcium concentration was not affected by the stress challenges, fluctuating in the range of 2.8-3.19 $mmolL^{-1}$ (P>0.05; Figure 2c). In fish on 1% NT, Ca^{+2}

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

showed significant fluctuations (P<0.05; Figure 2c). The lowest level of calcium concentration was 2.41+0.01mmolL^{-1} at 3h post-stress which was significantly lower than those measured for the control (P<0.05; Figure 2c). Serum Ca^{+2} content increased significantly and reached its maximum level (3.3 + 0.1 mmolL^{-1}) at 24h (P<0.05; Figure 3c). In most of the sampling times, the control fish showed higher levels of serum calcium than the fish on 1% NT. However, a significant difference was only observed at 8 h (P<0.05; Figure 3c).

DISCUSSION

The results of this study indicate that dietary NT has no uniform effect on serum enzymes. Each enzyme is a vital component of a biological pathway. Elevation in the activity of serum enzymes including ALT, AST, ALP, and LDH is usually considered as a symptom of liver dysfunction (Shi *et al*., 2006). In this study, only the level of LDH was significantly lower in fish fed the 0.25- 0.75% NT diet, but NT supplementation did not reduce the serum levels of other enzymes. Generally speaking, limited information is available on the effects of dietary NT on serum enzyme levels. In agreement with our results, Glencross and Rutherford (2010) showed that LDH level was significantly lower in barramundi (*Lates calcarifer*) which was on a diet containing NT. The decline in most of the above enzymes has also been reported by Tahmasebi-Kohyani *et al*. (2012) who investigated rainbow trout, *Oncorhynchus mykiss* and Abedian-Kenari *et al*. (2012) who experimented with Caspian brown trout, *Salmo trutta caspius*. The differences among these studies can be explained by the form of NT supplied, duration of administration, or even the special characteristics of the species studied (Li and Gatlin III, 2006). The decline observed in this study in LDH as one of the most important serum enzymes may show the potential positive effects of dietary NT on

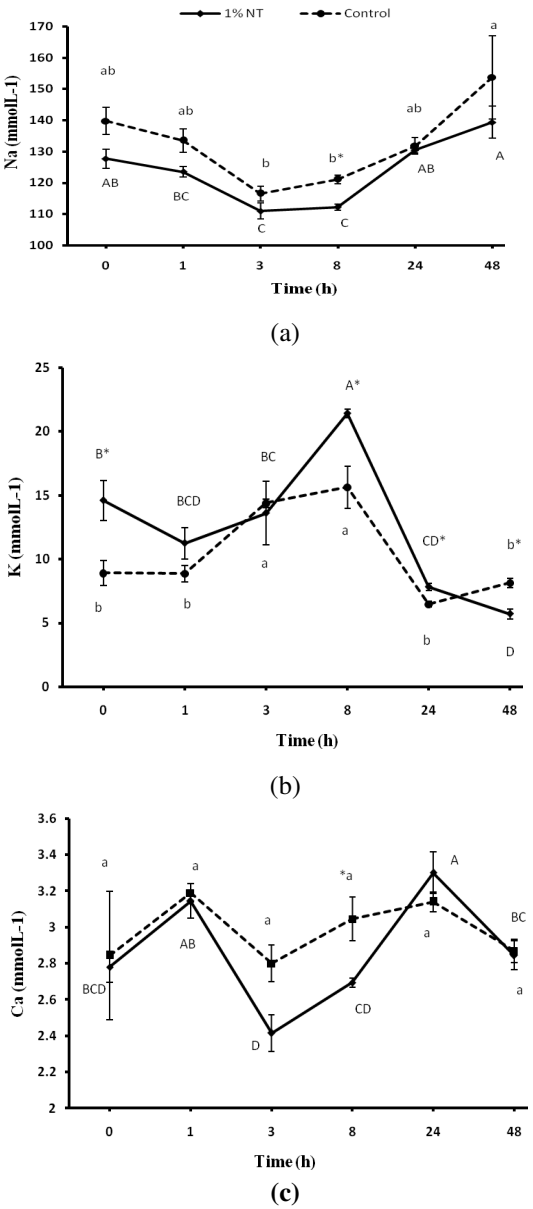


Figure 2. Effects of dietary nucleotide on Na^{+} (a), K^{+} (b), and Ca^{+2} (c) ion concentrations in iridescent catfish subjected to acute handling followed by 3 h of crowding stress. Data are presented as means±standard error as error bars. Significant differences between different times of blood sampling in the same group are indicated by unlike letters (P< 0.05; Tukey's test) while significant differences between two groups (0 and 1% of NT) at the same time (P< 0.05; t-test) are shown by asterisk (*).

