

Dynamics of Body Composition, RNA:DNA Ratio and Elemental Concentration of Rohu *Labeo rohita* (Ham.) in Relation to Stress Induced by Fasting

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Abstract

The present study was undertaken to examine composition changes in rohu during feed deprivation. Rohu fingerlings (initial weight = 12.86 ± 2.81 gm, initial length = 11.26 ± 2.59 cm) were subjected to feed deprivation for 0 (control), 15, 30 and 45 days at temperature of $32 \pm 2^\circ\text{C}$. Results revealed that % water, % ash increased, % organic contents and RNA:DNA ratio decreased significantly while % protein remained fairly constant on percent dry weight basis. Results also revealed that among elements when analyzed on absolute amount basis only calcium was significantly affected by starvation showing linear increase while all other elements showed a trend of increase in initial days of starvation and then decreased later. The overall effect of starvation on sodium, magnesium, zinc and iron was nonsignificant.

Introduction

Many fish undergo periods of undernutrition or anorexia e.g., during wintering, spawning, migration or when local food abundance diminishes, (Jobling 1994, Ali et al. 2003) and energy demand is reduced during such periods (Weatherley and Gill 1987). Nevertheless, metabolism is maintained at the expense of energy reserves, which results in a progressive depletion (Love 1980).

Lipid reserves may be utilized preferentially over protein stores in the early stages of feed deprivation (Blake and Love 1986; Love and Blake 1990; Navarro and Gutierrez 1995), but fish do not appear to utilize carbohydrate to any great extent (Weatherley and Gill 1987).

The RNA:DNA ratio for selected body tissues has frequently been used as an indicator of nutritional status and growth rate in fish (Ferron and Leggett 1994). In a variety of taxa, fish being fed a high ration had a higher RNA:DNA ratio than fish maintained on low rations or deprived of food for a few days. Positive correlations between short term growth rates and RNA:DNA ratios or concentration have also been reported (see review in Ferron and Leggett 1994)

The vital role of trace elements in catalyzing biochemical, metabolic and enzymatic reactions in the living cells of plants and animals is well understood (Awadslah et al. 1985). The weathering of soil and rocks and a variety of human activities like mining, processing or use of metals or substances that contain metal containment are important sources of addition of metal in aquatic ecosystem (Laws 1981).

During recent years, the role and importance of fish towards studying the problems of pollution of aquatic environment due to heavy metals have been actively considered (Jaffar et al. 1988). Fish may clearly reflect the status of water quality, as they are located at the end of aquatic food chain, and may act as indicator of water pollution in terms of these metals. Thus heavy metal contamination and retention of metal by fish may be monitored through fish analysis, so that health risk of the consumer may be avoided (Salam et al. 1996). There is a growing interest in carrying out studies on elemental concentration of wild and cultured food fishes throughout the world (Shearer 1984; Jaffar et al. 1988; Rottiers 1993; Shackley et al. 1994; Salam et al. 1996).

The present study was undertaken to examine composition, RNA:DNA ratio and elemental concentration changes in rohu during feed deprivation.

Materials and Methods

Rohu fingerlings were obtained from Qadir Fish Seed Hatchery, Matital Road Multan, Pakistan. Prior to experimentation, one group of 25 fingerlings was taken out and sacrificed for analysis of body composition parameters to provide baseline data. The rest of the four groups of fish were transferred to separate experimental aquaria (36"x12"x15") and acclimatized for 10 days. Temperature of the aquaria during experiment was kept constant at $32 \pm 2^{\circ}\text{C}$. The amount of dissolved oxygen (DO) and pH of the water were also monitored daily as 6.05 ± 0.55 ml/l and 8.0 ± 0.5

respectively by using oxygen meter (JENWAY 9071) and pH meter (JENWAY 3071) (Table 1).

After the experiment, each group was removed from the experimental aquaria, sacrificed after receiving a heavy dose of anesthetics and weighed on a top pan electronic digital balance (MP-3000).

For calculating water content, each group of preweighed fish was placed as a whole in pre-weighed aluminum foil tray for drying till constant weight in an electric oven (Memmert 200-Germany) at 60-65°C. For further analysis each dry group was crashed in pestle and mortar, powdered and homogenized in a Moulinex Electric Blender. To calculate ash content in each individual from the group of fish, 50 mg of the same was taken in preweighed heat-resistant China Clay crucible and ashed in muffle furnace (RJM, 1000-China) for 7 hours at 550°C and reweighed after cooling. Analysis of each sample was made in triplicate.

