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Evaluation of growth and biochemical responses of *Cyprinus carpio* reared in freshwater and inland saline water

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Original Article

Abstract

An experiment was conducted for 60 days to evaluate the growth performance and survival of *Cyprinus carpio* in inland saline water (ISW). One hundred twenty acclimatized fingerlings (average body weight- 5±0.5 g) were distributed between two treatments (FW-0.5 ppt and ISW-5ppt) in four replicates fed with control diet. The fishes reared in ISW showed significantly lower ($p<0.05$) weight gain percentage, and PER than the fishes reared in freshwater (FW). The metabolic enzyme activities (AST and ALT and glucose-6 phosphatase) in the liver was significantly higher ($p<0.05$) in the ISW group. These results revealed that the rearing of common carp in 5 ppt ISW results in significant reduction ($p<0.05$) in growth and increased metabolic stress. Even though there was a 10% reduction ($p<0.05$) in growth in common carp after rearing in ISW, the study reveals 100% survival of the fish. Thus, it can be inferred that common carp can be reared in inland saline water, which shows a prospective way to utilize the ISW for aquaculture.

Keywords*: Digestive enzymes, metabolic enzymes, oxidative stress enzymes*

Introduction

Inland saline aquaculture is a novel production system for the culture of fishes and shrimps. The ionic profile of inland saline water varies which is rich in sodium ions and deficient in potassium ions (Allan *et al.*, 2009). Potassium ion plays an important role in the physiology of aquatic animals (Shiau and Hsieh, 2001). Common carp (*Cyprinus carpio*) is the most preferred and delicious fish in south-east Asian countries and cultured throughout the world. It is a well-known example of a stenohaline freshwater fish and can tolerate low levels of salinity (Gupta and Hanke, 1982).

Many physiological changes occur when stenohaline freshwater fish is exposed to salt stress, those are depending on the salinity range, time of exposure to salinity changes and the effect of other ecological factors (Bailey *et al.*, 2002) which leads to increase energy demands in order to maintain the internal balance (Evans, 2002). The gills of teleost fish play an important role in ion regulation (Ay *et al.*, 1999; Weng *et al.*, 2002). The adaptation of *C. carpio* to saline water involves some functional changes in gill epithelium chloride cells and Na^+-K^+ ATPase activities. The adaptive capacity of Common carp to different salinities depends on the integrated osmoregulatory function of numerous organs, mainly the gills, digestive tract, and kidney (Cioni *et al.*,

1991). Changes in water salinity alters vital processes such as osmoregulation, rates of protein synthesis and oxygen uptake, the scope for growth, and extracellular acid-base balance (Guerin and Stickle, 1997; Whiteley *et al.*, 2001). Many studies showed the increased energy requirements and reduced growth in fresh water teleosts at higher salinity levels (De Boeck *et al.*, 2000; Luz *et al.*, 2008). The present study aimed to investigate the impact on growth and various enzyme activities of *C. carpio* reared in inland saline water.

Material and methods

Preparation and proximate composition of experimental diet

The experimental diet was formulated to contain 30% crude protein and 5% lipid level. Feed formulation was done using practical ingredients (defatted soybean meal, groundnut oil cake, wheat flour, vegetable oil, fish oil, vitamin-mineral mixture) in the laboratory. The additives like choline chloride, butylatedhydroxytoluene (BHT), carboxymethyl cellulose were used as attractant, anti-oxidant and binder respectively. The proximate composition of experimental diets (% dry matter basis) was carried out by following standard methods (AOAC, 1995).

Experimental design and acclimatisation

The experiment was carried out at ICAR-CIFE Rohtak Centre, Lahli, Haryana, India. The experimental fish, *C. carpio* (average weight 5±0.5 g) were procured from a private farm, located at Baniyani village, Rohtak, Haryana, India. The fishes were acclimatised and kept in 1000 l capacity FRP tanks. There were two experimental groups, i.e. fresh water and Inland saline water with four replicates in each followed completely randomized design (CRD). Thus, the fishes were acclimatised in two groups, one group in FW and other in 5 ppt ISW. Raw inland saline groundwater of 15 ppt pumped from borewell, was diluted with fresh water to 5 ppt and was used for rearing the fingerlings. They were maintained for ten days with continuous aeration and ad libitum feeding. After acclimatisation, the experiment was performed in 8 uniform size FRP tanks of each 200 l capacity. Out of these four tanks were filled with freshwater (~0.5 ppt), and rest of the tanks with inland saline water (5 ppt). Each tank was stocked with 15 nos. of *C. carpio* fingerlings and reared for 60 days.

