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The effects of dietary nucleotide type (Hilyses and Augic[15]) on growth performance and salinity resistance of kutum, *Rutilus kutum* (Kamensky, 1901) fingerlings

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Abstractt

The aim of this research was to evaluate the effects of dietary nucleotides on performance, immune system and salinity resistance of kutum fingerlings during migration to the sea. Five experimental diets were prepared by addition of Hilyses (0.75 and 1.5%) and/or Augic[15] (0.3 and 0.6%) to a control diet. After eight weeks adding nucleotide diets, fish were exposed to saline water for 48h. Supplementation of both nucleotide sources improved fish growth parameters (p<0.05). Fish mortality was also decreased by the additives in both fresh and sea water. Glucose and cortisol levels significantly decreased with the administration of both nucleotide sources (62 to 69 versus 75 mg dL^{-1} for glucose and 6 to 8.5 versus 9.8 mg dL^{-1} for cortisol (p<0.05). Supplementation of Hilyses and /or Augic[15] increased lysozyme activity in the blood (p<0.05) and maximum activity has observed in fish fed at high doses of Hilyses and Augic[15]diets. In conclusion, an improved performance of *R. kutum* fingerlings fed on or with both nucleotide sources may suggest that kutum fingerlings require a larger dietary nucleotide at early life stage to control stress related parameters such as cortisol and glucose.

Keywords: Kutum, Saline water, Stress resistance, Growth, Fingerling, Glucose, Cortisol

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Introduction

Caspian kutum, *Rutilus kutum* is an important commercial endemic fish species in the southern coast of the Caspian Sea. This species is a migratory anadromous fish (Heidari *et al*., 2009) which migrates from the Caspian Sea to freshwater inlets and spawns on aquatic weeds, graveled and sandy substrates in rivers and lagoons between March to April (Emadi, 1979; Azari Takami, 1990). Due to sharp decline observed in natural population during 1970s and early 1980s, The Iranian Fisheries Organization (Shilat) produces up to 192613000 fingerlings to restock the Caspian Sea population annually (Abdolhay *et al.,* 2016; Heyrati *et al.,* 2006).

The main challenge for this species is the environment of nursery sites (point of release up to river estuary) which apparently do not support fingerlings' growth before migration into the sea. Fish larva grow in fresh water while the fingerlings should adapt itself to the Caspian Sea brackish water with 13 ppt salinity after release. Dietary nucleotide supplementation can support fish to improve immune responses (Fuhr *et al.,* 2016; Hossain *et al.,* 2016; Valipour *et al.,* 2018) in order to tolerate such a harsh environmental conditions before adjusting to the seawater.

Nucleotides are low molecular weight intracellular compounds that play key roles in nearly all biochemical processes (Cosgrove, 1998.) Previous studies have showed that dietary supplementation of nucleotides enhanced resistance of salmonids to viral, bacterial and parasitic infections as well as improved efficacy of vaccination, osmoregulation capacity and also declined stress responses through a reduced serum cortisol levels of fish (Burrells *et al.,* 2001a, b; Li and Gatlin III, 2006).

Nucleotide requirements of fish increase during stress periods, low food availability and fast growth period (Low *et al.,* 2003). Stressful factors are associated with normal aquaculture conditions and practices such as poor water quality, crowding and handling, which cause additional demands on available nucleotides beyond those provided in typical aqua feeds such as exogenous supply may result in beneficial effects (Burrells *et al.,* 2001a, b). Most tissues including liver (Boza, 1998) can synthesize nucleotides and some cells such as important immune system cells such as lymphocytes, red blood cells, hematopoietic cells and the intestinal mucosa cells need the nucleotides due to high volume of cell metabolism rate and quick reactions (Quan and Uauy, 1991; Burrells *et al.,* 2001a). Dietary supplementation of nucleotides reduced serum cortisol levels of healthy rainbow trout after infection by diseases (Leonardi *et al.,* 2003).

The commercial nucleotide products which have been used in most of previous studies are derived from yeast and generally contain other components such as polysaccharides and trace elements (Lin *et al.,* 2009; Nazari *et al.,* 2016). This supplement can provide immune-stimulation in fish (Sakai, 1999). The yeast type i.e. the source of

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nucleotides may also play a role in the efficiency of nucleotides due to level and type of immune-stimulating components such as β-glucan, nucleic acids, mannan oligosaccharides and chitin (Gopalakannan and Arul, 2010).

