Journal of Animal Research: v.10 n.4, p. 563-568. August 2020

DOI: 10.30954/2277-940X.04.2020.13

Study on Alteration of Critical Water Quality Parameters and Selected Metabolic Response of *Labeo rohita* Fingerling Subjected to Transportation Stress

Abhilipsa Biswal, Prem Prakash Srivastava*, Subodh Gupta, Manish Jayant and Tincy Varghese

Division of Fish Nutrition, Biochemistry and Physiology, ICAR - Central Institute of Fisheries Education, Versova, Mumbai, INDIA

**Corresponding author: PP Srivastava; E-mail: ppsrivastava@cife.edu.in*

Received: 19 June, 2020 **Revised:** 25 July, 2020 **Accepted:** 29 July, 2020

ABSTRACT

The changes in physico-chemical characteristics of ambient water during transportation of fish causes stress and mortality. In this prelude, the present investigation involved the measurement of Physico-chemical characteristics of water (TAN, pH, and dissolved oxygen), biochemical parameters (serum total protein; serum glutamic-oxaloacetic transaminase, SGOT) during simulated transportation of *Labeo rohita* fingerlings using water additives. Four treatments of water additives were used in combination as T_1 (1g glucose + 2g sodium chloride)/L, T_2 (2g glucose + 2g sodium chloride)/L, T_3 (1g glucose + 4g sodium chloride)/L, and T_4 (2 g glucose + 4g sodium chloride)/L along with a control group, an experiment was conducted in triplicate. Before and after 12 h transportation, water quality and serum sampling were carried out. The analysis of the water quality parameters and serum sample showed a significantly higher ($p<0.05$) level of total ammonia nitrogen, lower pH, lower serum total protein, and higher SGOT activity in the control group after transportation. Whereas, a significantly ($p<0.05$) lower stress response and water quality values were observed in the treatment group indicating the potency of water additives in ameliorating the transportation stress, and in turn increases the survival rate of the IMC, *Labeo rohita* fingerlings after the transportation.

HIGHLIGHTS

- Transportation stress causes a significant alteration of water quality and serum biochemical parameters.
- Use of water additive (1g glucose+ 4g sodium chloride) provides higher survival and lower stress response during transportation.

Keywords: Transportation stress, Total ammonia nitrogen, Serum protein, Water pH, *Labeo rohita*

The success of the fish culture system is dependent upon the stocking of healthy and quality seeds. The seed is the fundamental requirement in a fish culture system. The aquaculture sector is regarded as one of the fastest-growing food production sectors continuing to gain its significance in providing food and nutrition globally (Food and Agriculture Organization, 2018). Although the aquaculture sector is growing at a fast pace, the major obstacle responsible for impeding the growth of this sector is 'stress'. The occurrence of various kinds of stressors is very frequent in an aquaculture system ultimately causing the elevated expenditure of energy, obstruction in growth, disease susceptibility, reproduction failure, and even mortality (Schreck, 2010; Tort, 2011). Due to this, mitigation of stress from the aquaculture sector emerges as a budding researchable issue in the current scenario. Among all the different kinds of stress in aquaculture sector, transportation stress occupies a prominent position since transportation of fish seed from hatchery to geographically distant culture system is an indispensable yet severely stressful process (Koolhaas *et al*., 2011).

Transportation of fish seeds in plastic bags is a popular

How to cite this article: Biswal, A., Srivastava, P.P., Gupta, S., Jayant, M. and Varghese, T. (2020). Study on alteration of critical water quality parameters and selected metabolic response of *Labeo rohita* fingerling subjected to transportation stress. *J. Anim. Res.,* **10**(4): 563-568.

Source of Support: None; **Conflict of Interest:** None

method and due to its economic feasibility for long-distance transportation, it is a widely accepted method worldwide (Jhingran and Pullin, 1985; Becker *et al*., 2012). However, it is reported that during such a mode of transportation, fishes experience several negative effects in their body physiology. This is due to alteration in physicochemical characteristics of transport water such as elevation in water ammonia, decrease in dissolved oxygen, decrease in water pH (Iversen *et al*., 2009; Parodi *et al.,* 2014). On a note, the severity of transportation stress depends upon the duration of transport and the physico-chemical characteristic of water (Das *et al*., 2015).

