Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2024.131429 2024, 23(5) 685-693

Research Article

Dietary effect of nucleotide supplement (Ascogen®) on growth performance and intestinal microbial flora in Siberian sturgeon (*Acipenser baerii*)

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Keywords

Nucleotide,
Siberian sturgeon,
Growth,
Gut bacterial community

Article info

Received: November 2022
Accepted: January 2024
Published: September 2024

Abstract

The present study was conducted to evaluate the dietary effect of nucleotide on growth performance and intestinal microbial flora in Siberian sturgeon (*Acipenser baerii*). Four hundred fifty fish of *A. baerii* (39.77±10.24 g initial weight) were distributed into 15 tanks (300 L) and they were fed with different levels of nucleotide (Ascogen®) including 0.0 (control), 0.2 (diet 1), 0.4 (diet 2), 0.6 (diet 3), and 0.8 (diet 4) g kg $^{-1}$ in triplicates for nine weeks. The fish were fed daily until apparent satiety. The results showed no significant differences in the final length and survival rate among the experimental groups (p>0.05). However, the final weight, weight gain, daily growth rate, specific growth rate, and condition factor were significantly enhanced in the groups fed with diet 3 and diet 4 (p<0.05). The count of intestinal lactic acid bacteria (LAB) was the same among the experimental groups (p>0.05), while the total count of the intestinal bacteria was significantly decreased compared to the control group (free from nucleotide) (p<0.05). In conclusion, most of the growth indices were improved in the fish fed with nucleotide at 0.6 and 0.8 g kg $^{-1}$. However, the treated diets could not affect the intestinal LAB population in Siberian sturgeon.

Introduction

Sturgeon farming has recently gained special attention in aquaculture industry worldwide due to the production of valuable meat and caviar (Billard and Lecointre, 2000). Furthermore, sturgeon aquaculture species are easily adapted to artificial feeds and it is possible to produce successful hybrids for farming (Hung, 2017). Hence, many recent studies have focused on the nutritional aspects of these fish, particularly Siberian sturgeon (*Acipenser baerii*), to develop sturgeon aquaculture and to reduce the pressure on the natural sturgeon resources by overfishing. *A. baerii* is one of the well-known sturgeon species (Shi *et al.*, 2009) and can be a suitable fish for farming due to its ability to live in freshwater, adapt to environmental changes, feed on formulated diets, and grow and mature in the captivity. In aquaculture, fish nutrition plays a key role in the product's cost and quality. In addition to the effect on economic efficiency, sustainable aquaculture production can be achieved by improving aquafeed. In recent years, various additives have been used to increase quality, productivity, and profitability in aquaculture nutrition. In this context, nucleotides play fundamental roles in the biological functions of cells as they participate in the structure of DNA and RNA (Li and Gatlin, 2006). They can also improve the immune system, stress responses, intestinal microbial flora, digestive system, growth performance, and disease resistance in fish. Although nucleotide biologically produces in the cells, there is a limited capacity for synthesizing nucleotides in the body, especially under stress and disease conditions (Li *et al.*, 2004). Therefore, providing exogenous nucleotide in aquafeed may lead to maintain health status and proper growth performance. Ascogen® (trade name: Vanagen and Optimum) as one of the feed supplements containing nucleotide is available in the market to use in aquafeed (Adamek *et al.*, 1996). The diversity and microbial community structure of the fish intestinal tract are affected by various biota and abiotic factors (Skrodenyte-Arbaciauskiene *et al.*, 2006; Denev *et al.*, 2009). Meanwhile, nutrition is one of the most critical factors that cause significant changes in the fish intestinal microbiota (Burr *et al.*, 2005). Since intestinal microbial flora is essential in the physiological and growth performances of aquatic organisms (Nayak, 2010; Roeselers *et al*., 2011; Wu *et al.*, 2012), there is various nutritional research to increase the number of beneficial bacteria (Ringø and Gatesoupe, 1998). So far, there have been many studies on the effect of nucleotides on different aquatic organisms. The positive effects of supplementing Nile tilapia (*Oreochromis niloticus*), hybrid tilapia, hybrid tilapia (*O. niloticus* × *O. aureus*), and turbot (Scophthalmus maximus) diets with an exogenous nucleotide on growth, blood biochemical parameters, immunity, and intestinal morphology have been investigated (Barros *et al.*, 2015; Xu *et al.*, 2015; Meng *et al.*, 2017). However, studies in supplementing sturgeon fish with nucleotide are limited and are being

completed. The objective of this study is to assess different dietary levels of a commercial nucleotide (Ascogen®) on growth performance and intestinal bacterial count in Siberian sturgeon fry.