Fish has to regulate internal osmotic pressure when migrate between fresh water and seawater during life cycle (Gholampoor *et al*., 2011). This condition increasea stress level leading to a higher level of glucose and cortisol in fish (Santos and Pacheco, 1996). Therefore, nucleotide supplementation might help fish to tolerate migration period.

To date, there are not too much data available on the impacts of nucleotides as feed additives on immune system and stress level during the migration period. Therefore, the main goal of the current study is to evaluate the influence of two dietary nucleotide types on performance, immune system and salinity resistance of *R. kutum* during migration to the sea.

Materials and methods

Experimental system and animal

This study was carried out at the experimental facility of Shahid Rajaee fish propagation center located in southern coast of the Caspian Sea during spring 2016. Fingerling of *R. kutum* have been bred at the reproduction facility of the fish station and adapted to the experimental conditions 10 days before the start of the experiment. Fish were fed with a control diet during the adaptation. Afterward, the fingerlings with an average weight of 1.0±0.02 g were divided randomly into fifteen 1000 L tanks with an initial stocking density of 300 fish per tank. The experiment lasted for 56 days.

In the current study, two nucleotide sources, Hilyses and Augic[15], tested in *R. kutum*. Both additives, available as a fine powder, are derived from a commercial product from specific strains of *Saccharomy cescerevisiae* and *Candida utilis* (Augic[15]) and only *S. cerevisiae* (Hilyses). Augic[15] contains more nucleotides than Hilyses (15 versus 6.1%; ICC. Indl. Co., Brazil).

Five experimental diets were prepared by addition of Hilyses and/or Augic[15] to a control diet: I. control (with no supplementation); II. control diet with 0.75% of Hilyses; III. control diet with 1.5% of Hilyses, IV. control diet with 0.25 % of Augic[15]; and V.control diet with 0.50 % of Augic[15]. The composition of the experimental diets has shown in Table 1.

Table 1: The percentage of ingredients used in the experimental diet on % dry matter weight basis.

Experimental diet	Control	Hilyses0.75%	Hilyses1.5 %	Augic 0.25 %	Augic 0.5%
Soybean meal	19	19	19	19	19
Fish meal	35	35	35	35	35
Meat meal	14	14	14	14	14
Corn	10	10	10	10	10

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Table 1 continued:					
Wheat	10	10	10	10	10
Cottonseed meal	6.5	6.5	6.5	6.5	6.5
Molasses	3	2.25	1.5	2.75	2.5
DL-methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Hilyses	0	0. 75	1.5	0	0
Augic	0	0	0	0.25	0.5
[1]Premix	2	2	2	2	2
[2]Nutrient composition of the experimental diet in g kg^{-1}					
Dry matter	915.0±6.5	912.3±7.6	908±4.1	917.3±2.1	919.3±7.8
Crude protein	375.6±3.2	376.9±3.4	380.1±1.8	371.9±2.4	382.3±2.9
Crude fat	107.1±1.9	109.3±1.6	108.6±2.1	106.8±1.5	109.2±1.0
Carbohydrate	299.1±2.9	288.6±3.1	290.3±4.2	302.3±3.8	284.6±2.6
Crude ash	133.6±1.8	139.1±1.9	129.1±2.0	137.2±1.4	141.2±2.1

[1]premix consisted of equal proportion of vitamins and minerals
[2]Diet composition values are means of three replicates
Vitamin premix consisted of (g kg^{-1} premix):1200000 IU Vitamin A, 400000 IU Vitamin D3, 3000 IU Vitamin E, 1200 mg Vitamin K3, 5400 mg Vitamin C, 200 mg Vitamin B1, 3360 mg Vitamin B2, 7200 mg Vitamin B3, 9000 mg Vitamin B5, 2400 mg Vitamin B6, 600 mg Vitamin B9, 4 mg Vitamin B12, 500mg Antioxidant, up to 1kg carrier.
Mineral premix consisted of (g kg^{-1} premix): 2600 mg Mn, 600 mg Cu, 6000mg Fe, 4600mg Zn, 50mg Se, 100mg Iu, 50 mg Co, 100000 mg choline chloride, up to 1 kg carrier (composed of wheat bran) .