A small sample (approximately 10 mg fresh weight) of white muscle was taken for measurement of RNA:DNA ratio. RNA and DNA of the muscle were extracted and quantified using the method of Clemmesen (1988) as modified by Steinhart and Eckmann (1992) and Grant (1996).

The fat contents were estimated using the dry tissue by dry extraction method in which a mixture of 1:2 chloroform and methanol was used following the method of Bligh and Dyer (1959). The protein content was calculated by difference from mass of other main constituents like ash, fat and water following Caulton and Bursell (1977); Salam and Davies (1994).

Carbohydrates do not form a major component of fish and are usually present in negligible amount (Elliott 1976; Caulton and Bursell 1977; Salam and Davies 1994). No attempt was made to estimate this constituent.

Organic contents were determined by subtracting ash content from dry body weight.

The data thus obtained was subjected to statistical analysis using computer package of Minitab and Excel for ANOVA. All weights and lengths were log transformed and percentages arc-sine were transformed before analysis to stabilize the variances (Ali and Wootton 1998).

Table 1. Principal characteristics of the experiments on which the study was based

Temperature (°C)	Mean initial fish size (cm)	Mean initial fish weight (gm)	No. of fish	Starvation level (days)
32±2	13.8±1.7	15.71±2.11	6	0 (control 45 feeding)
32±2	10.5±3.4	12.41±3.58	9	15
32±2	11.0±3.0	12.33±3.32	6	30
32±2	9.75±2.25	11.0±2.23	6	45

Results

Water content (%)

There was a highly significant effect of starvation on percent water content (df = 3, 23, n = 26, f = 42.12, P < 0.001***). There was a trend of increase in percent water content with an increase in the number of days of starvation. A rapid increase in percent water content was observed till 30 days and the rate of increase became slow between 30-45 days of starvation (Table 2).

Dry content (%): There was a highly significant effect of starvation on percent dry content (df = 3, 23, n = 26, f = 41.86, P < 0.001***). There was a trend of decrease in percent dry content with an increase in the number of days of starvation. A sharp decline in percent dry content was observed till 30 days and this decrease became slow between 30-45 days of starvation (Table 2).

Organic content (%)

There was also highly significant effect of starvation on organic content (df = 3, 23, n = 26, f = 264.77, P < 0.001***). There was a trend of decrease in organic content with an increase in the number of days of starvation. A gradual decrease in organic content was observed till 45 days, however, this decrease was relatively sharp till 15 days of starvation.

Ash content (%)

There was a highly significant effect of starvation on ash content (df = 3, 23, n = 26, f = 325.56, P < 0.001***). There was a trend of increase in ash content (% dry weight) with an increase in the number of days of starvation. A rapid increase in ash content was observed between 0-15 days and this decrease became slow between 15-45 days of starvation.

Table 2. Effect of starvation on body composition (% dry weight) and RNA: DNA ratio of Rohu, *Labeo rohita*

Body composition parameters	Control	Starvation (days)		
	45 days feeding	15	30	45
Water content	77.53±2.27	80.4±6.97	91.27±0.64	92.88±1.52
Dry content	22.5±2.30	19.9±6.43	8.77±1.0	7.11±1.52
Organic content	85.34±2.16	70±2.0	64.6±3.0	62.5±0.5
Ash content	13.96±1.48	30.36±1.66	35.7±2.7	37.8±0.5
Lipid content	35.5±05	18.4±0.4	12.8±0.4	10.4±0.6
Protein content	50.4±2.62	51.8±2.2	51.4±3.0	51.6±0.4
RNA:DNA ratio	4.56±1.08	3.38±0.72	2.31±0.86	1.66±0.81

Lipid content (%)

There was a highly significant effect of starvation on lipid content (% dry weight) ($df = 3, 23, n = 26, f = 4362.06, P < 0.001^{***}$). There was a trend of decrease in lipid content with an increase in the number of days of starvation. This decrease was sharp between 0-15 days and then became gradual.

Protein content (% dry or wet weight)

There was no significant effect of starvation on protein content (% dry weight) ($df = 3, 23, n = 26, f = 0.57, P > 0.1^{n.s}$). It was observed that protein content increased slightly during 45 days of starvation but the overall change was nonsignificant.

RNA:DNA ratio

There was a highly significant effect of starvation on RNA:DNA ratio ($df = 3, 23, n = 26, f = 42.12, P < 0.001^{***}$). There was a trend of decrease in RNA:DNA ratio with an increase in the number of days of starvation. A gradual decrease in RNA:DNA ratio was observed till 45 days (Table 2).