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

liver function. However, further study is required to determine the exact effects of NT on liver.

Compared to other groups, the iridescent catfish supplied with 0.75% or 1% NT had significantly higher triglycerides, while other blood biochemical parameters were not affected by dietary NT. Inconsistent results have been reported on the effects of dietary NT on certain blood biochemical parameters such as total protein, albumin, cholesterol, glucose, and triglycerides. For example, Tahmasebi-Kohyani *et al*. (2012) observed significant increases in total protein, albumin, and globulin levels in response to NT doses in rainbow trout but no such dose response was reported by Abedian-Kenari *et al*. (2012) who studied Caspian brown trout. Rather, they observed the highest levels of these serum components in the fish supplied with a medium NT dose of 0.25%. Yousefi *et al*. (2011) found that dietary NT did not affect total protein, albumin, and globulin levels or cholesterol and triglycerides in beluga sturgeon, *Huso huso*. Similar results were reported by Abedian-Kenari *et al*. (2012) who investigated these same components in Caspian brown trout. Contrary to our results, Fontana *et al*. (1998) reported that dietary nucleotide could normalize the level of cholesterol but did not affect triglycerides concentration in rats. These differences in findings can be explained by the NT level and the duration of administration as well as the variety in NT digestion and absorption potential of different aquatic species under distinct environmental conditions (Roald, 1978). However, a comprehensive research is needed to clarify the possible effects of NT on certain blood biochemical parameters in fish.

In the present study, the primary (cortisol) and secondary (glucose and ion fluctuation) stress responses were not as typical as expected in fish subjected to handling and crowding stress (Karakatsouli *et al*., 2008). This may be due to the resistance of iridescent catfish to the common handling and crowding stressors. Although published research suggests that dietary NT enhances fish resistance to a variety of stressors such as salinity in Atlantic salmon (Burrells *et al*., 2001b) and Caspian brown trout (Abedian Kenari *et al*., 2012) or handling and crowding stress in beluga sturgeon (Yousefi *et al*., 2011) and rainbow trout (Tahmasebi-Kohyani *et al*., 2012), the phenomenon failed to be validated by studies of red drum, *Sciaenops ocellatus* (Li *et al*., 2005), barramundi (Glencross and Rutherford, 2010), and Nile tilapia (Barros *et al*., 2013). These studies even suggested that inclusion of NT had a negative effect on fish health under stressful conditions. A possible explanation for such unlike results among different fish species is extreme variation among individual fishes which makes the data insignificant (Li and Gatlin III, 2006). It is also well documented that high levels of NT bioavailability or NT administration over long periods may lead to undesirable side effects on disease resistance (Matsuo and Miyazano, 1993) and growth (Adamek *et al*., 1996) so that these side effects may be responsible for the stress responses observed. After all, the matter remains far from clear before adequate study is carried out to shed more light on the specific effects of NT on primary and secondary stress responses. In conclusion, the results of the present study demonstrated that dietary NT has very limited positive effects on iridescent catfish welfare and stress responses which may indicate the inappropriateness of the use of NT in some aquatics.

ACKNOWLEDGEMENTS

This study was financed by Isfahan University of Technology, Isfahan, Iran, under grant number 502.92.53949 awarded to Dr. Fatemeh Paykan Heyrati. The authors would like to thank Sepanta Mahianab Aria Pars, Isfahan, Iran, for supplying the stripped catfish. Thanks also go to Chemoforma, Augst, Switzerland, for having kindly supplied the commercial

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

nucleotide "Optimun". The authors would also like to extend their gratitude to Dr. Ezzatollah Roustazadeh from Isfahan University of Technology for editing the final English version of this manuscript.