Experimental procedure

Fish were weighed on the 1st and the last days of the experiment. The experimental diets were given to the fish at a ratio of 10% of biomass twice per day (8.30 and 16.30 hrs.). Every two weeks the absolute ration size of the experimental diets has been corrected as the fish grew. The diets were randomly assigned to one of 15 tanks with three replications per diet. Water quality parameters were monitored daily to ensure they were in appropriate ranges for the fish. The water temperature and pH were 21.6±1.6 °C and 7.5± 0.55, respectively, during the experiment. Oxygen concentration was measured in a randomly selected tank by a digital oxygen detector, which always remained above 6.1 mg L^{-1}.

On the fifty sixth day, all fish were weighed and then 10 fish were randomly taken from each tank and sacrificed using overdosed (400 mg L^{-1}) clove essential oil solution (Amirkolaie, 2015) for measuring of the body composition. For analyzing blood parameters, 15 fish were captured quickly and blood samples were taken from the caudal vein using a syringe containing 3 mg of Na_2EDTA stored at 4 °C after gently shaken for further examination.

For glucose and cortisol measurements on the 56 th day, around one ml of the collected samples were placed in cooled, 1.5 ml plastic tubes,

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mixed and centrifuged at 6000 g for 5 min at 4 °C. Plasma glucose estimation has been measured colorimetrically according to Trinder (1969). Plasma cortisol levels measured by radio immunoassay (RIA) according to Rottlant et al. (2001) and expressed as ng ml^{-1}. Plasma lysozyme activity has been determined by aturbidimetric assay as described by Jorgensen *et al*. (1993) using a suspension of *Micrococcus lysodeikticus* and hen egg white lysozyme as an external standard. A lysozyme activity unit was described as the quantity of enzyme producing a decrease in absorbance of 0.001min^{-1}. Red and white blood cells counts were estimated following the method of Schalm *et al*. (1975). Haemoglobin (Hb) and Haematocrit (Ht) concentrations were determined based on Barros *et al.* (2015).

Chemical analysis and bacteria count

Feed and fish body have been analyzed for dry matter through drying samples for 24 h at 103 °C until constant weight (ISO 6496, 1983). Ash content has been determined by incineration in a muffle furnace for 4 h at $550^{\circ C}$ (ISO 5984, 1978). Crude protein (N×6.25) has measured by the Kjeldahl method after acid digestion according to ISO 5983 (1979). Lipid has been extracted by petroleum ether extraction in a Soxhlet apparatus (ISO 6492, 1999). Carbohydrate fraction was determined as dry matter minus fat, protein, and ash in the feed.

At the end of the experiment, six fish were collected randomly from each treatment and their intestine samples were tested for bacteria counts. Prior to dissection and homogenization, the juveniles were rinsed with sterilized distilled water, cleaned with ethanol (70.0%) and then washed up again with sterilized distilled water to eliminate all exterior bacteria. The intestine samples have been dissected out in sterile conditions. For microbiological analysis, three samples from the middle part of the intestine were taken. All samples were diluted using sterilized normal saline solution (0.85% NaCl w/v) and then placed into nutrient agar plates for bacterial counts (Ebrahimi *et al.*, 2012).

Stress resistance measurement

After weighting, bacterial sampling and blood measurements, the rest of *R. Kutum* fingerlings were exposed to saline water. Thirty fish were randomly collected from each tank (replicate) and distributed to the tanks containing saline water for salinity stress test. The salinity level (13 ppt) were based on fingerling releasing sites of kutum. The saline water prepared from the Caspian Sea. The fish were kept in saline water tanks for 48h without feeding and mortality in different treatments has been recorded.

Fish performance

Weight gain has determined by the difference between initial and final body weights. Daily gain calculated by dividing the weight gain over experimental days. Feed conversion ratio (FCR) has calculated for each tank from feed intake data and weight gains: FCR=feed consumed (g)/wet body

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weight gain (g). Specific growth rate (SGR) has calculated as follows and expressed as a percentage: SGR=100 (Ln W_{final}-Ln $W_{initial}$)×days^{-1}. Mortality data in fresh water referred to deaths of fish during 56 days of growth trail.