Elemental concentration (Absolute basis)

Results revealed that among elements only calcium is significantly affected by starvation showing linear increase ($df = 3, 20, n = 23, f = 10.87, P < 0.001^{***}$). All other elements showed a trend of increase in initial days of starvation and then decreased later. The overall effect of starvation on sodium, magnesium, zinc and iron was nonsignificant because of very large variations (Table 3).

Discussion

Periods of starvation affect the feeding and the digestive processes in fish (Fange and Grove 1979; Love 1980). Studies of fish starvation are important in understanding the growth biology of fish in wild state. In the present study it was observed that during starvation fish utilized the fat first (Table 2). The quantity of fat decreased progressively as the number of days of starvation increased. When the body composition of starved fish was compared with the control fish, it was observed that there was a significant decrease in fat content after 45 days. Similar findings are

reported in the literature (Salam et al. 2000, Ali et al. 2001). Many investigators are of the view that the first effect of starvation is mobilization of fat (Salam et al. 2000, Ali et al. 2001). Apart from carps, biological studies of lipid on other teleosts as an energy source have revealed its importance during period of starvation (Jezierska et al. 1982; Salam et al. 2000) due to utilization of body constituents as an energy source during starvation.

In the present case the amount of percent water was inversely related to the quantity of fat inside the body of fish. When compared with the percent water content, it was found that maximum quantity of water was present in starved fish and minimum in the control group (Table 2). A similar trend was documented by various species during starvation (Niimi 1972, Jobling 1980, Weatherley and Gill 1987, Salam et al. 2000, Ali et al. 2001). During starvation depletion of the body constituents results in tissue rehydration i.e. increase in water contents. Salam and Davies (1994) reported that the weight loss difference in fish during starvation may be partly due to change in water contents.

During starvation it was observed in the present study that no significant change in protein content (% dry weight) occurred in 45 days of starvation as compared to control group, but a decrease in protein content (% wet weight) occurred. This decrease was very sharp during the first 15 days and then became gradual. This is largely due to the inverse relationship of protein with water in starving fish, which is well documented (Salam and Davies 1994).

Quinton and Blake (1990) found that in rainbow trout, starved for three weeks, a reduction in fat and an increase in moisture and protein had occurred as compared to control. While in long term starvation, structured protein can also be drawn for energy used as lipid reserved become depleted (Love 1980). Ince and Thorpe (1976) found that the effect of starvation periods of 1 or 3 months on Pike, *Esox lucius*, main body losses were due to lipid and glycogen rather than muscle protein.

A rapid increase in ash content (% dry weight) was observed during the first 15 days of starvation and then became slow until 45 days as compared to control group. Ash content (% wet weight) also followed the same pattern.

Table 3. Effect of starvation on elemental concentration (dry weight) of Rohu, *Labeo rohita*

Concentration of element Ug g ⁻¹	Control	Starvation (days)		
	45 days feeding	15	30	45
Na	110.7±57.6	374.7±402.5	5027.3±9217.6	2584±1751.6
K	38.5±26.4	137.9±112.8	967.7±1344.1	237.2±116
Ca	520±271	9696.6±7783	19892±14048	27006±6256.1
Mg	93.6±38	1029.2±877.2	5716.4±9149.8	2496.1±507.1
Zn	2.4±1.1	34.3±25.5	263.3±399.5	142.5±39.0
Fe	4.0±2.3	34.4±17	754.7±1232.2	326.7±69.7

This is due to rapid utilization of body constituents. A similar trend was observed by Ali et al., (2001) in grass carp, *Ctenopharyngodon idella* (Val.).

Niimi (1972) reported 14% weight loss in large mouth bass during 40 days of starvation period at 25°C, for which the energy requirement was derived from lipid and protein in the ratio of 60:40. The confirmation of our results also comes from increases in total ash contents during starvation in *Sardia pilchardus* as reported by Herrera and Munoz (1957) and *Salvelinus fontinalis* by Phillips et al., (1960).

In the present study the RNA:DNA ratio responded very sharply during the first week of starvation and it went on gradually decreasing during the total experimental period. Tissue concentrations of nucleic acids and protein are proving to be valuable biochemical indices of nutritional status and recent growth rate for fishes (Ferron and Leggett 1994). In the stickleback, ration size had a highly significant effect on the RNA/DNA ratio. Grant (1996) reported that ration size had a significant effect on RNA/DNA ratio of white muscle tissue of brown trout. He suggested that RNA/DNA ratio in white muscle tissue reflected recent growth. In a variety of taxa, fish fed a high ration have had higher RNA/DNA ratios than fish maintained on low rations or those deprived of food for a few days (Bulow 1987; Clemmensen 1988; Steinhart and Eckmann 1992; Wang et al. 1993). Positive correlations between recent growth rate and the RNA:DNA ratios or the concentration of RNA have also been reported (Wilder and Stanley 1983; Miglavs and Jobling 1989; Ferguson and Danzmann 1990; Mathers et al. 1992).

