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RESEARCH PAPER

Effect of Stress Conditions on Body Composition Parameters of Farmed Rohu (Labeo rohita)

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Abstract

Various stressors affect the body composition of fish. The current study was undertaken to investigate the effect of stress conditions on the body composition of farmed *Labeo rohita*. Sixty fingerlings were subjected to experimentation in aquaria after being acclimatized. The specimens were divided into control, starvation stress and double stress (pH 8 and starvation) groups with 20 individuals in each group. Fish samples for the estimation of body composition were taken after 12 days, 24 days, 36 days and 48 days. Standard procedures and protocols were used for analysis. There was a trend of gradual increase in ash contents (% dry body weight) in starvation and double stress group with increase in number of days. However, fat contents (% dry body weight) considerably decreased and protein contents remained unchanged. The inter-comparison of three groups showed that there was significant effect of starvation and double stress on water contents (%) after 36 days; on ash contents (% dry body weight) after 12 days, 24 days, 36 days and 48 days; on fat content (% dry body weight) after 12 days and 24 days; and on protein contents (% dry body weight) after 24 days. The lipids were the major constituents of *Labeo rohita* that responded to starvation being main energy source for fish.

Keywords: Stress response, starvation, pH, body composition, *Labeo rohita*.

Introduction

The physiological system of a fish can be stressed by a wide range of biological, chemical and physical factors. When fish is exposed to environmental stressors, a hierarchy of responses is initiated. If stress is severe or long lasting, successively higher levels of biological organs are affected (Jobling, 1995). Severe stressors lead to decreased growth, increased metabolic exhaustion, disease incidence and possible mortality (Barton and Iwama, 1991; Jobling, 1995). Additionally, starvation stress has been reported to reduce oxygen consumption (Yengkokpalm *et al*. 2008), and growth rate (Hussain and Shah, 2016); and density stress during transportation affects the functioning of liver and kidneys and modulates metabolic activities in *Labeo rohita* (Pakhira, Nagesh, Abraham, Dash, & Behera, 2015).

The body components of fish include water, protein, lipid, much smaller amounts of carbohydrates and minerals, frequently termed as ash (Weatherley & Gill, 1987). The analysis of proximate body composition helps in assessing the nutritional status of fishes (Shackley, Talbot, Cowan, & Watt, 1994; Dempson, Schwarz, Shears, and Furey 2004). Many fish undergo period of starvation e.g. during wintering, spawning, migration or when local food abundance diminishes. There are several different types of responses reflected by variations in body composition parameters of starved fish species. Stored lipids especially triglycerides constitute the main energy source for maintenances of normal activity of fish during starvation (Shul'man, 1974). Some fishes utilize muscle protein as a major energy source (Stimpson, 1965; Butler, 1968), while others appear to conserve body proteins at the expense of lipid or glycogen stores (Love, 1980; Weatherley & Gill, 1987). For example, *Esox lucius* utilizes lipid and glycogen in early starvation (Ince and Thorpe, 1976) while there is a reduction in cell size of liver in *Cyprinus carpio* (Bastrop, Spangenberg and Jürss, 1991) and mobilization of liver tissue as initial response and muscle tissue in long term response of starvation in *Macquaria ambigua* (Collins & Anderson, 1997).

Increasing industrial and urban emission of the oxides has contributed considerably in water acidification. Thereby, studies on the effect of pH on fish growth have become important (Wootton, 1990).

The main effect of low pH on the fish seems to be an inhibition of oxygen uptake at the gills (Love, 1980), whereas high pH may cause higher levels of unionized ammonia resulting in increased toxicity (Popma & Lovshin, 1995).

Therefore, studies on the effects of various stressors like starvation and pH are important to better understand the biology and growth of fish. *Labeo rohita* (Rohu) is one of the major carps of Indo-Pak sub-continent being an inhabitant of natural freshwaters (Talwar & Jhingran, 1991). The aim of this study was to investigate the effects of stress responses on body composition parameters of farmed *Labeo rohita* at early stages of growth.