Statistical analysis

Data are presented as means of each treatment with standard deviation. All data were verified for normality (Kolmogorov-Smirnov test) after ArcSine transformation One-way ANOVA was used to determine the effects of two nucleotide sources on growth parameters, blood indices and salinity resistance. Tukey's test was used to compare differences between the means. For all statistical analyses, each tank was considered as the experimental unit.

Results

Fish growth performances details have been listed in Table 2. Supplementation of both nucleotide sources improved fish growth parameters. The highest final weights and SGR were attained when fish treated with either 1.5% Hilyses or 0.5% Augic^{15} (p<0.05).

FCR and SGR values were also significantly improved in *R. kutum* fed with Hilyses and Augic^{15} diets in comparison with the control diet (p<0.05). Fish mortality rates were also reduced by the both nucleotide sources (p<0.05). Maximum survival rate has observed in fish fed diet supplemented with either 1.5% Hilyses or 0.5% Augic^{15} (p<0.05) at both fresh and brackish water environment.

Body composition of *R. kutum* fingerlings was not affected by both nucleotide sources (Table 3; p>0.05) and the measured parameters were almost alike. Similar to the body composition, administration of the two nucleotide sources did not influence the intestinal microflora (p<0.05; Table 4). Gram negative and gram positive bacterial count in the nucleotide diets were similar to control fish (p>0.05).

Table 2: Growth performance in *Rutilus kutum* fingerlings fed on two types of nucleonic sources (Hilyses and Augic) over 56 days' experimental period.

Growth parameters	Diets				
	Control	Hilyses0.75 %	Hilyses1.5 %	Augic 0.25%	Augic 0.5%
Initial weight (g)	1.01 ±0.03	0.99± 0.02	1.02 ± 0.03	1.02 ± 0.03	1.01 ±0.02
Final weight (g)	3.50 ± 0.54^{c}	5.07± 1.33^{b}	5.34 ± 1.29^{a}	5.08± 1.19^{b}	5.38 ± 1.30^{a}
SGR (%/day)	2.39± 0.10^{c}	2.57 ± 0.17^{ab}	2.67 ± 0.11^{a}	2.49 ± 0.14^{b}	2.65± 0.12^{a}
FCR	4.3 ± 0.12^{c}	3.87 ± 0.11^{b}	3.70 ± 0.20^{a}	3.90 ± 0.10^{b}	3.66± 0.16^{a}
Mortality% (fresh water)	15.4 ± 2.1^{a}	7.2± 1.8^{b}	2.3 ± 0.8^{c}	6.4± 1.1^{b}	2.1± 0.6^{c}
Mortality% (brackish water)	32.4± 2.3^{a}	20.6± 1.8^{b}	7.2±1.8^{c}	18.6±2.4^{b}	6.1± 2.3^{c}

Values are means of triplicate groups±SD. Means with the different letters are significantly different (p<0.05).

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Table 3: Body composition of *Rutilus kutum* fingerlings fed on two types of nucleonic sources (Hilyses and Augic) over 56 days' experimental period.

Parameters	Diets				
	Control	Hilyses0.75%	Hilyses1.5%	Augic 0.25%	Augic 0.5%
Dry mater	24.32±1.32	25.04±1.01	24.69±2.77	25.00±1.24	24.62±1.32
Protein	18.97±0.59	19.96±0.46	18.98±0.91	18.81±0.09	18.09±0.09
Fat	2.50±0.30	2.74±0.24	2.63±0.48	2.36±0.36	2.57±0.18
Ash	3.08±0.23	2.98±0.26	2.84±0.38	3.15±0.21	3.15±0.21

Values are means of triplicate groups±SD. Means with the different letters are significantly different (p<0.05).

Table 4: Bacterial counts in the intestine of *Rutilus kutum* fingerlings fed on two types of nucleotide sources (Hilyses and Augic) over 56 days experimental period.

Bacterial community	Diet				
	Control	Hilyses 0.75%	Hilyses 1.5%	Augic 0.25%	Augic 0.5%
Gram negative (CFU g^{-1})	4.99×10^{4}	4.98×10^{4}	5.00×10^{4}	4.99×10^{4}	4.97×10^{4}
Gram positive (CFU g^{-1})	1.78×10^{4c}	1.75×10^{4}	1.77×10^{4}	1.79×10^{4}	1.78×10^{4}

Values are means of triplicate groups±SD. Means with the different letters are significantly different (p<0.05).

