Growth performance and some haematological parameters of ornamental striped catfish (*Pangasianodon hypophthalmus*) fed on dietary nucleotide

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(Received 19 Aug 2013; revised version 29 Oct 2013; accepted 9 Dec 2013)

Summary

The effects of dietary nucleotide (NT) on growth performance and some haematological parameters of striped catfish (*Pangasianodon hypophthalmus*) were investigated. Different levels of the NT (0, 0.25, 0.5, 0.75, 1%) in the diet were used for 10 weeks starting on the fish weighted (1.52 ± 0.11 g). The results showed that NT supplementations did not improve final weight, weight gain, food conservation ratio as well as specific growth rates of striped catfish (P>0.05). Among all growth evaluated parameters, only the condition factor was significantly improved in fish fed on 0.75 and 1% of NT in comparison to control (P<0.05). There were no significant differences in hemoglobin, hematocrit, mean erythrocytic hemoglobin, mean erythrocytic volume, and mean erythrocytic hemoglobin concentration, white blood cell (WBC) counts and WBC/RBC ratio among fish fed on dietary nucleotide and control diet (P>0.05). However, some significant fluctuations were observed in the haematological parameters within groups of fish fed on different levels of NT (P<0.05). The results showed that feeding striped catfish with commercial NT had very limited effect on growth parameters and some haematological characteristics.

Key words: Weight gain, Dietary component, Blood indices, Fresh water catfish

Introduction

Striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) is an ornamental and endangered native freshwater fish species of inland water of the Southeast Asia mainly Thailand, Viet Nam, Laos and Cambodia. This species has been introduced to different countries such as Singapore, the Philippines, the USA as well as Iran as a cultivated food fish or an ornamental hobby species. Some major threats such as over exploitation, habitat degradation in water quality and flow cause decreasing native and wild population. Despite the declining wild population, this species has a key role in aquaculture industry in many countries such as Thailand and Viet Nam. Fish cultivation practices under intensive condition such as grading, handling, transportation as well as poor water quality can reduce fish welfare and increase the risk of fish disease. Nowadays, different practices could be used to improve aquatic welfare, among them food additives use in commercial scale. Nucleotides are one of the most important food components which are frequently found in intracellular fluid of different organisms. These chemicals have varieties of physiological and biochemical roles in organisms such as fish (Li and Gatlin III, 2006) and shellfish (Shankar *et al*., 2012). Although most animals can synthesize NT, recent research has shown that dietary nucleotide supplementation could improve growth performance (Burrells *et al*., 2001b; Borda *et al*., 2003; Li and Gatlin III, 2007), immune gene expression and immune response (Low *et al*., 2003; Tahmasebi-Kohyani *et al*., 2011), disease resistance (Burrells *et al*., 2001a), intestinal morphology (Yaghobi *et al*., 2014) as well as disease resistance (Shankar *et al*., 2012) in different aquatic species (Burrells *et al*., 2001a, b; Li and Gatlin III, 2006). However, there is limited information regarding the beneficial effect of dietary NT on different aquatic species such as striped catfish, age/size-related responses and appropriate doses and timing of administration (Li and Gatlin III, 2006; Barros *et al*., 2013). Hence, the present study was carried out to evaluate the effect of dietary NT on growth performance and haematological indices in striped catfish, *P. hypophthalmus* under laboratory condition.

Materials and Methods

Fish and feeding trail

Eight hundred striped catfish fry (0.7 ± 0.05 g) were bought from a commercial supplier, Sepanta Mahianab Aria Pars, Isfahan, Iran. The fish were acclimatized to laboratory conditions for two weeks before starting the experiment. At the beginning of the experiment, the fish were randomly weighted (1.52 ± 0.11 g) and then stocked into 15 150-L aquaria (50 fish aquarium^{-1}) in triplicate per dietary treatment. The water level in aquaria was stable and about 50% of the water body was changed every two days to maintain the water quality parameters. Water temperature, pH, and dissolved oxygen were 30 ± 2.0°C, 6.5 ± 0.2 and 5 mg L^{-1}, respectively. Fish were fed about 5% of body weight, three times a day at 10:00, 13:00 and 16:00 for 70 days. A practical diet formulated based on fish meal, soybean oil, soybean meal, corn, corn gluten and fish oil containing 39% crude protein, 14% fat, 21.7% carbohydrate, 3% of fibre and less than 10% moisture 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^{-1} diet. Optimun contained inosine monophosphate (IMP), adenosine monophosphate (AMP), guanosine monophosphate (GMP) and ribonucleic acid (RNA).