Materials and methods

The present study was conducted using a mixture of well water and Sefid-Rud River in the Caspian Sea Sturgeon International Research Institute in an indoor environment for nine weeks. For this purpose, 450 Siberian Sturgeon (average weight of 39.77±10.24 g) were randomly distributed in 15 tanks (300 L). A commercial nucleotide metabolite mixture (Ascogen®, Chemoforma Augst, Switzerland) was used to supplement five experimental diets in three replications as follows: control treatment (0 g kg^{-1}), diet 1 (0.2 g kg^{-1}), diet 2 (0.4 g kg^{-1}), diet 3 (0.6 g kg^{-1}) and diet 4 (0.8 g kg^{-1}). The average water quality variables were 22.90±0.70 °C temperature, 5.37±1.54 mg L^{-1} dissolved oxygen, and 7.79±1.83 pH. After preparing the required feed ingredients, they were powdered using a grinder, mixed with a blender, and passed through a sieve. Ascogen® (an exogenous nucleotide supplement) was added to the blend in certain amounts. In the next step, hot water and oil were added to the mixture and the produced paste was pelleted with a diameter of 2 mm. The proximate composition of the basal diet (control diet with no nucleotide supplement) was presented in Table 1. The basal diet formulation was done with Lindo software based on sturgeon's nutritional requirements. The fish were fed by hand four times a day until apparent satiety (Şener *et al.*, 2006).

Table 1: The dietary composition and chemical composition of the basal diet (free from exogenous nucleotide) for Siberian sturgeon in this experiment.

Diet composition	% in dry basis	Proximate content	% in dry basis
Fish meal	54	Moisture	10.34
Wheat gluten	5	Protein	42.75
Wheat flour	5	Fat	11.86
Soybean oil	24	Total ash	12.59
meal Soybean flour	9	Fiber	2.63
Lysine	0.25	Phosphor	1.53
Methionine	0.25	Calcium	2.48
A mixture of minerals	1.25	A variety of vitamin substances	1.25
Total	100		

The fish biometry was done once every two weeks. The fish feeding was stopped the night before and after the sampling to reduce stress in the fish. The length was measured with an accuracy of 0.01 m using a biometric board and the weight was measured with an accuracy of 0.001 g using a digital scale (Jafarinejad *et al.*, 2020). Growth indices were calculated according to the biometry data and the following formulas:

Weight gain (g) = BW_f – BWi

Daily growth rate (DGR, g day^{-1}) = (BW_f – BWi) / n

Body weight increase (BWI, %) = [(BW_f – BWi) / BWi] ×100

Specific growth rate (SGR, % day^{-1}) = (Ln(BW_f) – Ln(BWi)) ×100 / n

Condition factor (CF, %) = [(BW_f / TL^3] ×100

BWi is the initial body weight (g), BW_f is the final body weight (g), TL is the final total length (cm), FN is the number of fish alive at the end of the period, IN is the total number of fish at the beginning of the experiment, and n is the number of farming days.

At the end of the feeding trial, three fish from each replication was sampled to investigate the intestinal bacterial count. Briefly, the sampled fish were washed with a 0.1% benzalkonium chloride solution for 60 seconds to remove all the bacteria on the external surface of the fish. Then, they were rewashed with sterile water (Olsen *et al.*, 2001). A sterile scalpel was used to split the midline of the abdomen. Intestinal contents were also collected from 1 cm of the end of the intestine under hygienic conditions. The intestinal samples were prepared using serial dilutions (10^{-1} to 10^{-7}) and subjected to culture on tryptic soy agar (TSA) and de man, rugosa, and Sharpe (MRS) media for five days at room temperature under aerobic conditions (Mahious *et al*., 2006; Ringø *et al.*, 2006). After the end of the incubation, the bacteria of each dish were counted by the separator machine and calculated based on the logarithm of the colony unit g^{-1} and the results were expressed as colony-forming units (CFU g^{-1}) (Merrifield *et al.*, 2011). The research was conducted in the form of a completely randomized design. SPSS software (version 22) was used to analyze all the data. Data were expressed as mean±standard deviation (SD). After controlling their homogeneity by Kolmogorov-Smirnov, the means of the data were analyzed by a One-way ANOVA, and the means were compared using Tukey's test to distinguish the statistical differences among the experimental groups at the 95% confidence level (p<0.05).

Results

There was no significant difference between the treated fish and the control fish in terms of initial weight and length, final length, and survival rate. However, the CF value was improved in all nucleotide-added groups, except for diet 2, which was significantly lower than the control group. Besides, the final weight, DGR, BWI, and SGR levels were significantly decreased in the fish fed with 0.4 g kg^{-1} nucleotide (diet 2) compared to the fish fed with diets 3 and 4 (p<0.05; Table 2). The population sizes of lactic acid bacteria and total intestinal bacteria are displayed in Table 3. The total number of intestinal bacteria in the treated groups with different dietary levels of nucleotide was decreased compared to the control group (free from nucleotide) (p<0.05). However, the count of intestinal lactic acid bacteria was the same in all experimental groups (p>0.05).