There are several reports presently evaluating the efficiency of various kinds of water additive in the transport water (Iversen *et al*., 2009; Becker *et al*., 2012). In this present experiment, the idea behind the addition of sodium chloride is to decrease the osmotic gradient difference between the body fluid and ambient water (Kamalam *et al*., 2017), and glucose was used to act as an energy source for the fish body. As herbivorous fishes are more tolerant to the monosaccharide glucose, it was expected it will not affect the fishes negatively and will supplement the extra energy requirement (Polakof *et al*., 2012).

The change in the water quality parameters leads to an increase in the metabolic rate (Oyoo-Okoth *et al*., 2011). This elevated metabolic rate leads to the increased degradation of body protein to sustain the increased energy demand of the body via gluconeogenesis (Chatterjee *et al*., 2006). Thus, the ultimate change in the water quality such as pH, ammonia, and dissolved oxygen along with biochemical parameters such as serum total protein value and SGOT level will depict the efficiency of this particular water additive. Thus, the objective of this experiment was to understand the efficacy of these aforementioned water additives in combination based on physicochemical parameters of water, biochemical parameters, and survival post 12 h transportation.

MATERIALS AND METHODS

Experimental animal

Commercially procured *L. rohita* fingerlings (4.5±0.5 g) (mean weight + SD) were acclimatized in laboratory conditions for three weeks. A circular plastic tank of 1000 L capacity was used for rearing them in acclimatized condition, in which optimal water quality (water temperature 25-27 °C, pH 7.5-7.7, dissolved oxygen 5.7 ± 0.8 mg. L^{-1}) was maintained. During that period fishes were fed at 3% of their body weight with a diet containing 30% protein. However, before the transportation, the fishes were starved for 24 hours to release the fecal waste, which could prevent affecting the water quality of transport bags to some extent.

Experimental procedure and sampling

Experimental fishes were collected and packed in plastic bags (35 x 20 cm**)** containing 1 L of water and oxygen up to two-third volume. Twenty fishes were packed in each plastic bag and additives were added in four treatments and control groups. The treatments administered were T_1-1g glucose + 2g sodium chloride in 1 L of water, T_2- 2g glucose + 2g sodium chloride in 1 L water, T_3- 1g glucose + 4g sodium chloride in 1 L water, and T_4-2 g glucose+ 4g sodium chloride in 1 L water. Simulated transportation of fishes was carried for 12 h by keeping them on a mechanical shaker platform rotating at a speed of 110 rpm. To prevent the outer environmental temperature from affecting the transported fishes, the fish packets were placed in a Styrofoam box.

The sampling of transport water was done once before packing and after the 12 h transportation. Similarly, the blood and tissue sampling were carried out both before and immediately after the 12 h transportation. The sampled blood was collected, and after clotting, it was centrifuged at 5000 rpm for 5 minutes to collect the serum. Dead fishes were counted and discarded.

Physicochemical parameters of water

Water quality parameters were analyzed before the transportation (0 h) and after 12 h of transportation. The dissolved oxygen method was measured following Winkler's method. Following the standard protocol of APHA (2005), the total ammonia-nitrogen value was measured with the help of a spectrophotometer in the phenate method. Water pH was measured by using digital pH meter (LABINDIA). Water temperature was determined using a dry bulb mercury-in-glass thermometer.

Biochemical parameters

The level of serum total protein and serum glutamic pyruvic transaminase (SGOT) was analyzed by CK-NAC kits (NAC activated method, kinetic, Erba Mannheim, TransAsia Bio-medicals, Daman, India).

Survival

Immediately after transportation, the dead fish numbers were counted. Dead fish were discarded and the survival data was reported.

Statistical analysis

Statistical analysis of different serum biochemical was done using one-way analysis of variance (ANOVA) in SPSS 16.00 using Duncan's multiple range tests. Data is presented in terms of mean ± standard error with 5% level of significance.