Estimation of physico-chemical parameter of water

The hand held refractometer (ATAGO), digital refractometer (HANNA Instruments, Model HI 96822) was used to determine the salinity of water. The flame photometry (ESICO Micro-processor flame photometer, Model 1382) was used to estimate the Na^+ and K^+ ions levels in water. Calcium ionic concentration of the water was estimated by coplexometric titration following the standard methods (APHA, 2005) using murexenic acid as indicators. Magnesium ionic concentration of water was estimated by total hardness less calcium hardness multiplied by a factor 0.243.

Growth and nutrient utilisation parameters

Fish from each tank were collectively weighed at every 15 day interval and accordingly feeding rate was adjusted. Fishes were starved overnight before taking the weight and sampling. Growth performance and nutrient utilisation in *C. carpio* fingerlings were evaluated in terms of percent weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and percent survival based on the following standard formulae:

Weight gain % (WG) = 100 (final weight-initial weight)/initial weight

Specific growth rate (SGR) = 100 (ln final weight-ln initial weight)/ no. of days of feeding trial)

Feed conversion ratio (FCR) = Feed Intake/Weight Gain

Survival % = 100 (final no. of fish harvested/ initial no. of fish stocked)

Sample collection and processing

After 60 days of the experiment trial, three fishes were collected from each tank and anaesthetised with clove oil (50μl^{-1}). The different tissues, such as gill, liver, intestine and muscle, were dissected, and the weight was recorded accordigly. The tissues were homogenised in 0.25M chilled sucrose solution to prepare 5% homogenate using Teflon coated mechanical homogeniser REMI Equipment's, Mumbai, India. The homogenised samples were centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected and stored in the deep freezer (-20°C) for enzyme assay. The tissue protein was quantified by Lowry's method (Lowry, 1951) using bovine serum albumin (BSA) as the standard.

Digestive Enzyme assay

The intestine tissue homogenate was used for digestive enzymes assay. Amylase activity was measured using the method of Rick and Stegbauer (1974). Protease enzyme activity was estimated following the casein digestion method (Drapeau, 1974). Lipase

activity was determined by the titrimetric method (Cherry and Crandell, 1932) where the measurement of fatty acids released is done by the enzymatic hydrolysis of triglycerides present in a stabilised olive oil emulsion.

Metabolic Enzyme assay

AST and ALT activity was assayed from muscle and liver tissues following the method of Wooton (1964) using substrates 0.2M DL-aspartic acid and 0.2M DL-alanine in 0.05 M phosphate buffer, respectively. The lactate dehydrogenase (LDH) activity was analysed by the process of Wrobleiuski and Laude (1955) by observing oxidation of NADH at 340 nm (pH 7.5). The Glucose 6-Phosphatase activity in the tissues (liver) was assessed by the method of Marjorie (1964). ATPase activity was assayed in tissues by enzyme catalyzed liberation of 1 μmole of Pi per minute under the conditions specified. Pi was determined colorimetrically by the method of Fiske and Subbarao (1925).

Oxidative stress enzymes

Superoxide dismutase (SOD) was assessed based on the oxidation of epinephrine-adrenochrome transition by the enzyme (Mishra and Fridovich, 1972). Catalase assay was measured by observing the decomposition of H_2O_2 into oxygen and water (Takahara *et al.*, 1960). The decrease in absorbance was measured at 240 nm at 15-sec intervals for 3 min.

Statistical analysis

The statistical tests were done using SPSS software version 22.0, the means were compared by Independent-Samples T-Test at 95% confidence interval. Levene's test for equality of variances was used for testing the homogeneity of variances.

Results

Water quality parameters

The ionic composition of FW (0.5 ppt) was Na^+ (112±22 mgL^{-1}), K^+ (3.2±0.5 mgL^{-1}), Mg^+ (42.2±22 mgL^{-1}), Ca^+ (17.1±6.4mgL^{-1}), and that of ISW (5 ppt) was Na^+ (1351±32 mgL^{-1}), K^+ (5.7±0.4 mgL^{-1}), Mg^+ (289±28mgL^{-1}), Ca^+ (154±13 mgL^{-1}).