Table 2: Growth indices of Siberian sturgeon fed with different levels of nucleotide treatments for 8 weeks.

Characteristics	Dietary levels of nucleotide (g kg^{-1})				
	0 (control)	0.2 (diet 1)	0.4 (diet 2)	0.6 (diet 3)	0.8 (diet 4)
Initial weight (g)	39.07 ± 3.87^{a}	40.17 ± 3.99^{a}	39.67 ± 3.90^{a}	40.23 ± 3.88^{a}	39.75 ± 3.60^{a}
Initial length (cm)	24.48 ± 1.16^{a}	24.74 ± 1.13^{a}	24.68 ± 1.20^{a}	24.85 ± 1.89^{a}	24.66 ± 1.08^{a}
Final weight (g)	110.66 ± 12.47^{ab}	109.83 ± 11.38^{ab}	98.79 ± 11.96^{b}	122.64 ± 15.44^{a}	117.91 ± 16.46^{a}
Final length (cm)	32.90 ± 4.11^{a}	33.43 ± 3.80 ^{a}	32.62 ± 2.82 ^{a}	33.29 ± 5.09 ^{a}	33.85 ± 3.17 ^{a}
Weight gain (g)	73.07 ± 3.40^{ab}	69.58 ± 11.13 ^{ab}	59.56 ± 10.31 ^{b}	82.42 ± 15.17 ^{a}	78.12 ± 16.56 ^{a}
Daily growth rate (g day^{-1})	1.15 ± .23^{ab}	1.10 ± 0.19 ^{ab}	0.94 ± 0.10 ^{b}	1.30± 0.25 ^{a}	1.24 ± 0.28 ^{a}
Body weight increase (%)	187.41 ± 31.91^{ab}	175.17 ± 20.17 ^{ab}	149.87 ± 5.02 ^{b}	207.56 ± 30.46 ^{a}	197.51 ± 32.34^{a}
Specific growth rate (percentage day^{-1})	1.50 ± 0.26^{ab}	1.53 ± 0.19 ^{ab}	1.40 ± 0.12 ^{b}	1.70 ± 0.19 ^{a}	1.67 ± 0.21 ^{a}
Condition factor (%)	0.31 ± 0.01^{a}	0.28 ± 0.03^{ab}	0.27 ± 0.02^{b}	0.30 ± 0.02^{ab}	0.29 ± 0.03^{ab}
Survival rate (%)	95.00 ± 4.30^{a}	90.00 ± 5.50^{a}	96.66 ± 2.70^{a}	90.00 ± 5.70^{a}	93.33 ± 3.50^{a}

The non-similar letters in each column indicate a significant difference among the treatments (p<0.05).

Table 3: Changes in the intestinal microbial flora of Siberian sturgeon in response to different dietary levels of nucleotide for 8 weeks.

Indicators (CFU g^{-1})	Dietary levels of nucleotide (g kg^{-1})				
	0 (control)	0.2 (diet 1)	0.4 (diet 2)	0.6 (diet 3)	0.8 (diet 4)
Total count of lactic acid bacteria	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Total count of intestinal bacteria	more 100000.00 ± 1165.00^{a}	99.00 ± 15.40^{b}	1362.33 ± 115.64^{b}	25.33 ± 3.46^{b}	675.67 ± 64.08^{b}

The non-similar letters in each column indicate a significant difference between the treatments (p<0.05).

Discussion

The overuse and misuse of chemotherapeutics such as antibiotics in fish farms cause serious problems namely antibiotic resistance crisis (da Silva *et al.*, 2013). Also, overprescribing antibiotics can alter the intestinal microbial community and increase production costs. Therefore, the attention of aquaculturists is more on the use of immune-stimulating substances to improve non-specific immunity and create resistance against pathogenic agents in aquatic animals. In general, it is common to add immunostimulants to aquafeed to increase resistance to infectious diseases and reduce the consumption of antibiotics (Parodi *et al.*, 2014). In this regard, nucleotide supplements can be practical to promote growth performance, intestinal absorption rate, fat metabolism, and immune responses in fish (Burrells *et al.*, 2001; Li and Gatlin, 2006). In the present study, supplementation of Siberian sturgeon fry diet could significantly improve growth performance. This study showed that the final weight, weight gain, daily growth rate, body weight increase, specific growth ratio, and condition factor had significantly changed among different experimental groups, especially in the fry fish fed with diets 3 and 4 (0.6 and 0.8 g kg^{-1} nucleotide, respectively). The higher growth indices in the nucleotide-supplemented groups may represent higher rates of feed digestion and absorption efficiency. It was also shown that the addition of exogenous nucleotide did not adversely affect the survival rate of *A. baerii*. In a similar study, Yunzhang *et al.* (2009) investigated the dietary effect of 0.0, 0.15, 0.25, 0.35,