Materials and Methods

Sixty specimens (fingerlings) of farmed rohu (*Labeo rohita*) were utilized for the experiment. The fishes were acclimatized to laboratory conditions in glass aquaria (36"×12"×5") for duration of 20 days. Meanwhile they were regularly fed on commercial fish diet. The experiment was conducted for a period of 48 days. The fish were divided into three groups i.e. control, starvation and double stress (pH 8 and starvation), each consisting of 20 individuals. The control group was continuously fed throughout the experimental period. The starvation group was kept under starvation stress at neutral pH. The double stress group was kept under starvation stress at pH 8. The pH was maintained at 8 by using lime water (calcium hydroxide) and monitored from time to time with the help of pH meter (3071, Jenway). The aquaria were regularly cleaned after every four days by partial replacement of water.

The experiment was conducted for a period of 48 days. Fish specimens were sampled after every 12 days for estimation of body composition parameters. Each individual fish was weighed on an electronic digital balance (MP 3000 – Ohyo, Japan) to the nearest 0.01 g. For estimation of water contents, pre-weighed fish specimen was placed in a pre-weighed aluminium foil tray for drying in an electronic oven (Memmert U-25) at 70°C for several days till constant weight. The weight of obtained dry body mass was subtracted from initial wet body weight to determine the total amount of water evaporated during drying process and described as percent water using the formula; Percent water = (Total amount of water/Wet body weight) x 100

For further analysis, each dry carcass was crushed, powdered, homogenized and preserved in glass bottles. For estimation of ash content, 50 mg of homogenized sample was taken in pre-weighed heat resistant china clay crucibles and put in a Muffle furnace (RMJ-1000-CHINA) for 15 hours at 550°C, cooled in desiccator, reweighed on an analytical balance (IBRORA EG 220 SHIMADZU) to the nearest 0.0001 g to calculate ash content and described as percent ash (dry body weight) by using formula; Percent ash (dry body weight) = (Total amount of ash in fish/Dry body weight) x 100

For estimation of lipid, dry extraction method developed by Bligh and Dyer (1959) and later modified by Salam and Davies (1994) was adopted. For this purpose 20 mg of homogenized sample was mixed in 10 ml of chloroform and methanol mixture (1:2 v/v), stirred well, kept overnight and then centrifuged. The supernatant was removed into pre-weighed small glass bottles and placed in an oven at 70°C to evaporate the solvent. The lipids present in glass bottle were weighed and total lipids in dry body mass were determined and described as % fat (dry body weight) using the formula; Percent fat (dry body weight) = (Total weight of fat/Dry body weight) x 100

Protein contents were determined by subtracting mass of other main constituents like ash and fat from dry body mass following Salam and Davies (1994) and described as % protein (dry body weight) using the formula; Percent protein (dry body weight) = (Total protein content/Dry body weight) x 100

Statistical Analysis

The data obtained was subjected to one-way ANOVA for statistical analysis using statistical software Minitab. The data are presented as Mean ± Standard Deviation (SD). The significance level was set at $P<0.05$.

Results

There was insignificant effect ($P>0.05$) of starvation on water contents (%) of starvation group; however, the double stress group (pH and starvation) showed a significant decrease ($P<0.05$; df=3,5; F=7.2) in water contents (%). The inter-comparison of control, starvation and double stress groups suggested that there was insignificant effect of starvation and double stress on water contents (%) after 12 days, 24 days and 48 days, however, significant effect ($P<0.01$; df=2,5; F=11.78) was observed after 36 days (Table 1).

There was insignificant effect of starvation on dry body mass (%) of starvation group; however, the double stress group (pH and starvation) showed a significant decrease ($P<0.05$; df=3,5; F=7.2) in dry body mass (%). The inter-comparison of control, starvation and double stress groups suggested that there was insignificant effect of starvation and double stress on dry body mass (%) after 12 days, 24 days and 48 days, however, significant effect ($P<0.01$, df=2, 5; F=11.78) was observed after 36 days (Table 1).