Proximate composition of the diet

The diet used in the experiment was formulated to contain 30% crude protein and 5% lipid for both the experimental groups. After proximate analysis of the diet, the level of crude protein, crude fat, nitrogen-free extract, crude fibre, and ash was found to be 30.81, 5.31, 49.47, 7.16, and 7.25% respectively. The calculated digestible energy of the feed was 368.91 Kcal/100g.

Growth and nutrient utilisation parameters

The weight gain percentage (WG) and specific growth rate (SGR) was found to be significantly higher ($P<0.05$) in FW group as compared to ISW group. Food conversion ratio (FCR) was found to be significantly lower in the FW group (Table 1, Fig. 1).

Table 1. Growth and nutrient utilisation parameters of *C. carpio* fingerlings reared in inland ground saline water

Treatments	WG%[1]	SGR[2]	FCR[3]
FW	106.15^b±1.04	1.20^b±0.01	2.21^a±0.03
ISW	97.20^a±0.95	1.13^a±0.01	2.41^b±0.03
P value	0.003	0.003	0.017

Data is expressed as Mean ± SE (n=3), different superscripts in the same column signify statistical differences ($P<0.05$). [1]Percentage Weight Gain, [2]Specific Growth Rate, [3]Feed Conversion Ratio

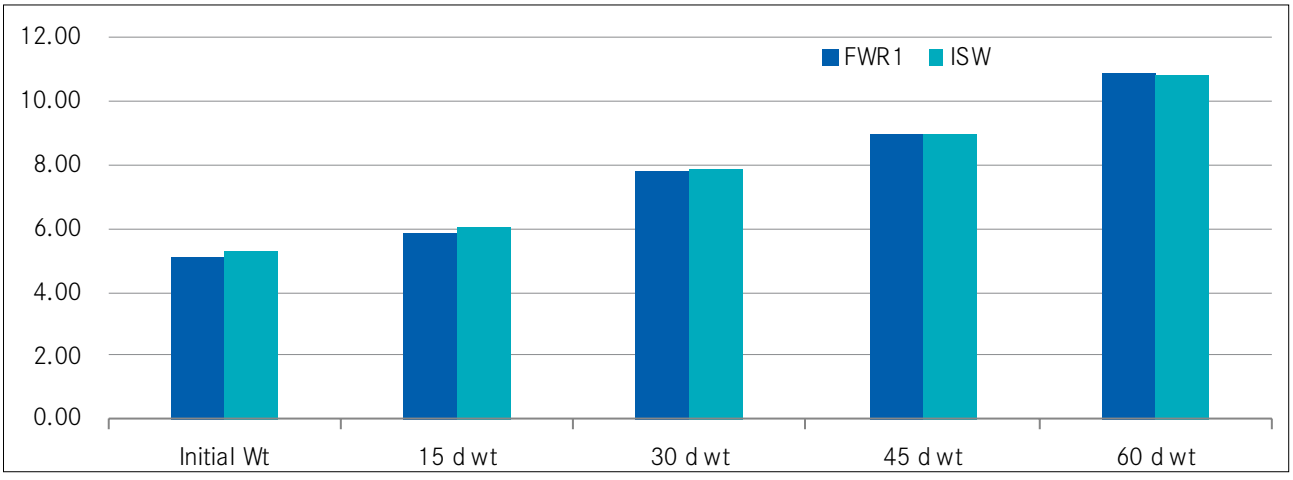

Fig. 1. Body weight gain (g) of the different experimental groups at 15 days interval during the experimental period of 60 days

Digestive enzyme activities

The digestive enzymes (amylase, protease, and lipase) activity of *C. Carpio* varied significantly between the groups and was found significantly higher ($P<0.05$) in the ISW group than the FW group (Table 2).

Metabolic enzymes

The amino-transferase enzymes (AST and ALT) activity of muscle and liver tissues varied significantly ($P<0.05$) between the experimental groups. The AST and ALT activity in liver and muscle of ISW reared fishes was significantly higher ($P<0.05$) than FW reared group. The liver and muscle LDH activity varied significantly ($P<0.05$) and was found to be higher in the ISW group. A significant difference ($P<0.05$) in the liver glucose 6-phosphatase activity was observed between the experimental groups, with significant higher activity in the ISW group (Table 2).