and 0.5% nucleotide on growth performance and survival rate of common grouper and indicated the highest growth rate in the group received 0.35% nucleotide. Another study pointed out that common carp growth attributes were increased by feeding with 0.2% nucleotide due to an increase in the palatability of the supplemented diets (Koehn, 2004). Moreover, Adamek *et al.* (1996) reported that feeding rainbow trout with a diet containing 0.26 and 2.5 g kg^{-1} commercial nucleotide (Gascogne) led to increase growth attributes. Amal and Zamri-Saad (2011) investigated the effect of different levels of dietary nucleotide in Nile tilapia (*O. niloticus*) and stated that showed that these compounds improve growth indicators and reduce the food conversion ratio, which is in agreement with our results. Abtahi *et al.* (2013) showed the beneficial effect of adding nucleotide in Beluga (*Huso huso*) diets at 0.35% to improve growth indices, however, the higher level of nucleotide (0.5%) deteriorated the growth performances due to physiological disorders (*e.g.* nitrogen imbalance in cells and reduction in uricase activity). Furthermore, purine (which is not easily eliminated from the body) and the low ability of fish to break down purine in response to high levels of nucleic acids can elevate serum uric acid (Oliva-Teles *et al.*, 2006). According to the present study and other studies, it seems that the dietary of nucleotide causes positive effects in fish, but higher levels can induce remarkable side effects. Therefore, it is necessary to find an appropriate dietary level of nucleotide in different fish species.

In general, the dietary requirement of nucleotide increases by increasing the somatic growth of fish and shellfish, especially in the early stages of life (Borda *et al.*, 2003). In the present study, the results of growth data noticed that fry Siberian sturgeon need exogenous nucleotide for higher growth rate, especially at 0.6 and 0.8 g kg^{-1}, compared to the control group. Nucleotide in fish nutrition improves the absorption rate in the initial stages of growth by affecting intestinal bacterial flora (Xu *et al.*, 2015). Rumsey *et al.* (1992) illustrated a significant increase in feed consumption in rainbow trout (*Oncorhynchus mykiss*) due to the high absorption rate of nucleotide, which makes feed palatable and leads to higher growth performance. One of the beneficial mechanisms of dietary nucleotide on the physiological responses of fish is probably the prevention of stress-induced cortisol release by nucleotide (Reddy and Leatherland 1998). Burrells *et al.*, (2001) reported that dietary nucleotide compensates for growth reduction during stress. However, the effect of nucleotide on growth indices is not constant and can be influenced by the type of fish species (Barros *et al.*, 2015), the length of the feeding period (Reddy and Leatherland 1998), and the dosage (Borda *et al.*, 2003).

Recently, the manipulation of intestinal bacterial population to increase the share of beneficial bacteria has been on the agenda of fish nutrition researchers. Fish intestinal microbial community plays a fundamental role in electrolyte balance, metabolism, and fighting against pathogens (Denev *et al.*, 2009; Merrifield

et al., 2011). The change in fish intestinal microbial flora can be related to nutritional status, intestinal structure and health status, age, geographic location, environmental factors, and stress (Denev *et al.*, 2009). The increase in growth efficiency can be also related to the intestine architecture and microbial community to increase the absorption of nutrients (Ringø *et al.*, 2010). From the obtained results in this study, only the total number of aerobic intestinal bacteria had a significant difference among the experimental groups and the nucleotide-added groups showed a significant decrease in the count of intestinal bacteria compared to the control group. There was no significant difference in the total count of intestinal lactic acid bacteria in Siberian sturgeon fed with different levels of nucleotide. The effect of supplementing a hybrid tilapia diet with 0.6 % nucleotide on the intestinal bacterial community was considered to be negligible compared to the control group (Xu *et al.*, 2015). To compare the results of this study with similar studies, molecular assays are needed to distinguish the changes in the bacterial flora in Siberian sturgeon in response to the dietary levels of nucleotide.

In conclusion, the present study showed that dietary nucleotide, especially at 0.6 and 0.8 g kg^{-1}, in Siberian sturgeon posed positive effects on growth performance. However, it is suggested to investigate the effects of different dietary levels of nucleotide on the serum physiometabolic responses, immune system, and gut histomorphological aspects as well as molecular intestinal microbial pattern.

Acnowledgments

The authors would like to thank the cooperation of Dr. Shanawar Masuleh, the president of the Caspian Sea Sturgeon International Research Institute, and Mr. Maleki, the honorable head of the iroMed Laboratory in Rasht, Iran.

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