There was insignificant effect of starvation on ash contents (%) of starvation group. However, the double stress group (pH and starvation) showed a significant increase ($P<0.05$; df=3,5; F=6.99) in ash contents (%). In both starvation and double stress

group, there was a trend of increase in ash contents (%) with increase in number of days (Table 1). There was an increase of 12.9% in ash contents (%) of starvation group after 48 days compared to 12 days. And an increase of 32.9% in double stress group after 48 days compared to 12 days (Table 1). The inter-comparison of control, starvation and double stress groups showed that there was significant effect of starvation and double stress on ash contents (%) after 12 days ($P<0.05$; df =2,6; $F=7.89$), 24 days ($P<0.01$, df=2,6; $F=17.64$), 36 days ($P<0.01$; df=2,5; $F=11.63$) and 48 days ($P<0.05$; df=2,8; $F=4.45$) (Table 1).

There was insignificant effect of starvation and double stress on fat contents (%) for dry body weight (Table 1). However, there was trend of considerable decrease in fat contents (%) in all the three groups i.e. control, starvation and double stress. There was a decrease of 29.3% in control group, 53.5% in starvation group and 41.3% in double stress group after 48 days compared to 12 days. In control, there was a sharp decrease observed after 24 days compared to 12 days, however, in starvation and double stress group, decrease in fat contents was steady and uniform. The inter-comparison of control, starvation and double stress groups suggested that there was a significant effect of starvation and double stress on fat contents (%) after 12 days ($P<0.05$; df=2,6; $F=6.45$) and 24 ($P<0.05$; df=2,6; $F=5.15$) days, however, after 36 days and 48 days it was insignificant (Table 1).

There was an increase in protein content from 12 days to 24 days in control group, and then contents again decreased. However, in starvation group, there was a gradual increase in protein content, while in double stress group, there was a slight decrease in protein content (%). The inter-comparison of control, starvation and double stress groups suggested that there was no significant effect of starvation and double stress on protein contents (%) after 12 days, 36 days and 48 days, while a significant effect ($P<0.05$; df=2,6; $F=6.10$) was observed after 24 days (Table 1). However, these changes were insignificant ($P>0.05$). A significant effect ($P<0.05$; dF=3,5; $F=10.46$) was observed for double stress group for wet body weight (data not shown).

Discussion

During starvation stress and double stress, fish lost its body weight as demonstrated by gradual decrease in dry body mass (%) between 24 to 36 days (Table 1). The weight loss in *Labeo rohita* could be due to utilization of fats as results of present study demonstrated decrease in fat reserve of *Labeo rohita*. Decrease in body mass (%) seems to be compensated by water intake in the present study as significant decrease in body mass (%) after 36 days had resulted in significant increase in water contents (Table 1). This is consistent with previous studies which indicated that the quantities of body fat and protein are significantly reduced in the starved fish Moyle & Cech, 1996). The body composition analysis of *Labeo rohita* revealed that quantity of fat decreased progressively as the number of days of starvation increased (Table 1). The stored lipids especially triglycerides constitute the main energy source for maintenance of normal activity of fish during over wintering starvation. The lipid losses may amount to 40-50% decrease in the pre-starvation weight of the fish (Shul'man, 1974). The period of food deprivation imposed on juvenile Atlantic salmon in winter significantly reduced fat level in comparison to control fish (Bull & Metcalfe, 1997).

In addition to fat contents, proteins have also been reported to be utilized in starved fish. Starvation in *Channa punctatus* leads to the depletion of protein from skeletal muscle and liver (Ayub & Cheema, 1985). Protein may be increasingly utilized with the progress of starvation in *Oreochromis rendalli* (Caulton & Bursell, 1977). However, in present study, there was no definite trend of decrease in protein content in *Labeo rohita* (Table 1). Similar trend was also observed in grass carp (% dry body mass) in which no change in protein content was observed (Ali, Salam & Ali, 2001). The ash contents increased with starvation in *Labeo rohita* in present study. There was an increase in ash contents in starved and double stressed fish compared to control group (Table 1). Many researchers showed that ash contents increased during starvation (Herrera & Munoz 1957; Phillips, Livingston & Dumas, 1960; Salam, Ali & Iqbal, 2000b). Previously, it has been observed that the response of fish body constituents i.e. proteins, fats and glycogen, to starvation seems to be species specific (Ince & Thorpe, 1976; Mehner & Wieser, 1994). The results of our study on effects of starvation on body composition of *Labeo rohita* also verify this observation.