Oxidative Stress enzymes

The branchial and hepatic SOD activity varied significantly ($P<0.05$) between the experimental groups. The SOD activities of gills and liver were significantly higher ($P<0.05$) for the ISW group compared to the FW group. The branchial catalase and hepatic catalase activity varied significantly ($P<0.05$) between the experimental groups, with the ISW group bearing the significantly higher ($P<0.05$) activities (Table 2).

ATPAse activity

ATPase activity in the gills and liver of different experimental groups were measured, there was a significant difference ($P<0.05$) observed in the ATPase activity of gills whereas the liver ATPase activity did not vary significantly ($P<0.05$) between the treatment groups. ATPase activity of gill was higher in the ISW than in FW.

Discussion

In the present experiment, the effect of salinity on WG%, SGR, FCR and enzyme activities were studied in the experimental groups. The fishes reared in ISW showed lower WG%, SGR, and PER than the fishes reared in FW. The FCR of ISW group

Table 2. Digestive, metabolic and oxidative stress enzyme activities of *C. carpio* fingerlings reared in inland ground saline water

Enzymes	FW	ISW	p value
Amylase[1]	$0.22^{a}\pm0.02$	$0.27^{b}\pm0.01$	0.004
Protease[2]	$0.33^{a}\pm0.06$	$0.48^{b}\pm0.05$	0.034
Lipase[3]	$0.22^{a}\pm0.04$	$0.38^{b}\pm0.05$	0.048
AST (Muscle)[4]	$15.35^{a}\pm0.69$	$23.76^{b}\pm1.02$	0.002
AST (Liver)	$16.86^{a}\pm1.87$	$24.96^{b}\pm0.51$	0.014
ALT (Muscle)[5]	$15.79^{a}\pm2.39$	$24.39^{b}\pm1.66$	0.042
ALT (Liver)	$21.49^{a}\pm1.02$	$26.13^{b}\pm0.56$	0.017
LDH (Muscle)[6]	4.58 ± 0.65	6.24 ± 0.89	0.117
LDH (Liver)	$14.09^{a}\pm0.49$	$17.59^{b}\pm0.47$	0.035
G 6-phosphatase[7]	$2.64^{a}\pm0.59$	$6.39^{b}\pm1.10$	0.040
ATPase (Gill)[8]	$0.02^{a}\pm0.003$	$0.04^{b}\pm0.002$	0.015
ATPase (Liver)	0.02 ± 0.008	0.03 ± 0.003	0.110
SOD (Gill)[9]	$31.95^{a}\pm0.62$	$37.95^{b}\pm1.26$	0.013
SOD (Liver)	$38.84^{a}\pm0.92$	$60.02^{b}\pm.08$	0.001
Catalase (Gill)[10]	$3.49^{a}\pm0.27$	$6.15^{b}\pm0.75$	0.005
Catalase (Liver)	$23.83^{a}\pm2.60$	$39.09^{b}\pm3.58$	0.026

Data is expressed as Mean $\pm$ SE ($n=3$), different superscripts in the same row signify statistical differences ($P<0.05$).

[1]Amylase activity is expressed in micromole maltose released / min/mg protein at 37°C.

[2] Protease activity is expressed in millimoles of tyrosine released / min/mg protein at 37°C.

[3] Lipase activity is expressed in units/hour/mg protein at 37°C.

[4]AST (Aspartate aminotransferase), specific activities expressed as nano-moles of oxaloacetate released / min/mg protein at 37°C.

[5]ALT (Alanine aminotransferase), specific activities expressed as nano-moles of sodium pyruvate released / min/mg protein at 37°C.

[6] LDH (Lactate dehydrogenase), specific activities expressed as μM of pyruvate utilised / min/mg protein at 37°C.

[7]Gluose-6-phosphatase, specific activities expressed as nano-moles phosphorus release/min/mg protein.

[8]ATPase (adenosine triphosphatase), enzyme activity expressed in μg of phosphorus released/mg protein/minute at 37°C.

[9]SOD (superoxide dismutase): μmol/mg protein/min at 37°C.

[10] Catalase: mmol H_2O_2 decomposed/min/mg protein at 37°C.

was also higher than the FW group. These results conclude that the rearing of common carp in 5 ppt ISW results in a significant reduction in growth (approximately 10%) as well as feed conversion. The results are in concurrence with studies of Wang *et al.*, 1997; De Boeck *et al.*, 1999, who reported that the growth of *C. carpio* decreased with increase in salinity. When fish is exposed to saline conditions the energy requirement is increased to maintain the body ionic concentration. Therfore, increased salinity or ionic imbalance has a negative impact on growth performance (Likongwe *et al.*, 1996; Luz *et al.*, 2008).