RESULTS AND DISCUSSION

Water quality parameters

During transportation alteration of physicochemical characteristics of water, particularly changes in critical parameters such as dissolved oxygen, total ammonia nitrogen, pH, and temperature are responsible for impacting the seed survival (Portz *et al*., 2006). The physicochemical parameters of transport water were recorded both before and after transportation. The dissolved oxygen level of all the treatments and the control group was found to be insignificant to each other (Table 1). This finding is corroborated by the previous reports where the prolonged 48 h transportation of the high oxygen demanding rainbow trout fingerlings did not show any significant difference in the dissolved oxygen content of the transport water (Kamalam *et al*., 2017). It has been reported earlier that during transportation stress, the fish changes its behavior and due to hyperactivity related to increased respiration rate, the dissolved oxygen concentration tends to deplete in the transport water (Kutty, 1987). However, in closed system transportation under a pressurized oxygen atmosphere, the dissolved oxygen content is not usually fluctuated (Lim *et al.,* 2003).

Table 1: Dissolved oxygen and water temperature value in transport water (before and after transportation) with addition of glucose+ sodium chloride in combination. Data is represented in mean ± SE, p value ≤ 0.05 and various superscripts represent the significant levels

Dissolved Oxygen (mg. L^{-1})		
Before Transportation	**Treatment**	**After transportation**
	Control	$8.1^b \pm 0.35$
$8.5^a \pm$ **0.**24	T_1	$7.9^c \pm 0.33$
	T_2	$8.3^b \pm 0.37$
	T_3	$8.2^b \pm 0.25$
	T_4	$8.3^b \pm 0.34$
Water Temperature (ºC)		
	Control	30 ± 1
30 ± 1	T_1	30 ± 1
	T_2	30 ± 1
	T_3	30 ± 1
	T_4	30 ± 1

Further, there was no significant difference in the water temperature of ambient water among the control and treatment groups after the transportation (Table 1). The level of total ammonia nitrogen was found to be significantly ($p<0.05$) higher in control fishes in comparison to the treatment groups after transportation. However, among the treatments, there was no statistically significant difference between T_3 and T_4 group (Table 2) existed.

Table 2: Total ammonia nitrogen and water pH value in transport water (before and after transportation) with addition of glucose+ sodium chloride in combination. Data is represented in mean ± SE, p value ≤ 0.05 and various superscripts represent the significant levels

Total ammonia nitrogen (mg-N. L^{-1})		
Before Transportation	**Treatment**	**After transportation**
	Control	$3.56^b \pm 0.14$
$0.14^a \pm 0.01$	T_1	$2.20^c \pm 0.17$
	T_2	$2.00^c \pm 0.11$
	T_3	$1.54^d \pm 0.16$
	T_4	$1.43^d \pm 0.12$
Water pH		
	Control	$6.4^b \pm 0.20$
$7.3^a \pm 0.02$	T_1	$6.6^a \pm 0.38$
	T_2	$6.7^a \pm 0.49$
	T_3	$7.1^a \pm 0.35$
	T_4	$6.9^a \pm 0.24$

Due to metabolic excretion in a small confined environment and exaggerated movement pattern in plastic bags, transport water changes its water quality. Transportation is a stressful process, which increases the metabolism of fishes during that time. Increased metabolism leads to the deamination of protein, resulting in increased excretion of ammonia (Randall and Tsui, 2002). After reaching a certain level of total ammonia nitrogen content in the water, the fish body will impede the further excretion of ammonia, which will lead to the deposition of ammonia nitrogen in the body fluid and thus hinders its oxygen-carrying capacity. This condition leads to the mortality of fishes during transportation (Hong *et al.,* 2019). Thus, in this study increased total ammonia nitrogen in the control group transport water may indicate higher stress and higher metabolic activity during transportation, leading to increase excretion of ammonia. Similarly, Das *et al*. (2015) have reported a change in total ammonia levels in the transport water during the transportation of *L. rohita* seeds. However, decreased ammonia levels in the treatment groups transport water might suggest decreased ammonia excretion and decreased metabolic activity due to the effect of the additive.