The mobilization of endogenous energy resources during starvation is an apparently sequential process in fish (Balck & Love, 1986; Collin & Anderson, 1997). In the present study fats seems to be the initial reserves used by the fish during starvation up to 24 days (Table 1), thus demonstrating a significant decrease. Several studies report that lipids are the first reserves to be mobilized when food becomes scarce in many fish species (Jezierska, Hazel & Gerking, 1982; Satoh, Takeuchi & Watanabe, 1984; Van Dijk, Hardewig, & Hölker, 2005). It has been demonstrated that in different fish species lipids are broken down early in the starvation like in *Cyprinus carpio* (Nagai and Ikeda, 1971), rainbow trout (Jezierska *et al*., 1982), *Esox lucius* (Ince & Thorpe, 1976) and *Oreochromis niloticus* (Satoh *et al*., 1984). Our study results confirm such observation as data indicates that fats were continuously decreased i.e. utilized in starvation up to 48 days in *Labeo rohita*. On the other hand, some fish utilized muscle protein as a major energy source. For example, American eel utilized structural muscle protein and maintained

Table 1. Comparison of body composition parameters within and between control, starvation and double stress groups after 12 days, 24 days, 36 days and 48 days

Variables	Treatment	12 days	24 days	36 days	48 days	≠P value
Water	Control	82.74±0.91	83.75±3.57	80.82±1.28	81.90±1.62	0.32
	Starvation	81.80±0.93	81.02±1.47	83.10±0.21	83.07±0.24	0.09
	Double stress	82.24±0.36	81.53±1.13	85.43±1.45	84.16±0.43	0.02*
	£P value	0.40	0.37	0.01**	0.33	
Dry body mass	Control	17.25±0.91	16.24±3.57	19.17±1.28	18.10±1.62	0.32
	Starvation	18.19±0.93	18.98±1.47	16.89±0.21	16.93±0.24	0.09
	Double stress	17.75±0.36	18.46±1.13	14.56±1.5	15.84±0.43	0.02*
	£P value	0.40	0.37	0.01**	0.33	
Ash	Control	26.76±1.94	28.52±0.72	25.80±4.02	31.36±3.478	0.06
	Starvation	31.45±2.15	28.96±2.69	35.24±3.81	35.53±3.38	0.10
	Double stress	31.23±4.06	35.55±0.47	40.71±0.88	41.53±0.97	0.03*
	£P value	0.02*	0.003**	0.01**	0.05*	
Fat	Control	33.72±0.59	21.18±1.77	23.68±2.47	23.81±6.29	0.11
	Starvation	31.90±4.42	26.21±4.64	21.56±5.03	14.82±6.29	0.12
	Double stress	20.23±5.27	17.47±2.98	13.76±1.45	11.87±1.23	0.27
	£P value	0.03*	0.05*	0.30	0.21	
Protein	Control	39.89±4.78	50.28±1.05	46.07±2.47	44.80±3.79	0.34
	Starvation	36.62±1.19	44.80±1.95	40.38±8.16	49.64±10.72	0.27
	Double stress	48.51±3.64	46.93±2.51	45.47±0.51	45.58±1.04	0.70
	£P value	0.06	0.03*	0.79	0.52	

£– P values for one way ANOVA for comparison of body composition values between groups; ≠–P values for one way ANOVA for comparison of body composition values between days within groups.
*P <0.05; **P <0.01

glycogen and lipid reserve. Similar is the case in European eel, however, it utilized muscle proteins at much later stages in starvation (Butler, 1968). In our study, after 12 days, protein did not seem to be utilized, however, the decrease in %age contents of proteins after 48 days compared to 12 days indicate that proteins were also utilized in starvation in *Labeo rohita*. Therefore, the inter relationship of various body constituents during starvation are very complex which make it difficult to define a predominant metabolic strategy employed by various species during starvation (Weatherley & Gill, 1987). In *Labeo rohita*, it is evident that fats were utilized at early stage as well as at later stage during starvation, while proteins were utilized to a smaller extent and only at later stage during starvation.