The enzymes present in the digestive tract of fishes helps in breaking down of large nutrient molecules into small absorbable subunits (Cho, 1987). A significant increase in digestive enzyme activities of *C. carpio* fingerlings was observed in ISW reared fishes than FW. The higher amylase, protease and lipase enzyme activities of ISW group in this study suggests that digestive activities are activated at higher salinities (Li *et al.*, 2008). The enhanced digestive enzyme activity provides direct evidence that when ambient salinity deviates from the optimum physiological salinity, fishes derive extra energy from food to recompensate the energy lost for osmoregulation.

The transaminases or amino-transferases *viz.* AST and ALT are involved in the interconversion of amino acids by transferring amino groups. When the activity of these enzymes increases, there is more transfer of amino groups or the higher metabolic utilisation of amino acids in the body. In fishes the amino acids are utilised as the substrates for gluconeogenesis and for the fulfilment of energy (Demeal, 1978). The higher activity in the ISW group indicates the amino acids are mobilised to produce glucose for the production of glucose. Therefore, the higher activity of AST and ALT indicates the mobilisation of aspartate and alanine via gluconeogenesis for glucose production to cope with stress (Knox and Greengard, 1965; Chatterjee *et al.*, 2006). It is also reported that an elevated level of transaminase activity during stress would lead to increased feeding of ketoacids into TCA cycle, thereby affecting oxidative metabolism. In the present study, the observed increases in the activities of aspartate aminotransferase and alanine aminotransferase suggest that the observed proteolysis is intended to increase the role of proteins in energy production during salinity stress.

Lactate dehydrogenase enzyme catalyses the conversion of pyruvate to lactate and concomitant conversion of NADH to NAD^+. The NAD^+ released helps in maintaing the glycolysis cycle to supply energy during the inadequate supply of oxygen to the tissues. The pyruvate enters the Krebs cycle when there is enough oxygen in the cells and it is oxidised to produce energy (Murray *et al.*, 2000). There is no significant difference in the LDH activity in muscle, but the LDH activity in the liver of ISW group fishes have higher activity than the FW group, which is due to salinity stress. When the organisms are under stress the LDH activity is increased (Vijayaraghavan and Rao, 1986).

The glucose-6 phosphatase activity significantly increased in the liver of ISW group compare to the FW group. The enhanced glucose-6 phosphatase activity indicated the increased formation of free glucose in liver from glycogenolysis and gluconeogenesis pathway, both of which comprise glucose-6 phosphatase as a terminal enzyme. The increased glucose-6 -phosphatase of ISW in the present study indicates the enhanced production of glucose to meet increased energy demand during stress.

It was observed that the ATPase activity of the liver increased in the ISW reared fishes. Adenylphosphatases are the enzymes which helps in the decomposition of ATP to ADP and free phosphate or the reverse reaction (Post and Sen, 1967). These are integral membrane proteins present within the biological membranes and help in the transport of solutes or ions across the membrane. The total ATPase activity was inclusive of several atpase enzymes activities, incvolved in osmoregulation, energy metabolism and the atpase activity is used as a sentivitive biomarker to assess the membrane fragility of gills (Stagg *et al.*, 1992). The fish gill Na^+ K^+-ATPase, Mg^{2+}-ATPase and H^+-ATPase are involved in active electrolyte transport, oxidative phosphorylation, ion uptake, regulation of cell volume and membrane permeability (Sancho *et al.*, 2003; Gerenscer and Lee, 1983; Evans, 2002). Thus, the most likely explanation of the enhanced ATPase levels in ISW is there may be higher activation of membrane-bound channel ATPases due to the ionic alterations in ISW.

The antioxidant enzyme systems (superoxide dismutase, catalase and glutathione peroxidase) are the body's endogenous defence mechanisms which protect the cell against free radicals and reactive oxygen species (ROS). The non-enzymatic antioxidants such as vitamin E, vitamin C, etc. are also useful in protecting the cell from oxidative damage (Birben *et al.*, 2012). The cells are affected by oxidative stress when there are pro-oxidants and the ROS are inadequately removed (Sies *et al.*, 1986). In our study, increase in superoxide dismutase and catalase were observed in both gill and liver of ISW reared fishes, i.e. there was a net effect of inland saline water on the activities of antioxidant enzymes The increased salinity of water induced stress in the fishes with the production of free radicals and ROS due to which the activity of antioxidant enzyms got enhanced to protect the oxidative damage of cells (Liu *et al.*, 2007).