It was observed that the concentrations of sodium and potassium were not significantly affected by starvation. However, they increased by increasing the number of days of starvation up to 30 days as the amount of ash increased (Table 3) and then decreased. Based on this trend, it can be concluded that during starvation the fish utilizes its stored lipid and body protein in the early stages (Weatherley and Gill 1987; Salam et al. 2000 and Ali et al. 2001) and then begin to utilize its inorganic constituents as sodium and potassium.

In the case of calcium, it was observed that its concentration increased in both dry and wet weight of fish and followed a linear pattern. Since calcium is a major component of skeleton (Guyton 1991) therefore starvation does not affect the skeleton but relative increase synchronizes with increase in ash.

The concentration of the other three elements (Mg, Fe, Zn) were also not significantly affected by starvation. But the trend of change in their concentrations also increased by increasing the number of days of starvation up to 30 days as the amount of ash increased (Table 3) and then decreased again showing that fish utilizes its inorganic constituents after a limited period of food deprivation.

Acknowledgments

The authors are grateful to Mr. Muhammad Fahim, owner of Qadir Fish Farm for the supply of experimental fish.

References

- Ali, M., A. Salam Z. Ali. 2001. Dynamics of body composition in relation to various starvation regimes of Chinese grass carp, *Ctenopharyngodon idella* (Val.). Pakistan Journal of Zoology 33: 47-51.
- Ali, M., A. Nicieza and R.J. Wootton. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries* 4:147-190.
- Ali, M., and R.J. Wootton. 1998. Do random fluctuations in the intervals between feeding affect growth rate in juvenile three-spined sticklebacks? *Journal of Fish Biology* 53:1006-1014.
- Awadallah, R.M., A.E. Muhammad and S.A. Gabr. 1985. Determination of trace elements in fish by Instrumental Neutron Activation Analysis. *Journal of Radional Nuclear Chemistry* 95:145-154.
- Blake, D. and R.M. Love. 1986. The sequential metabolism and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *Journal of Comparative Physiology B* 156: 469-479.
- Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification: *Canadian Journal of Biochemistry and Physiology* 37: 911-917.
- Bulow, F.J. 1987. RNA-DNA ratios as indicators of recent of growth rates of a fish. *Journal of the Fisheries Research Board of Canada* 27:2343-2349.
- Caulton, M.S. and E. Bursell. 1977. The relationship between changes in condition and body composition in Young Tilapia *Rendalli Boulenga*. *Journal of Fish Biology* 11:143-153.
- Clemmesen, C.M. 1988. RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larva. *Meeresforsch* 32:134-143.
- Clemmesen, C.M. 1993. Improvements in the fluorimetric determination of the RNA and DNA content of individual marine fish larvae. *Marine Ecology Progress Series* 100:177-183.
- Elliott, J.M. 1976. Body composition of brown trout (*Salmo trutta*) in relation to temperature and ration size. *Journal of Animal Ecology* 45:273-289.
- Fänge, R. and D.J. Grove. 1979. Digestion. In *Fish physiology*. Vol. 8 (Hoar, W.S., Randall, D.J. and Donaldson, E.M., eds), pp. 161-260.
- Ferguson, M.M. and R.G. Danzmann. 1990. RNA/DNA ratios in white muscle as estimates of growth in rainbow trout held at different temperatures. *Canadian Journal of Zoology* 68:1494-1498.
- Ferron, A. and W.C. Leggett. 1994. An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology* 30:217-303.
- Grant, G.C. 1996. RNA-DNA ratios in white muscle tissue biopsies reflect recent growth rates of adult brown trout. *Journal of Fish Biology* 48:1223-1230.
- Guyton, C. (1991). *Textbook of medical physiology*. 8th Edition. W.B. Saunders. USA.
- Herrera, J. and F. Munoz. 1957. Biological consideration on the chemical composition of the sardine (*Sardina pilchardus walb*) from Castellon. *Investigation Perq* 7:33-48.
- Ince, B.W. and A. Thorpe. 1976. The effects of starvation and force feeding on the metabolism of the Northern Pike, *Esox lucius* L. *Journal of Fish Biology* 8:79-88.
- Jaffer, M., M. Ashraf and A. Rasool. 1988. Heavy metal contents in some selected local fresh water fish and relevant waters. *Pakistan Journal of Scientific and Industrial Research* 31: 189-193.
- Jezierska, B., J.R. Hazel and S.D. Gerking. 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention of fatty acids. *Journal of Fish Biology* 21:681-692.
- Jobling, M. 1980. Effects of starvation on proximate chemical composition and energy utilization of Plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 17:325-334.
- Jobling, M. 1994. *Fish bioenergetics*. London: Chapman and Hall.
- Lambert, P. and P.A. Dehnel. 1974. Seasonal variations in biochemical composition during the reproductive cycle of the intertidal gastropod *Thias Lamellosa* Gmelin (Gastropoda, Prosobranchia). *Canadian Journal of Zoology* 52:305-318.
- Laws, E.A. 1981. *Aquatic pollution*. John Wiley and Sons, London.
- Love, R.M. 1980. *The chemical Biology of fishes*, Vol. II. Academic Press, London.
- Love, R.M. and D. Blake. 1990. Dynamics of stored energy in the North sea cod, *Gadus morhua* and cultured rainbow trout, *Salmo gairdneri*. *Comparative Physiological Zoology* 63:630-638.