During starvation; body weight is maintained by water uptake to compensate the fat/protein losses (Love, 1980; Weatherley & Gill, 1987; Moyle & Cech, 1996). Several researchers have demonstrated a rise in water contents in different fish species during starvation (Miglavs & Jobling, 1989; Craig, Smiley & Babaluk, 1989; Salam & Davies, 1994; Salam, Ali & Ali, 2000a; Ali, Salam & Masud, 2003). An inverse relation between lipids and water contents occur due to replacement of catabolised lipids by an equal volume of water (Sargent, Henderson & Tocher 1989). The similar trend was shown by the present study in which, there was an inverse relationship between water and lipid contents in starvation group ($r=-0.72$, $P<0.01$) as well as in double stress group ($r=-0.77$, $P<0.01$). An inverse relationship between protein content and water contents during starvation in fish species has also been reported earlier (Salam & Davies, 1994).

When we compare values of dry body mass and lipid contents of starved and double stressed groups with control group, maximum decrease in dry body mass and lipid contents was observed in double stressed group. This observation supports the idea that pH stress also had detrimental effects on the body composition of fish. Fish ponds with large carbonate alkalinity may result in relatively higher levels of un-ionized ammonia, which is toxic (Krom, Porter & Gordnin, 1985). Generally at pH 7, only less than 1% of the total ammonia is in the toxic un-ionized form, at pH 8 about 5% to 9%, at pH 9 about 30% to 50%, while at pH 10 is about 80% to 90%. The first mortalities from prolonged exposure to toxic ammonia begin at concentration as low as 0.2 mg/L and this un-ionized form of ammonia begin to depress appetite of tilapia at concentration as low as 0.08 mg/L (Popma & Lovshin, 1995).

Many fish species try to maintain body composition, probably by employing several types of biochemical and physiological strategies during starvation, which is an adaptation to seasonal periods of fasting that fish may face as a part of their natural life cycle (Weatherley & Gill, 1987). Body composition of *Labeo rohita* was different in starved and double stressed groups. The starved and double stressed fish showed a considerable decrease in fat contents. However this decrease was greater in double stressed group than that of starved fish. Whereas, protein contents increased in starvation group and decreased in double stress group. More lipids decreased as compared to the proteins which reflect that proteins are metabolized to a lesser extent.

Therefore, we conclude that fat reserves were the major constituents in *Labeo rohita* to compensate the effects of starvation.

References

Ali, M., Salam, A., & Ali, Z. (2001). Dynamics of body composition, in relation to various starvation regimes of Chinese Grass carp, *Ctenopharynqodon idella* (Val.). *Pakistan Journal of Zoology*, 33(1), 47-51.

Ali, M., Salam, A., & Masud, S. (2003). Effect of stress induced by fasting on specific growth rate, protein growth rate and condition factor of Thaila, *Catla catla*. *Pakistan Journal of Zoology*, 18, 13-21.

Ayub, M., & Cheema, I. (1985). Effects of starvation in a fresh water teleost, *Channa punctatus:* some biochemical aspects. *Pakistan Journal of Zoology*, 17(1), 1-9.

Balck, D., & Love, R.M. (1986). The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *Journal of Comparative Physiology*, 156(4), 469-479.