REFERENCES

1. Abedian-Kenari, A., Mahmoudi, N., Soltani, M. and Abedian-Kenari, S. 2012. Dietary Nucleotide Supplements Influence the Growth Hemato-immunological Parameters and Stress Responses in Endangered Caspian Brown Trout (*Salmo trutta caspius* Kessler, 1877). *Aqua Nutr.,* **938**: 1365-2095.
2. Adamek, Z., Hamackova, J., Kouril, J., Vachta, R. and Stibranyiova, I. 1996. Effect of Ascogen Probiotics Supplementation on Farming Success in Rainbow Trout (*Oncorhynchus mykiss*) and Wells (*Silurus glanis*) under Conditions of Intensive Culture. *Krmiva (Zagreb)*, **38**: 11-20.
3. Barros, M. M., Giumaraes, I. G., Pezzato, L. E., Orsi, R. O. D., Junior, A. C. F., Teixeira, C. P., Fleuri, L. F. and Padovani, C. R. 2013. The Effect of Dietary Nucleotide Mixture on Growth Performance, Hematological and Immunological Parameters of *Nile tilapia*. *Aqua Res*., 1-7. Doi:10.1111/are.12229.
4. Barton, B. A. 2002. Stress in Fish: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. *Integr. Comp. Biol.,* **42**: 517-525.
5. Barton, B. A. and Iwama, G. K. 1991. Physiological Changes in Fish from Stress in Aquaculture with Emphasis on the Response and Effects of Corticosteroids. *Annu. Rev. Fish. Dis.,* **1:** 3-26.
6. Benfey, T. J. and Biron, M. 2000. Acute Stress Response in Triploid Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*). *Aqua.*, **184**: 167-176.
7. Burrells, C., William, P. D. and Forno, P. F. 2001a. Dietary Nucleotides a Novel Supplement in Fish Feeds. 1. Effects of Resistance to Disease in Salmonids. *Aqua.*, **199**: 159-169.
8. Burrells, C., William, P. D., Southage, P. J. and Wadsworth, S. L. 2001b. Dietary Nucleotides: a Novel Supplement in Fish Feeds. 2. Effects on Vaccination, Salt Water Transfer, Growth Rate and Physiology of Atlantic Salmon. *Aqua.*, **199**: 171- 184.
9. Braun, N., Lima, R. L. D., Baldisserotto, B., Dafre, A. L. and Nuner, A. P. D. O. 2010. Growth, Biochemical and Physiological Responses of *Salminus brasiliensis* with Different Stocking Densities and Handling. *Aqua.*, **301**: 22-30.
10. Carver, J. D. 1994. Dietary Nucleotides: Cellular Immune, Intestinal and Hepatic System Effects. *J. Nutr*., **1S**: 144S–148S.
11. Carver, J. D. and Walker, W. A. 1995. The Role of Nucleotides in Human Nutrition. *J. Nutr. Biochem*., **6:** 58-72.
12. Citarasu, T. 2010. Herbal Biomedicine: A New Opportunity for Aquaculture Industry. *Aqua. Int.,* **18**: 403-14.
13. Davidson, I. and Nelson, D. A. 1977. The Blood. In: "*Clinical Diagnosis by Laboratory Methods*", (Eds.): Davidsohn, I. and Henry, J. B.. 15th Edition, WB Saunders Co., Philadelphia, PA, PP. 100-310.
14. Doumas, B. T., Watson, W. A. and Biggs, H. G. 1977. Albumin Standards and the Measurement of Serum Albumin with Bromcresol Green. *Clin. Chim. Acta*., **258**: 21-30.
15. Falahatkar, B. and Barton, B. A. 2007. Preliminary Observations of Physiological Responses to Acute Handling and Confinement in Juvenile Beluga (*Huso huso). Aqua. Res*., **38**: 1786-1789.
16. Glencross, B. D. and Rutherford, N. R. 2010. Dietary Strategies to Improve the Growth and Feed Utilization of Barramundi (*Lates calcaifer*) under High Water Temperature Conditions. *Aqua. Nutr*., **16**: 343-350.
17. Fontana, L., Moreira, E., Torres, M. I., Fernández, I., Sánchez de Medina, F. and Gil, A. 1998. Dietary Nucleotides Correct Plasma and Liver Microsomal Fatty Acid Alterations in Rats with Liver Cirrhosis Induced by Oral Intake of Thioacetamide. *J. Hepatol*., **28 (4):** 662-669.
18. Karakatsouli, N., Papoutsoglou, S.E., Panopoulos, G., Papoutsoglou, E. S., Chadio, S. and Kalogiannis, D. 2008. Effects of Light Spectrum on Growth and Stress Response of Rainbow Trout (*Oncorhynchus mykiss)* Reared under Recirculating System Conditions. *Aqua. Eng*., **38**: 42-63.
19. Kumar, S., Sahu, N.P., Pal, A. K., Choudhury, D., Yengkokpam, S. and Mukherjee, S. C. 2005. Effect of Dietary