Growth measurements

At least 30 fish from each replicate were weighted at the end of the 10-week experiment. Final weight, mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor were estimated for all groups. The following formula was used to calculate the growth parameters (Soosean *et al*., 2010).

MWG = (Mean final weight - mean initial weight)

SGR (% day^{-1}) = 100 × [($\ln W_1$ - $\ln W_0$)/t]

where,
W_0: Average initial
W_1: Final body weights
t: Time (days)

FCR = Food consumed (g)/weight gain (g)

CF (g cm^{-3}) = Weight (g)/[length (cm)]^3

Blood sampling and haematological analysis

At least 15 fish from each replicate, 45 individuals from each treatment were anaesthetized with clove powder (100 ppm) at the end of the experiment, day 70. Blood sampling (about 500 µl) was performed individually from caudal puncture (G.18 needle) placed in heparinised 1.5 vials for haematological analysis. Red blood cells (RBC) and white blood cells (WBC) were counted manually, using Neubaur haemocytometer (Paul Marienfeld Gmbh, Lauda, Koenigshofen, Germany) after the blood was diluted (200 times for RBC and 50 for WBC) with Diace solution containing brilliant cresyl blue 0.1 g, sodium citrate 3.8 g, and formaldehyde 37% 0.2 mL in 100 mL distilled water (Dorafshan *et al*., 2008). Haematocrit (Hct %) was measured using heparinised microhaematocrit capillary tubes after centrifugation (2500 rpm for 5 min). Haemoglobin concentration (Hb: g dL^{-1}) measured cyanomethemoglobin spectrophotometrically according to Houston (1990). Mean erythrocytic haemoglobin (MEH), volume (MEV), and haemoglobin concentration (MEHC) were calculated using the equations (Dorafshan *et al*., 2008):

MEHC (g Hb dL^{-1}) = Hb (g dL^{-1})/Hct (%)

MEH (µg Hb $cell^{-1}$) = Hb (g L^{-1})/RBC (10^6 mm^3)

MEV (nm^3 $cell^{-1}$) = Hct (%) × 10/RBC (10^6 mm^3)

Statistical analysis

Statistical analysis was performed by one way ANOVA at 5% significance level. A multiple comparison test (Turkey Multiple Comparison Test, TMCT) was conducted to compare the significant differences among the groups using SPSS V.19. Values are presented as mean ± SEM.

Results

Growth performance

In this experiment, the mean initial body weight of striped catfish was about 1.52 g, after 10 weeks of feeding the final weight and length of the fish was in the range of 16.34-17.59 g and 12.80-13.17 cm, respectively, without any significant differences among groups (P>0.05; Table 1). Dietary nucleotide could not cause significant changes in the weight gain of the striped catfish (P>0.05; Table 1), although an elevated trend in weight gain was observed by increasing the level of NT in the diet (Table 1). The lowest levels of FCR (1.03 and 1.05) were achieved in the groups fed on the diet containing 0.5 and 1% NT, respectively (Table 1), while the highest level of FCR (1.35 ± 0.04) belonged to the fish fed on control diet (Table 1). However, statistical analysis did not show any significant differences in the FCR value among experimental groups (P>0.05; Table 1). SGR was in the range of 2.08-2.26 and not affected by the dietary inclusion of NT (P>0.05; Table 1). The CF value was the only growth related parameter which showed significant elevation by NT inclusion in the diet of striped catfish (P<0.05; Table 1). The highest CF value was calculated as 0.89 for the fish fed on the diet

Table 1: Growth performance and feed utilization of juvenile striped catfish fed diets containing different doses of nucleotide for 10 weeks

Variable (units % w/w)	0 (Control)	0.25	0.5	0.75	1
Initial weight (g)	1.57 ± 0.04[a]	1.55 ± 0.01[a]	1.54 ± 0.20[a]	1.4 ± 0.17[a]	1.54 ± 0.15[a]
Final weight (g)	16.54 ± 0.45[a]	16.34 ± 0.26[a]	16.55 ± 0.31[a]	17.12 ± 0.51[a]	17.59 ± 0.43[a]
Final length (cm)	12.94 ± 0.09[a]	13.17 ± 0.10[a]	12.87 ± 0.09[a]	12.8 ± 0.15[a]	12.89 ± 0.11[a]
Weight gain (g)	13.36 ± 0.51[a]	13.69 ± 0.38[a]	14.05 ± 0.19[a]	14.31 ± 1.86[a]	15.17 ± 1.17[a]
FCR	1.35 ± 0.04[a]	1.2 ± 0.05[a]	1.03 ± 0.02[a]	1.18 ± 0.15[a]	1.05 ± 0.08[a]
SGR (% day^{-1})	2.12 ± 0.09[a]	2.08 ± 0.02[a]	2.09 ± 0.06[a]	2.26 ± 0.11[a]	2.18 ± 0.08[a]
CF (g cm^{-3})	0.86 ± 0.006[b]	0.85 ± 0.01[b]	0.88 ± 0.004[ab]	0.89 ± 0.005[a]	0.89 ± 0.005[a]