- Mathers, E.M., D.F. Houlihan and M.J. Cunningham. 1992. Nucleic acid concentrations and enzyme activities as correlates of growth rate of salthe *Pollachius virens*: growth rate estimates of open-sea fish. *Marine Biology* 112:363-369.
- Miglav, I. and M. Jobling. 1989. The effects of feeding regime on proximate body composition and patterns of energy deposition in Juvenile Arctic Charr, *Salvelinus alpinus*, *Journal of Fish Biology* 35:1-11.
- Navarro, I. and J. Gutierrez. 1995. Fasting and starvation. In: *Biochemistry and molecular biology of Fishes* (Hockachka, P.W. and Mommsen, T.P., eds), Vol. 4, pp. 393-433. New York: Elsevier.
- Niimi, A.J. 1972. Changes in the proximate composition of largemouth bass (*Micropterus salmoides*) with starvation. *Canadian Journal of Zoology* 50:815-819.
- Phillips, A.M., D.L. Livingston and R.F. Dumas. 1960. Effect of starvation and feeding on the chemical composition of brook trout. *Progressive Fish Culturist* 22:147-154.
- Quinton, J.C. and R.W. Blake. 1990. The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. *Journal of Fish Biology* 37:33-41.
- Rottiers, D.V. 1993. Relationship between the amount of bone, major conditions and body size in Atlantic Salmon, *Salmo salar*. *Copeia* 2:440-446.
- Salam, A. and P.M.C. Davies. 1994. Body composition of northern pike (*Esox lucius* L.) in relation to body size and condition factor. *Fisheries Research* 19:193-204.
- Salam, A., M. Naeem, T.M. Ansari, J.M. Akhtar and N. Tariq. 1996. Effect of body size on trace metal concentrations in hatchery reared *Oreochromis nilotica*. *Biologia* 42:19-26.
- Salam, A. and P.M.C. Davies. 1998. Studies on the energy budget of freshwater fish, *Esox lucius* (L.) in relation to abiotic and biotic variables in Nottinghamshire. *Proceeding of Pakistan Congress of Zoology* 18:165-176.
- Salam, A., M. Ali and F. Iqbal. 2000. Body composition dynamics of mrigal, *Cirrhinus mrigala* in relation to various starvation regimes. *Pakistan Journal of Fisheries* 1:65-74.
- Shackley, P. E., C. Talbot, A. Gawon and A. Watt. 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* 44:693-704.
- Shearer, K.D. 1984. Changes in the elemental composition of hatchery reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. *Canadian Journal of Fisheries and Aquatic Sciences* 41:1592-1600.
- Steinhart, M. and R. Eckmann. 1992. Evaluating the nutritional condition of individual whitefish (*Coregonus spp.*) larvae by RNA/DNA ratio. *Journal of Fish biology* 40:791-799.
- Wang, S. Y., J.L. Lum, M.G. Carls and S.D. Rice. 1993. Relationship between growth and total nucleic acids in juvenile pink salmon, *Oncorhynchus gorbuscha*, fed crude oil contaminated food. *Canadian Journal of Fisheries and Aquatic Science* 50:996-1001.
- Weatherley, A.H. and H.S. Gill. 1987. *The biology of fish growth*, Academic Press, London.
- Wilder, I.B. and J.G. Stanley. 1983. RNA-DNA ratio as an index of growth in salmonid fishes in the laboratory and in streams contaminated by carbaryl. *Journal of Fish Biology* 22:165-172.