Barton, B.A., & Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of carticosteriods. *Annual Review of Fish Diseases*, 1, 3-26. doi:10.1016/0959-8030(91)90019-G

Bastrop, R., Spangenberg, R., & Jürss, K. (1991). Biochemical adaptations of juvinile (*Cyprinus carpio* L.) to food deprivation. *Comparative Biochemistry and Physiology*, Part A 98(1), 143-149. doi: 10.1016/0300-9629(91)90592-Z

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification: *Canadian Journal of Biochemistry and Physiology*, 37(8), 911-917. doi: 10.1139/o59-099

Bull, C.D., & Metcalfe, N.B. (1997). Regulation of hyperphagia in response to varying energy deficit in overwintering juvenile Atlantic salmon. *Journal of Fish Biology*, 50(3), 498-510. doi: 10.1111/j.1095-8649.1997.tb01945.x

Butler, D.G. (1968). Hormonal control gluconeogenesis in the Northern American eel (*Anguilla rostrata*). *General and Comparative Endocrinology*, 10(1), 85-91. doi: 10.1016/0016-6480(68)90012-9

Caulton, M.S., & Bursell, E. (1977). The relationship between changes in condition factor and body composition in young *Tilapia rendalli* Boulenger. *Journal of Fish Biology*, *11*(2), 143-150. doi: 10.1111/j.1095-8649.1977.tb04107.x

Collins, A.L., & Anderson, T.A. (1997). The influence of change in food availability on the activities of key degradative and metabolic enzymes in the liver and epaxial muscles of the golden perch. *Journal of Fish Biology*, *50*(6), 1158-1165. doi: 10.1111/j.1095-8649.1997.tb01644.x

Craig, J.F., Smiley K., & Babaluk, J.A. (1989). Changes in the body composition with age of Goldeye *Hiodon alosoides*. *Canadian Journal of Fisheries and Aquatic Sciences*, 46(5), 853-857. doi: 10.1139/f89-107

Dempson, J. B., Schwarz, C. J., Shears, M., & Furey, G. (2004). Comparative body composition of atlantic salmon with emphasis on parr from fluvial and lacustrine habitats. *Journal of Fish Biology*, 64(5), 1257-1271. doi: 10.1111/j.0022-1112.2004.00389.x

Herrera, J., & Munoz, F. (1957). Biological considration of the sardine *Sardina pilchardus walb* from Castellon. *Investigation Pesquera*, 7, 33-48.

Hussain, M. Z. & Shah, S. Q. A. (2016). Effect of stress conditions on condition factors and growth of farmed rohu (*Labeo rohita*). *Journal of Entomology and Zoology Studies*, 4(5), 258-261.

Ince, B. W., & Thorpe, A. (1976). The effects of starvation and force feeding on the metabolism of the Northern pike, *Esox lucius L*. *Journal of Fish Biology*, *8*(1), 79-88. doi: 10.1111/j.1095-8649.1976.tb03909.x

Jezierska, B., Hazel, J. R., & Gerking, S. D. (1982). Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention of fatty acids. *Journal of Fish Biology*, 21(6), 681-692. doi: 10.1111/j.1095-8649.1982.tb02872.x

Jobling, M. (1995). *Environmental Biology of Fishes*. London: Chapman and Hall.

Krom, M. D., Porter, C., & Gordnin, H. (1985). Causes of fish mortality in semi intensively operated seaweed ponds in Eilat, Israel. *Aquaculture*, 49, 159-177. doi: 10.1016/0044-8486(85)90122-X

Love, R. M. (1980). The Chemical Biology of Fishes, Vol. II. London: Academic Press, 943 pp.

Mehner, T., & Wieser, W. (1994). Energetics and metabolic correlates of starvation in juvenile perch (*Perca fluviatilis*). *Journal of Fish Biology*, 45(2), 325-33. doi: 10.1111/j.1095-8649.1994.tb01311.x

Miglavs, I., & Jobling, M. (1989). The effects of feeding regime on proximate body composition and patterns of energy deposition in juvenile Arctic charr, *Salvelinus alpinius*. *Journal of Fish Biology*, *35*(1), 1-11. doi: 10.1111/j.1095-8649.1989.tb03387.x

Moyle, P. B., & Cech, Jr., J. J. (1996). *An Introduction to Ichthyology*. (3rd ed.) Prentice Hall, New Jersey. pp.726.