Values are expressed as mean ± SEM. Mean with the same letter in the same row is not significantly different (P>0.05)

Table 2: Haematological parameters of striped catfish fed diet containing different levels of the nucleotide

Variable (units % w/w)	0 (Control)	0.25	0.5	0.75	1
Hb (g dL^{-1})	13.28 ± 0.25^{abc}	13.98 ± 0.23^{a}	12.58 ± 0.15^{c}	13.81 ± 0.37^{ab}	12.9 ± 0.28^{bc}
Hct (%)	40 ± 0.66^{ab}	41.88 ± 0.77^{a}	37.55 ± 0.6^{b}	40.75 ± 1.03^{ab}	38.33 ± 1.1^{b}
RBC (× 10^{6} cell mm^{-3})	3.05 ± 0.55^{a}	2.9 ± 0.65^{a}	2.39 ± 0.23^{a}	2.54 ± 0.41^{a}	3.81 ± 0.55^{a}
WBC (× 10^{3} cell mm^{-3})	123.75 ± 16.02^{a}	107.5 ± 15.66^{a}	70 ± 8.72^{a}	85.55 ± 15.19^{a}	74.28 ± 14.93^{a}
WBC/RBC (× 10^{-2})	29.22 ± 1.89^{a}	37.66 ± 6.59^{a}	28.58 ± 4.96^{a}	27.02 ± 5.52^{a}	21.13 ± 2.83^{a}
MEV (nm^{3})	142.56 ± 22.59^{a}	169.85 ± 21.21^{a}	169.89 ± 17.8^{a}	160.09 ± 20.45^{a}	125.68 ± 14.53^{a}
MEH (µg $cell^{-1}$)	47.44 ± 7.62^{a}	51.42 ± 8.06^{a}	57.06 ± 6.03^{a}	55.09 ± 7.14^{a}	42.12 ± 4.79^{a}
MEHC (g dL^{-1})	0.33 ± 0.001^{a}	0.33 ± 0.001^{a}	0.33 ± 0.002^{a}	0.34 ± 0.004^{a}	0.33 ± 0.003^{a}

Values are expressed as mean ± SEM. Mean with the same letter in the same row is not significantly different (P>0.05)

enriched with 0.75 and 1% NT (Table 1).

Haematology

Table 2 shows the haematological characteristics of the striped catfish fed the diet containing different level of nucleotide. Dietary NT could significantly change the Hb and Hct levels in the striped catfish, but not in a dose response trend (P<0.05; Table 2). The highest level of Hb (gdl^{-1}) (13.98 ± 0.23) was observed in group fed 0.25% NT followed by 13.81 ± 0.37 fed 0.75% NT (Table 2). Feeding catfish with diets containing 0.5 and 1% of NT reduced the Hb level in comparison to control group, but not in significant level (P>0.05; Table 2). Hct was in the range of 37.55-41.88% (Table 2). The highest Hct value (41.88 ± 0.77%) was observed in fish fed 0.25% NT which was significantly higher than those measured for fish fed 0.5% NT (37.55 ± 0.6%) and 1% NT (38.33 ± 1.1%) (P<0.05; Table 2). However there were no significant changes in Hct in groups of catfish fed diets containing NT in comparison to control (P>0.05; Table 2). The highest mean value of RBC (3.81 ± 0.55 × 10^{6} cell mm^{-3}) and WBC (107.5 ± 15.66 × 10^{3} cell mm^{-3}) were calculated for fish fed diets containing 1% and 0.25% NT, respectively (Table 2). Although significant differences and dose response trend were not observed regarding these primary haematological parameters (P>0.05; Table 2). The highest WBC/RBC ratio (×10^{-2}) (37.66 ± 6.59) was observed in group of fish fed 0.25% NT followed by control (29.22 ± 1.89) and 0.5% NT (28.58 ± 4.96) (Table 2). While the lowest level of the ratio was 21.13 ± 2.83 and calculated for the catfish fish fed the highest level of NT 1% in the diet (Table 2). Insignificant increasing trends were observed in the secondary haematological indices including MEV, MEH and MEHC by elevating the NT dose up to 0.75% (P>0.05; Table 2), while 1% inclusion of NT in the diet showed obvious but not significant reduction in all secondary haematological parameters in comparison to groups fed 0.75% NT (P>0.05; Table 2).