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

Carbohydrate on Hematology, Respiratory Burst Activity and Histological Changes in *L. rohita* Juveniles. *Fish Shellfish Immu*., **19**: 331-344.

20. Li, P., Burr, G. S., Goff, J., Whiteman, K. W., Davise, K. B., Vega, R. R., Neill, W. H. and Gatlin III, D. M. 2005. A Preliminary Study on the Effects of Dietary Supplementation of Brewer's Yeast and Nucleotides, Singularly or in Combination, on Juvenile Red Drum (*Sciaenops ocellatus*). *Aqua. Res*., **36:** 1120-1127.
21. Li, P. and Gatlin III, D. M. 2006. Nucleotide Nutrition in Fish: Current Knowledge and Future Applications. *Aqua.*, **251**: 141-152.
22. Matsuo, K. and Miyazano, I. 1993. The Influence of Long-term Administration of Peptidoglycan on Diseases Resistance and Growth of Juvenile Rainbow Trout. *Nippon Suisan Gakkaishi*., **59**: 1377-1379.
23. National Research Council (NRC). 1973. *Nutrient Requirement of Fish*. Academic Press, Washinton, DC, USA.
24. Nematollahi, M. A., de Van Pelt, H. and Komen, H. 2013. Response to Stress in 17α-Hydroxylase Deficient Common Carp (*Cyprinus carpio* L.). *J. Agr. Sci. Tech.,* **15 (2):** 303-310.
25. Peyghan, R. and Azary Takamy, G. 2002. Histopathological, Serum Enzyme, Cholesterol and Urea Changes in Experimental Acute Toxicity of Ammonia in Common Carp (*Cyprinus carpio*) and Use of Natural Zeolite for Prevention. *Aqua. Inter*., **10:** 317-325.
26. Pickering, A. D. and Pottinger, T. G. 1989. Stress Responses and Disease Resistance in Salmonid Fish: Effects of Chronic Elevation of Plasma Cortisol. *Fish Physiol. Biochem*., 7: 253-258.
27. Roald, S. O. 1978. Effects of Sublethal Concentrations of Lignosulphonates on Growth, Intestinal Flora and Some Digestive Enzymes of Rainbow Trout (*Salmo gairdneri*). *Aqua.*, **12**: 327-335.
28. Rowland, S. J., Mifsud, C., Nixon, M. and Boyd, P. 2006. Effects of Stocking Density on the Performance of the Australian Freshwater Silver Perch (*Bidyanus bidyanus*) in Cages. *Aqua.*, **253**: 301-308.
29. Shankar, R., Shivanada, H., Sujata, H. R., Jayaraj, E. G., Tejpal, C. S. and Chinthamani, V. S. 2012. Effect of Nucleotide on Growth, Immune Responses and Resistance of *Macrobranchium rosenbergii,* Nodavirus and Extra Small Virus and *Aeromonas hydrophila* Infection. *Aqua.*, **20**: 1-12.
30. Shi, X., Li, D., Zhuang, P., Nie, F. and Long, L. 2006. Comparative Blood Biochemistry of Amur Sturgeon (*Acipenser schrenckii*) and Chinese Sturgeon (*Acipenser sinensis*). *Fish Physiol. Biochem*., **32:** 63-66.
31. Tahmasebi-Kohyani, A., Keyvanshokooh, S., Nematollahi, A., Mahmoudi, N. and Pasha-Zanoosi, H. 2012. Effects of Dietary Nucleotides Supplementation on Rainbow Trout, *Oncorhynchus mykiss* Performance and Acute Stress Response. *Fish Physiol. Biochem*., **38**: 431-440.
32. Tietz, N.W. 1986. *Textbook of Clinical Chemistry*. WB Saunders, London.
33. Urbinati, E. C. and Carneiro, P. C. F. 2001. Metabolic and Hormonal Responses of Matrinxa, *Brycon cephalus* (Teleost: Characidae) to Transport Stress under Influence of Benzocaine. *J. Aqua. Trop*., **16**: 75-85.
34. Vidthayanon, C. and Hogan, Z. 2011. *Pangasianodon hypophthalmus*. In: "*IUCN 2013*". IUCN Red List of Threatened Species, Version 2013.1. <www.iucnredlist.org>.
35. Yousefi, M., Abtahi, B. and Abedian Kenari, A. 2012. Hematological, Serum Biochemical Parameters, and Physiological Responses to Acute Stress of Beluga Sturgeon (*Huso huso*, Linnaeus (1785) Juveniles Fed Dietary Nucleotide. *Comp. Clin. Pathol*., **21**: 1043-1048.