In the present study, a significant difference ($p<0.05$) in pH value was observed in the control group in comparison to the treatments after transportation (Table 2). Transportation usually leads to a decrease in pH of transport water.

Here, the decrease in pH of transport water might be attributed due to the increased metabolic rate followed by an increased level of carbon dioxide as a respiration by-product (Hong *et al*., 2019). Carbon dioxide leads to the formation of carbonic acid in the water, which further dissociates to produce more hydrogen ion, and thus, it acidifies the transport water by decreasing its pH (Wurts, 2003; Singh *et al.,* 2004).

All these above changes of increased TAN concentration, carbon dioxide concentration, and decreased pH have shown to synergistically affect and decrease the oxygen-binding affinity of hemoglobin (Biswas, 1990). These kinds of alterations of water quality generally occur in the transportation of almost all fishes, which ultimately disturbs the homeostasis process and affects the body physiology (Sampaio and Freire, 2016).

Serum biochemical parameters

The serum total protein level was found to be significantly ($p<0.05$) lower in the control group fishes in comparison to treatments after transportation (Fig. 1). This finding goes per the experiment where transportation stress led to the decrease of serum total protein of *Cyprinus carpio* (Dobšíková *et al*., 2006). Alteration in the level of serum total protein is often used as an indicator of fish health (Tahmasebi-kohyani *et al*., 2012). However, it's reported that ammonia is the by-product of protein metabolism. Thus, the result of higher total ammonia level supports the finding of significantly decreased protein concentration in control fish. It signifies that the control group fishes were in severe stress which led to an extra energy requirement leading to protein degradation. The highest value of serum total protein was observed in T_3 group in comparison to other treatments. This might be attributed to efficacy of water additives which might have decreased the energy demand of treatment group fishes, leading to decreased protein degradation in comparison to the control group.

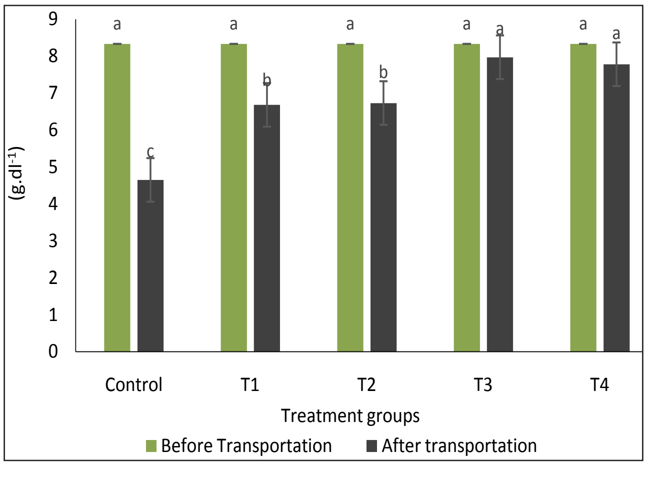

Fig. 1: Serum total protein level (before and after transportation) with addition of glucose+ sodium chloride in combination. Data is represented in mean ± SE, p value ≤ 0.05 and various superscripts represent the significant levels

SGOT is an important enzyme that signifies the liver function and often used as an indicator of hepatocellular damage (O'Brien *et al*., 2000). This transaminase enzyme is responsible for the transamination of amino acid. After transportation serum SGOT level was found to be significantly ($p<0.05$) higher in the control group compared to treatments. During the onset of stress, increased activity of SGOT suggests the mobilization of aspartate for gluconeogenesis (Chatterjee *et al*., 2006). Elevated concentration of SGOT in transported fishes of the control group indicates the liver damage (Refaey *et al*., 2018). Among the treatments, the SGOT level was found to be lowest in the T_3 group (Fig. 2). Decreased SGOT levels in the treatment fishes can signify the efficacy of the additives, which decreased the energy demand to cope with transportation stress. Also, the increased SGOT level goes in accord with decreased serum total protein level in the control group, indicating the increased occurrence of transamination and gluconeogenesis.