The rearing of *C.carpio* in inland saline water caused a reduction in growth and increased metabolic enzyme activities to meet the energy demand for osmoregulating in the ion imbalanced

environment and for routine body maintenance. Although there is nearly 10% reduction in growth in *C. carpio* reared in ISW, the utilisation of these vast water resources for fish culture could generate income and employment for the people. It would also help in the prevention of secondary salinisation and produce quality protein for the growing population. Hence, it can be concluded that even though reduced growth and higher FCR can be seen in Common carp in ISW, there is a scope of rearing this fish species in ISW in the light of their undisturbed survival in ISW. The study also reveals further scope of research using high energy diets in ISW compared to FW, as these diets compensate for the energy loss and result in similar growth response in the species

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References

Allan, G. L., D. S. Fielder, K. M. Fitzsimmons, S. L. Applebaum and S. Raizada. 2009. Inland saline aquaculture. In: New technologies in aquaculture. Woodhead publishing, p. 1119-1147.

Ay, O., M. Kalay, L. Tamer and M. Canli. 1999. Copper and lead accumulation in tissues of a freshwater fish Tilapia zillii and its effects on the branchial Na, K-ATPase activity. *Bull. Environ. Contamin. Toxicol.*, 62(2): 160-168.

AOAC. 1995. Official methods of analysis of the association of official analytical chemists. In: Cunniff P (ed.) Association of official analytical chemists, 16th edn. Arlington, USA.

APHA. 2005. Standard Methods of Water and Wastewater. 21st Edn., American Public Health Association, Washington, DC., ISBN: 0875530478, p. 2-61.

Bailey, P., P. Boon and K. Morris. 2002. Australian biodiversity salt sensitivity database. A report prepared for Land and Water Australia, Monash University and Victorian University of Technology, Melbourne, 75 pp.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum and O. Kalayci. 2012. Oxidative stress and antioxidant defense. *World Allergy Organ. J.,* 5(1): 9-15.

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

Cherry, I. S. and L. A. Crandall. 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *Am. J. Physiol.,* -Legacy Content, 100(2): 266-273.

Cho, C. Y. 1987. La energıa en la nutricion de los peces. In: Nutricion en Acuicultura, Espinosa de los Monteros (Labarta, J. U. ed.), Vol. II: 197-244. CAICYT, Madrid, Spain.

Cioni, C., D. Merich, E. Cataldi and S. Cataudella. 1991. Fine structure of chloride cells in freshwater-and seawater-adapted Oreochromis niloticus (Linnaeus) and Oreochromis mossambicus (Peters). *J. Fish Biol.*, 39(2): 197-209.

De Boeck, G., A. Vlaeminck, A. Van der Linden and R. Blust. 2000. The energy metabolism of common carp (*Cyprinus carpio*) when exposed to salt stress: an increase in energy expenditure or effects of starvation? *Physiol. Biochem. Zool.,* 73(1): 102-111.

Demeal, N. A. 1978. Some characteristics of carbohydrate metabolism in fish. Oceanis, DOC. *Oceanography*, 4: 35-365.

Drapeau, G. R. 1976. Protease from Staphylococcus aureus. *Methods in Enzymology*, 45: 469-475.

Evans, D. H., 2002. Cell signaling and ion transport across the fish gill epithelium. *J. Exp. Zool.*, Part A: Ecological Genetics and Physiology, 293(3): 336-347.

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.*, 66(2): 375-400.

Gerencser, G. A. and S. H. Lee. 1983. Cl—stimulated adenosine triphosphatase: Existence, location, and function. *J. Exp. Biol.*, 106(1): 143-161.

Guerin, J. L. and W. B. Stickle. 1997. Effect of salinity on survival and bioenergetics of juvenile lesser blue crabs, *Callinectes similis*. *Mar. Biol.*, 129(1): 63-69.

Gupta, O. P. and W. Hanke. 1982. The effects of osmotic stressors on the stenohaline carp (*Cyprinus carpio*). *Comp. Biochem. Physiol. Part A: Physiology*, 71(2):165-173.