[DOR: 20.1001.1.16807073.2015.17.5.9.8]

تغییرات بیوشیمیایی سرم و پاسخ به تنش حاد در گربه‌ماهی رنگین‌کمان در حال انقراض (*Pangasianodon hypophthalmus*) تغذیه شده با جیره حاوی نوکلئوتید

م. یعقوبی، ف. پیکان حیرتی، س. درافشان، و ن. محمودی

چکیده

تاثیر جیره محتوی نوکلئوتید بر برخی پارامترهای بیوشیمیایی و پاسخ به استرس حاد در گربه‌ماهی *Pangasianodon hypophthalmus* ارزیابی شد. آزمایش در ۵ گروه با جیره‌های غذایی متفاوت حاوی ۰، ۰/۲۵، ۰/۵، ۰/۷۵ و ۱٪ نوکلئوتید افزوده شده به جیره به مدت ۱۰ هفته اجرا شد. در پایان آزمایش، ماهیان گروه شاهد و تغذیه شده با جیره محتوی ۱٪ نوکلئوتید تحت تاثیر استرس دستکاری و تراکم قرار گرفتند. نتایج نشان داد که سطح آنزیم لاکتات دهیدروژناز (LDH) سرم ماهیان تغذیه‌شده با جیره محتوی ۰/۷۵-۰/۲۵ نوکلئوتید به طور معنی‌داری کم‌تر بود در حالی که تفاوت معنی‌داری در سطوح سایر آنزیم‌های سرمی شامل آلکالین فسفاتاز، آسپارات ترانسمیناز و آلانین ترانسمیناز مشاهده نشد ($P > 0.05$). ماهیان تغذیه شده با بالاترین سطوح نوکلئوتید در جیره، ۰/۷۵ و ۱٪ مقادیر بالاتری از تری‌گلیسرید را در مقایسه با سایر گروه‌ها نشان دادند در حالی که تفاوت معنی-داری را در سایر پارامترهای بیوشیمیایی سرم شامل پروتئین کل، آلبومین، گلوبولین، کلسترول و گلوکز نشان ندادند ($P > 0.05$). به منظور ارزیابی پاسخ به استرس حاد، میزان کورتیزول (پاسخ اولیه) و گلوکز و غلظت یون‌های سدیم، پتاسیم و کلسیم سرم (پاسخ‌های ثانویه) سنجش شد. نتایج بیانگر تغییرات معنی‌دار در تمامی پارامترهای مورد بررسی در خلال زمان‌های نمونه‌برداری، تا ۴۸ ساعت پس از اعمال استرس بود. با این وجود، میزان گلوکز در گروه تغذیه شده با ۱٪ نوکلئوتید و میزان کورتیزول و کلسیم سرمی در گروه شاهد بدون تغییر باقی ماند. براساس نتایج می‌توان بیان داشت که استفاده از نوکلئوتید افزوده شده به جیره شاید بتواند منجر به بهبود عملکرد کبد در گربه‌ماهی رنگین‌کمان شود اما فاقد تاثیر مشخص بر سایر پارمترهای سرمی و پاسخ به استرس در این گونه است.

[DOR: 20.1001.1.16807073.2015.17.5.9.8]