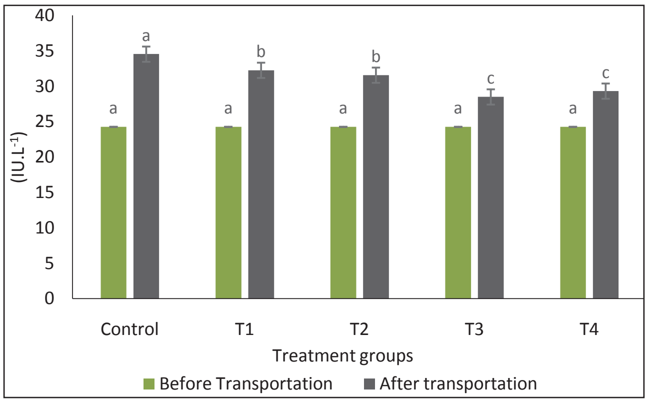

Fig. 2: SGOT level (before and after transportation) with addition of glucose + sodium chloride in combination. Data is represented in mean ± SE, p value ≤ 0.05 and various superscripts represent the significant levels

In the present experiment, a significantly ($p<0.05$) lowest percentage survivability was noticed in control groups after 12 h simulated transportation (Table 3). This observation goes in accordance with the findings of the previous parameters, where the highest ammonia level, lowest pH value, lowest serum total protein concentration, and highest SGOT level were seen in the control group fishes. The highest percentage survival of *L. rohita* was obtained in the T_3 (1 g/L of glucose+4 g/L of sodium chloride) and T_4 (2 g/L of glucose+4 g/L of sodium chloride) group. The probable mechanism of higher survivability may be due to the addition of additive such as glucose which acts as an extra source of energy supplementation and salt might have helped in decreasing ion loss by lessening the osmotic gradient difference from body fluid to an aquatic environment. This indicates the stress-relieving potential of these water additives in particular concentrations.

Table 3: Percentage survivability of *L. rohita* fingerlings after transportation (mixed with glucose + sodium chloride in ambient water). Data is represented in mean ± SE, p value ≤ 0.05 and various superscripts represent the significant levels

Treatment	Survival (%)
Control	$87^{c} \pm 2.15$
T_1	$91^{bc} \pm 2.18$
T_2	$95^{b} \pm 2.25$
T_3	$100^{a} \pm 0.00$
T_4	$100^{a} \pm 0.00$

CONCLUSION

Based on the previously mentioned observations, it can be concluded that the optimum dose of the particular water additive for ameliorating the transportation stress of *Labeo rohita* fingerlings is 1g/L glucose+ 4/L g sodium chloride. Further investigations are required to check the potency of these water additives in case of other Indian major carps, and other commercially important fishes.

ACKNOWLEDGMENTS

The authors are thankful to The Director and Vice-chancellor, ICAR-Central Institute of Fisheries Education (CIFE), Mumbai, and The Head of Department, Fish Physiology and Biochemistry Division, ICAR- CIFE, Mumbai for providing all possible resources to complete the research work successfully.

REFERENCES

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Becker, A.G., Parodi, T.V., Heldwein, C.G., Zeppenfeld, C.C., Heinzmann, B.M. and Baldisserotto, B. 2012. Transportation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. *Fish Physiol. Biochem*, **38**(3): 789-796.

Biswas, K.P. 1990. *A Text Book of Fish, Fisheries & Technology*. Narendra Publishing House.

Chatterjee, N., Pal, A.K., Das, T., Mohammed, M.S., Sarma, K., Venkateshwarlu, G. and Mukherjee, S.C. 2006. Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. *Aquac. Res.*, **37**(5): 472-476.

Das, P.C., Mishra, B., Pati, B.K. and Mishra, S.S. 2015. Critical water quality parameters affecting survival of *Labeo rohita* (Hamilton) fry during closed system transportation. *Indian J. Fish*, **62**(2): 39-42.

Dobšíková, R., Svobodová, Z., Blahová, J., Modrá, H. and Velíšek, J. 2006. Stress response to long distance transportation of common carp (*Cyprinus carpio* L.). *Acta Veterinaria Brno.*, **75**(3): 437-448.

FAO. 2018. The state of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. Rome. Licence, CC BY-NC-SA 3.0 IGO.