Some blood parameters in *R. kutum* fingerlings were influenced by the inclusion of nucleotide in the diets (p<0.05; Table 5). While red blood cells and hemoglobin were not influenced by both nucleotide sources, hematocrit percentage wsa increased by addition of the nucleotides. White blood cells count were increased with both nucleotide sources (p<0.05) and the highest values observed in fish fed high doses of Hilyses and Augic[15]. Neutrophils and lymphocyte values showed similar trends being maximized at 1.5% Hilyses and 0.5% Augic[15] (p<0.05).

Table 5: Blood parameters and plasma analyses in *Rutilus kutum* fingerlings fed on two types of nucleotide sources (Hilyses and Augic) over 56 days` experimental period.

Blood parameters	Diets				
	Control	Hilyses 0.75%	Hilyses 1.5%	Augic 0.25%	Augic 0.5%
Red blood cells (10^{6} µl)	1.31± 0.05	1.33± 0.11	1.28 ± 0.20	1.29 ± 0.10	1.27± 0.22
Haemoglobin (g dl^{-1})	8.53 ± 0.07	8.51± 0.14	8.55 ± 0.28	8.50 ± 0.49	8.57± 0.28
Haematocrit (%)	29.5 ± 0.7^{ab}	31.5 ± 0.2^{b}	34.5 ± 0.4^{a}	31.5 ± 0.2^{b}	34.5 ± 0.2^{a}
White blood cells (10^{3} µl)	8.80 ± 0.4^{c}	9.20 ± 0.07^{b}	10.0 ± 0.05^{a}	9.9 ± 0.9^{a}	10.2 ± 0.2^{a}
Neutrophil %	4.50 ± 0.17^{c}	5.50 ± 0.11^{b}	6.50 ± 0.07^{a}	5.50 ± 0.21^{b}	6.50 ± 0.09^{a}
Lymphocyte %	64.0 ± 1.14^{c}	79.5 ± 1.0^{b}	89.5 ± 0.33^{a}	78.9 ± 0.51^{b}	89.6 ± 0.2^{a}
Monocyte %	0.44 ± 0.01^{ab}	0.54 ± 0.14^{b}	0.65 ± 0.02^{a}	0.53 ± 0.03^{b}	0.66 ± 0.12^{a}

Values are means of triplicate groups±SD. Means with the different letters are significantly different (p<0.05).

24 h after stress, glucose and cortisol levels significantly decreased with the administration of both nucleotide sources compared to the control (p<0.05; Table 6), and higher levels of the nucleotides led to the lowest estimates of these parameters. Supplementation of Hilyses and/or Augic[15] increased blood lysozyme activity (p<0.05) and maximum blood lysozyme activity was observed in fish fed high levels of Hilyses and Augic15.

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Table 6: Stress related parameters in *Rutilus kutum* fingerlings after 24 hours' exposure to the sea water.

Blood parameters	Diets				
	Control	Hilyses0.75%	Hilyses1.5%	Augic 0.25%	Augic 0.5%
Glucose (mg dl^{-1})	75.1± 1.72^{a}	66.5± 2.93^{ab}	62.0± 1.0^{c}	69.0± 1.30^{b}	62.08± 1.99^{c}
Cortisol (mg dl^{-1})	9.8 ± 5.0^{a}	8.5± 4.0^{ab}	6.0± 5.5^{c}	7.0± 1.41^{b}	6.3± 1.6^{c}
Lysozyme (mg L^{-1})	1033± 101^{c}	1121± 105^{b}	1128± 100^{a}	1110± 95^{b}	1230± 99^{a}

Values are means of triplicate groups±SD. Means with the different letters are significantly different (p<0.05).

Discussion

The overall results revealed that both nucleotide sources had positive impacts on kutum fingerlings performance and its stress resistance although the sources of the used nucleotide were different. In other words, the nucleotide sources had no large impact on growth related parameters. Similarly, Fuhr *et al.* (2016) showed that *Artemia* enriched with both Augic[15] and Hilyse increased growth of *Odontesthes argentinensis* larvae. However, cumulative stress index was recorded lower for larvae fed Augic[15] diet compared to Hilyse one (Fuhr *et al.*, 2016). These contradictory results may suggest that the impact of nucleotide source on stress resistance depends on fish species.