Nagai, M., & Ikeda, S. (1971). Carbohydrate metabolism in fish. Effects of starvation and dietary composition on the blood glucose level and hepatopancreatic glycogen and lipid contents in carp. *Bulletin of Japanese Society of Scientific Fisheries*, 37, 404-409.

Pakhira, C., Nagesh, T.S., Abraham, T.J., Dash, G., & Behera, S. (2015). Stress responses in rohu, *Labeo rohita* transported at different densities. *Aquaculture Reports*, 2, 39-45. doi.org/10.1016/j.aqrep.2015.06.002

Phillips, Jr. A.M., Livingston, D.L., & Dumas, R.F. (1960). Effects of starvation and feeding on the chemical composition of brook trout. *The Progressive Fish Culturist*, *22*(4), 147-154. doi.org/10.1577/1548-8659(1960)22[147:EOSAFO]2.0.CO;2

Popma, J.T., & Lovshin, L.L. (1995). Worldwide prospects for commercial production of Tilapia, Department of Fisheries and Allied Aquacultures, Auburn University, 36849, Alabama 42 pp.

Salam, A., & Davies, P.M.C. (1994). Body composition of northern pike (*Esox lucius* L.) in relation to body size and condition factor. *Fisheries Research*, 19(3-4), 193-204. doi: 10.1016/0165-7836(94)90038-8

Salam, A., Ali, M., & Ali, Z. (2000a). Effect of starvation regime on growth rate of Chinese grass carp, C*tenopharyngodon idella* (Val.). *Pakistan Journal of Fisheries*, 1(2), 39-46.

Salam, A., Ali, M., & Iqbal, F. (2000b). Effect of starvation regime on growth rate of mrigal, *Cirrhinus mrigala*. *Pakistan Journal of Fisheries*, 1, 17-24.

Satoh, S., Takeuchi, T. Watanabe, T. (1984). Studies on

nutritive-value of dietary lipids in fish. 28. Effects of starvation and environmental-temperature on proximate and fatty-acid compositions of *Tilapia nilotica*. *Bulletin of the Japanese Society of Scientific Fisheries*, *50*(1), 79-84.

Sargent, J. R., Henderson, R. J. & Tocher, D. R. (1989). The Lipids. In J. Halver (Ed.), *Fish Nutrition* (2nd Ed.) (pp. 154-209). London: Academic Press.

Shackley, P. E., Talbot, C., Cowan, A., & Watt, A. (1994). The use of body water, sodium, potassium and calcium contents to investigate the nutritional status of first year Atlantic salmon parr in two Scottish Highland streams. *Journal of Fish Biology*, *44*(4), 693-706. doi: 10.1111/j.1095-8649.1994.tb01245.x

Shul'man, G.E. (1974). Life Cycles of Fish; *Physiology and Biochemistry*, New York: Wiley, 258 pp.

Stimpson, J.H. (1965). Comparative aspects of the control of glycogen utilisation in vertebrate liver. *Comparative Biochemistry and Physiology*, 15(2), 187-197. doi: 10.1016/0010-406X(65)90346-4

Talwar, P.K., & Jhingran, A.G. (1991). Inland Fishes of India and Adjacent Countries. New Delhi, Bombay: Oxford and IBH Publishing Company Private Limited, 1158 pp.

Van Dijk, P. L. M., Hardewig, I., & Hölker, F. (2005). Energy reserves during food deprivation and compensatory growth in juvenile roach: the importance of season and temperature. *Journal of Fish Biology*, 66(1), 167-181. doi: 10.1111/j.0022-1112.2005.00590.x

Weatherley, A. H., & Gill, H. S. (1987). *The Biology of Fish Growth*. London: Academic Press.

Wootton, R. J. (1990). Ecology of Teleost Fishes. London: Chapman and Hall.

Yengkokpalm, S., Pal, A. K., Sahu, N. P., Jain, K. K., Dalvi, R., Misra, S., & Debnath, D. (2008). Metabolic modulation in *Labeo rohita* fingerlings during starvation: Hsp70 expression and oxygen consumption. *Aquaculture*, 285(1-4), 234-237. doi.org/10.1016/j.aquaculture.2008.08.034