Knox, W. E. and O. Greengard. 1965. The regulation of some enzymes of nitrogen metabolism—an introduction to enzyme physiology. Advances in enzyme regulation, 3: 247-313.

Li, B., F. Lu, X. Wei and R. Zhao. 2008. Fucoidan: structure and bioactivity. *Molecules*, 13(8): 1671-1695

Likongwe, J. S., T. D. Stecko, J. R. Stauffer Jr, and R. F. Carline. 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). *Aquaculture*, 146(1-2): 37-46.

Liu, Y., W. N. Wang, A. L. Wang, J. M. Wang and R. Y. Sun. 2007. Effects of dietary vitamin E supplementation on antioxidant enzyme activities in *Litopenaeus vannamei* (Boone, 1931) exposed to acute salinity changes. *Aquaculture*, 265(1-4): 351-358.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193(1): 265-275.

Luz, R. K., R. M. Martínez-Álvarez, N. De Pedro and M. J. Delgado. 2008. Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture*, 276(1-4): 171-178.

Marjorie, A. S. 1964. Glucose-6-phosphatase in the liver. *Methods in enzymology*, 2, 541 pp.

Misra, H. P. and I. Fridovich. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247(10): 3170-3175.

Murray, R. K., D. K. Granner, P. A. Mayes and V. W. Rodwell. 2000. Glycogen metabolism. In: Harper's Biochemistry, 25th edition: p. 199-207.

Post, R. L. and A. K. Sen. 1967. Sodium and potassium-stimulated ATPase. In: Methods in Enzymology., Vol. X. Oxidation and phosphorylation (ed. R. W. Estabrook and M. E. Pullman): 762-768. New York and London: Academic Press, p. 762-768.

Rick, W. and H. P. Stegbauer. 1974. –Amylase measurement of reducing groups. Methods of enzymatic analysis: p. 885-889.

Sancho, E., C. Fernandez Vega, M. D. Ferrando and E. Andreu Moliner. 2003. Eel ATPase activity as a biomarker of thiobencarb exposure. *Ecotoxicology and environmental safety,* 56(3): 434-441.

Shiau, S. Y. and J. F. Hsieh. 2001. Quantifying the dietary potassium requirement of juvenile hybrid tilapia (*Oreochromis niloticus*× O. aureus). *Br. J. Nutr.*, 85 (2): 213-218.

Sies, H. 1986. Biochemistry of oxidative stress. Angewandte Chemie International Edition in English, 25(12): 1058-1071.

Stagg, R. M., J. Rusin and F. Brown. 1992. Na^+, K^+-ATPase activity in the gills of the flounder (*Platichthy sflesus*) in relation to mercury contamination in the Firth of Forth. *Mar. Environ. Res.*, 33(4): 255-266.

Takahara, S., H. B. Hamilton, J. V. Neel, T. Y. Kobara, Y. Ogura and E. T. Nishimura. 1960. Hypocatalasemia: a new genetic carrier state. *J. Clinical Invest.*, 39(4): 610-619.

Vijayaraghavan, S. and J. V. R. Rao. 1986. Starvational stress effects on tissue lactate and lactate-dehydrogenase activity in *Anabas scandens* (Cuvier). *Comp. Physiol. Ecol.*, 11(4): 233-236.

Wang, J. Q., H. Lui, H. Po and L. Fan. 1997. Influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. *Aquaculture*, 148(2-3): 115-124.

Weng, C. F., C. C. Chiang, H. Y. Gong, M. H. C. Chen, C. J. F. Lin, W. T. Huang, C. Y. Cheng, P. P. Hwang and J. L. Wu. 2002. Acute changes in gill Na^+-K^+-ATPase and creatine kinase in response to salinity changes in the euryhaline teleost, tilapia (Oreochromis mossambicus). *Physiol. Biochem. Zool.*, 75(1):29-36.

Whiteley, N. M., J. L. Scott, S. J. Breeze and L. McCann. 2001. Effects of water salinity on acid-base balance in decapod crustaceans. *J. Exp. Biol.*, 204 (5):1003-1011.

Wooten, I. D. P. 1964. Microanalysis. In: Medical Biochemistry. 4th edition, J. and A. Churchill, London: p. 101-107.

Wroblewski, F. and J. S. Ladue. 1955. Lactic dehydrogenase activity in blood. *Exp. Biol. Med.*, 90(1): 210-213.