Hong, J., Chen, X., Liu, S., Fu, Z., Han, M., Wang, Y., Gu, Z. and Ma, Z. 2019. Impact of fish density on water quality and physiological response of golden pompano (*Trachinotus ovatus*) flingerlings during transportation. *Aquaculture*, **507**: 260-265.

Iversen, M., Eliassen, R.A. and Finstad, B. 2009. Potential benefit of clove oil sedation on animal welfare during salmon smolt, *Salmo salar* L. transport and transfer to sea. *Aquac. Res.,* **40**(2): 233-241.

Jhingran, V.G. and Pullin, R.S. 1985. *A hatchery manual for the common, Chinese, and Indian major carps* (No. 252). WorldFish.

Kamalam, B.S., Patiyal, R.S., Rajesh, M., Mir, J.I. and Singh, A.K. 2017. Prolonged transport of rainbow trout fingerlings in plastic bags: optimization of hauling conditions based on survival and water chemistry. *Aquaculture*, **480**:103-107.

Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P. and Richter-Levin, G. 2011. Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.*, **35**(5): 1291-1301.

Kutty, M. N. 1987. Transport of fish seed and brood fish, In: Delince, G. A., Campbell, D., Janssen, J. A. I. and Kutty, M. N. (Eds.), *Seed Production-Working paper for senior Aquaculturist Course at African Regional Aquaculture Centre,* Port Harcourt, Nigeria, No. 13, pp. 118.

Lim, L.C., Dhert, P. and Sorgeloos, P. 2003. Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquacult. Res.*, **34**(11): 923-935.

O'Brien, P.J., Slaughter, M.R., Swain, A., Birmingham, J.M., Greenhill, R.W., Elcock, F. and Bugelski, P.J. 2000. Repeated acetaminophen dosing in rats: adaptation of hepatic antioxidant system. *Hum. Exp. Toxicol.*, **19**(5): 277-283.

Oyoo-Okoth, E., Cherop, L., Ngugi, C.C., Chepkirui-Boit, V., Manguya-Lusega, D., Ani-Sabwa, J. and Charo-Karisa, H. 2011. Survival and physiological response of *Labeo victorianus* (Pisces: Cyprinidae, Boulenger 1901) juveniles to transport stress under a salinity gradient. *Aquaculture*, **319**(1-2): 226-231.

Parodi, T.V., Cunha, M.A., Becker, A.G., Zeppenfeld, C.C., Martins, D.I., Koakoski, G., Barcellos, L.G., Heinzmann, B.M. and Baldisserotto, B. 2014. Anesthetic activity of the essential oil of Aloysia triphylla and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, *Rhamdia quelen*. *Fish Physiol. Biochem.*, **40**(2): 323-334.

Polakof, S., Panserat, S., Soengas, J.L. and Moon, T.W. 2012. Glucose metabolism in fish: a review. *J. Comp. Physiol. B.*, **182**(8): 1015-1045.

Portz, D.E., Woodley, C.M. and Cech, J.J. 2006. Stress-associated impacts of short-term holding on fishes. *Rev. Fish. Biol. Fisher.*, **16**(2):125-170.

Randall, D.J. and Tsui, T.K.N. 2002. Ammonia toxicity in fish. *Mar. Pollut. Bull.,* **45**(1-12): 17-23.

Refaey, M.M. and Li, D. 2018. Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of channel catfish *Ictalurus punctatus*. *Front Physiol.*, **9**: 1628.

Sampaio, F.D. and Freire, C.A. 2016. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish Fish*, **17**(4): 1055-1072.

Schreck, C.B. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. *Gen. Comp. Endocrinol.*, **165**(3): 549-556.

Singh, R.K., Vartak, V.R., Balange, A.K. and Ghughuskar, M.M. 2004. Water quality management during transportation of fry of Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). *Aquaculture*, **235**: 297-302.

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**(2): 431-440.

Tort, L., 2011. Stress and immune modulation in fish. *Dev. Comp. Immunol.*, **35**(12): 1366-1375.

Wurts, W.A. 2003. Daily pH cycle and ammonia toxicity. *World Aquacult.*, **34**(2): 20-21.