A number of previous studies demonstrated similar effects of nucleotides in fish e.g., in tilapia (Ramadan *et al.*, 1994), Pacific white shrimp (Murthy *et al.*, 2009), Grouper (Lin *et al.*, 2009), Rainbow trout (Ahmad *et al.*, 2011) and Common carp (Falahatkar *et al.*, 2012). A lowered oxidative stress induced by the consumption of dietary nucleotide may account for these positive impacts on growth and health performance in rainbow trout (Mohebbi *et al.*, 2013). In addition, nucleotide may play as an attractant and improve feed intake in fish. A diet supplemented by nucleotide increased growth and survival rates of turbot larvae because of an improved feed intake (Person-Le Ruyet *et al.*, 1983). Similarly, growth-enhancing effect induced by larger feed intake observed in tilapia larvae (Ramadan and Atef, 1991) and juveniles of rainbow trout (Adamek *et al.*, 1996) fed nucleotide diets.

It seems that internal production of nucleotide is not enough for physiological demands when fish is exposed to unfavorable environmental condition and/or disease (Hoffmann and Horne, 2008; Mohebbi *et al.*, 2013; Peng *et al.*, 2013). Inclusion of nucleotide can improve fish immune system such as phagocytosis activity (Grimble and Westwood, 2000; Sakai *et al.*, 2001; Gil, 2002), natural killer cells and macrophage activation (Carver, 1994; Sakai *et al*., 2001).An increased lysozyme activity in *R. Kutum* fingerlings with increasing nucleotide supplementation may confirm the idea that nucleotide supplementation improves innate immune system of fish when they are exposed to stressors like saline water. Similar observations in relation to dietary nucleotide and lysozyme activity were made by Sakai *et al.* (2001) nd Ahmad *et al.* (2011).

Increased resistance to environmental stress has been related to nucleotide inclusion in fish feed. Nucleotide administration beyond those existing in commercial fish feed may provide

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helpful effects on fish exposed to culture-related stressors such as poor water quality, crowding and handling, which place additional demands on the available nucleotide pool (Li and Gatlin, 2006). The current results also reveal higher survival rates of *R. kutum* fingerlings fed nucleotide diets both in fresh and saline water. There are evidences showing an improved immune system activities and resistance to brackish water after feeding with nucleotide diet. A lower mortality observed in nucleotide-fed salmonids after affecting by infectious diseases (Burrells *et al.,* 2001 a). Nucleotide diet also improved stress resistance in channel catfish (Welker *et al.,* 2011).

Stress related parameters such as cortisol and glucose were reduced in fish fed nucleotide 24 h after transforming to saline water. This finding is similar to those of Burrells *et al.*(2001 b) and Leonardi *et al.* (2003) who have observed significant decrease in levels of stress parameters in Atlantic salmon exposed to high salinity and rainbow trout infected by pancreatic necrosis, respectively, fed nucleotide supplemented diets after exposure to stressors. Dietary nucleotide may increase stress tolerance in fish by partially naturalizing the inhibitory impacts of cortisol release associated with stress (Li and Gatlin, 2006). This condition may lead to an enhanced resistance in fish challenged by infectious disease (Leonardi *et al.,* 2003). An enhanced resistance to a number of bacterial diseases were observed in several species such as salmonids (Burrells *et al.,* 2001a), common carp (Sakai *et al.,* 2001) and hybrid striped bass (Li *et al.,* 2004).

Red blood cells and hemoglobin of *R. kutum* fingerlings were not influenced by dietary nucleotide. This is similar to Barros *et al.* (2015) who observed no effect of nucleotide supplementation on hematological parameters in Nile tilapia. However, white blood cells and lymphocyte were increased by dietary nucleotide, which is similar to previous reports on the influence of dietary nucleotides on white blood cells and leucocytes (Leonardi *et al.,* 2003; Tahmasebi-Kohyani *et al.,* 2011; Barros *et al.,* 2015). Stress resulted from transferring to saline water seems to be the main reason for such higher white blood cells levels.

In conclusion, it has been demonstrated that the inclusion of both nucleotide types in the diets improved stress resistance and growth performance of kutum fingerlings. These effects are probably related to a higher nucleotide requirement at early life stage. The control of stress indices and improvement of immune response by nucleotide may suggest that the use of exogenous nucleotides as a protecting treatment before culture-related stress may prove beneficial by decreasing the immune suppressive effects of stress.

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