Discussion

In recent years, the active area of research has been conducted on the inclusion of dietary NT for different fish species such as red drum, *Sciaenops ocellatus* (Li *et al*., 2007), rainbow trout, *Oncorhynchus mykiss* (Tahmasebi-Kohyani *et al*., 2011, 2012), Beluga sturgeon, *Huso huso* (Yousefi *et al*., 2012) and Nile tilapia, *Oreochromis niloticus* (Barros *et al*., 2013). Regarding the result of this study, dietary NT up to 1% of the feed, did not show any significant improvement in growth performance characteristics of the striped catfish (CF as an exception). The lack of effect of dietary NT on the growth performance is in agreement with previous studies conducted with hybrid striped bass, *Morone chrysops* × *Morono saxatilis* (Li *et al*., 2004) and Nile tilapia (Barros *et al*., 2013). However, it is in opposite the results achieved on some other aquatic species such as Atlantic salmon, *Salmo salar* (Burrells *et al*., 2001b), rainbow trout (Tahmasebi-Kohyani *et al*., 2011) and Caspian brown trout, *Salmo trutta caspius* (Abedian Kenari *et al*., 2012). The difference in growth performance responses to dietary nucleotide may be due to the species-specific needs of NT, the aquatic growth phases, the kind of NT as well as the substantial contribution of NT from the dietary component, especially fish meal (Barros *et al*., 2013). It is also possible that omnivorous or herbivorous fish such as striped catfish, common carp and/or Nile tilapia require a lower amount of dietary NT, which may be due to the long intestine and enriched micro fauna of the digestive tract which could help the fish in providing NT, at least in partial ratio. Anyway, there is no information about the effect on structure, quantitative and qualitative parameters of the digestive micro fauna on the NT need of aquatics, so it would be useful to test the relationship between such parameters in different aquatic species.

In the present study, haematological parameters including Hb and Hct had maximum levels in fish fed NT at dose as low as 0.25% and control group, while other haematological parameters were not affected by dietary NT. There is a hypothesis regarding the positive effects of dietary NT on iron absorption and blood-forming or hemopoietic tissues such as head kidney, which has been proposed for rainbow trout by Tahmasebi-Koyani *et al*. (2012). There are also some scientific reports about the stimulatory effects of NT on hemopoietic tissues of juvenile beluga (Yousefi *et al*., 2012) and Caspian brown trout (Abedian Kenari *et al*., 2012). The results of the mentioned studies indicated that feeding beluga and Caspian brown trout by diet containing 0.35% NT significantly improve the Hb levels in comparison to control. On the other hand, some researchers such as Burrells *et al*. (2001b) and Welker *et al*. (2011) indicated that dietary NT did not have any significant effect on major haematological parameters of

Atlantic salmon and channel catfish, *Ichthalurus panctatus*, respectively. Indeed, Barros *et al.* (2013) showed that RBC, WBC, MCV, MCH and MCHC values in Nile tilapia were not affected by NT dietary supplementation which is in agreement with our results.

It is well documented that blood parameters may be affected by a very vast variety of internal and external parameters such as water temperature, age, sex, dietary nutrition components, pollution, as well as disease (Houston, 1990). Regarding the effects of NT as a dietary component on haematological parameters, it is completely obvious that the response to NT could be affected by several parameters such as dose and kind of NT, the duration of experiment as well as species specific response, and these parameters may be pursued in future research.

In conclusion, based on the results of this study, it could be concluded that the Optimun, as a commercial dietary supplementation of NT had very limited effect on growth performance and major haematological parameters in the striped catfish. It is recommended to that the effectiveness of this compound be tested on some other biological characteristics of the striped catfish such as immunological as well as stress response.

Acknowledgements

The authors would like to thank Sepanta Mahianab Aria Pars, Isfahan, Iran for supplying the striped catfish. Special thanks to Chemoforma, Augst, Switzerland which kindly provided us the commercial nucleotide "Optimun". This research was supported by funding from the Isfahan University of Technology under grant No. 502.92.53949 awarded by Dr. F. Paykan Heyrati. The authors would like to thank everyone who helped us in doing the experiment.

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