

Full Length Research Paper

Effect of feed cycling on specific growth rate, condition factor and RNA/DNA ratio of *Labeo rohita*

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A study was conducted to evaluate the effect of feed cycling on specific growth rate, condition factor and RNA/DNA ratio of *Labeo rohita*. Fingerling *L. rohita* were randomly collected from Qadria Fish Farm and Hatchery, Multan, Pakistan and divided into control, 5 days and 10 days feed cycling groups. Specific growth rate (% g day⁻¹), body composition, condition factor, and RNA/DNA ratio of individual fish and of each group were calculated. There was a highly significant (P<0.001) effect of feed cycling on specific growth rate and RNA/DNA ratio of *L. rohita*. However, the effect on condition factor was non-significant. A gradual decline was observed in specific growth rate and RNA/DNA ratio with increasing length of starvation. *L. rohita* was able to maintain its main body constituents such as fat, protein, organic and inorganic contents indicating a compensatory growth, which was independent of duration of starvation.

Key words: *Labeo rohita*, feed cycling, specific growth rate, body composition, condition factor, RNA/DNA ratio.

INTRODUCTION

Compensatory growth is a phase of rapid growth, greater than normal or control growth rates, associated with adequate re-feeding of animals following a period of under-nutrition (Weatherley and Gill, 1981). In the natural environment, many organisms exploit food supplies whose spatial, temporal or seasonal variability implies significant periods of near or actual famine. As an adaptation to such a lifestyle, many organisms exhibit faster growth during recovery from starvation than during access to constant food availability. When food supplies are increased following a period of starvation or restricted feeding, fish may display a growth spurt, often referred to as catch up or compensatory growth (Bulow, 1970; Dobson and Holmes, 1984; Weatherley and Gill, 1981). Fish populations may face reduced availability of food at various times of the year. A number of species are capa-

ble of withstanding prolonged periods of starvation during which accumulated reserves, mostly in the form of lipid, are utilized as metabolic fuel. Cycles vary in fishes according to age, ontogenetic stage, gender, photoperiod, season and temperature. Feed cycling consists of alternate starvation periods and feeding periods (Boujard and Leatherland, 1992; Van Dijk et al., 2005).

Body composition and energy content are good indicators of physiological condition of fish but their measure is relatively time consuming. The condition of a fish is frequently used to reflect the overall effects of physiological and environmental factors particularly the nutritional status. Indices of condition which can be determined easily and quickly are needed in routine fisheries surveys and are good predictors of the body composition and growth rate of fish (Cui and Wootton, 1988). Condition factor is one of the most important parameters, which throws light on the physiological state of the fish in relation to indication of the onset of the sexual maturity (Salam and Davies, 1994).

Nucleic acids play a major role in growth and development. The amount of DNA, the carrier of genetic information, remains stable under changing environmen-

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Table 1. Analysis of variance (ANOVA) of specific growth rate, RNA/DNA ratio and condition factor of *Labeo rohita* under different feed cycling regimes.

Source	df	SS	MS	F	P
Specific growth rate (% g day ⁻¹)	2,57	0.5603	0.2802	38.52	***
RNA/DNA ratio	2,57	6.5456	3.2728	133.98	***
Condition factor	2,57	0.0492	0.0246	1.94	n.s

Significance level: *** P<0.001, n.s. = Non significant.

tal situations and has been used as an indicator of biomass (Holm-Hansen et al., 1983). The concentrations of RNA in a tissue provide an estimate of ribosome numbers. The changes in nutritional status lead to alterations in ribosome numbers. Measurements of RNA/DNA ratios can provide useful information about the nutritional status of animals (Wang and Stickle, 1986; Wright and Hetzal, 1985). There is usually a significant correlation between nutritional status, RNA/DNA ratios and rates of growth (Lied et al., 1983, Loughna and Goldspink, 1984).

Feeding frequency is an important consideration as it affects growth, survival and fillet composition (Davies et al., 2006). Feeding at an optimum frequency can result in tremendous savings in feed costs. Diet cost represents 30-70% of total operating cost of an aquaculture enterprise thus overfeeding would mean economic waste and could adversely affect water quality by leaching of the nutrients. Underfeeding may suppress growth as a result of starvation (Davies et al., 2006). Determination of appropriate feeding frequency is necessary to give optimal growth and better survival rate. As the fisheries industry is expanding, there is need to know what feeding frequency would be optimal at the least cost for better production. The purpose of present study was to determine the effect of feed cycling on specific growth rate, condition factor and RNA/DNA ratio of *Labeo rohita*.

MATERIALS AND METHODS

Fingerling *Labeo rohita* were collected from Qadria Fish Farm and Hatchery, Multan, Pakistan. They were transported live in plastic containers to Applied Fisheries Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. Fishes were kept into fiberglass fish aquaria (60×60×150 cm). Fishes were acclimatized to experimental conditions for 10 days before the start of the experiment. The aquaria were divided into three treatments as control, 5 days feeding and 5 days deprivation (5D5F), and 10 days feeding and 10 days deprivation (10D10F) cycle. Twenty fishes were randomly used for each treatment and were fed with normal diet used in aquaculture and contained a maximum of 24% protein. All the fish in each treatment received the feed at 3% of their body weight. Fish in the control treatment were fed once a day while the fish in the other two treatments received the same diet after 5 and 10 days of starvation over 90 days experimental period. Each aquarium was constantly aerated by electric aerators. The temperature was maintained throughout the experimental period by air coolers. Water was changed regularly in each aquarium after 48 h maintaining the water depth.

After acclimation, initial weight and length were taken. All specimen of *L. rohita* were weighed on an electronic digital balance (Chyo, Japan) to the nearest 0.01 g. Total body length of each individual fish was measured to the nearest of 0.01 cm using a Perspex measuring tray fitted with a sheet of millimeter ruler. All the length measurements were taken from the tip of mouth to the longest caudal fin ray. Each fish was tagged with its initial weight and length. At the end of experiment, the fishes were left unfed for 24 h to empty their guts and their final weight and length were recorded. RNA/DNA ratio was determined following the method of Clemmesen (1988, 1993) as modified by Grant (1996) and Steinhart and Eckmann (1992).

All the weights were log transformed and specific growth rate was calculated by the method of Ali et al. (2001). Water content was determined by placing the whole fish in a pre-weighed aluminum foil tray for drying in an electric oven at 65-80°C till constant weight. Ash content was estimated by burning a 500 mg sample in a pre-weighed heat resistant China clay crucible placed in a Muffle furnace for 7 h at 500°C and reweighed after cooling. Lipid content was estimated by dry extraction following the method of Bligh and Dyer (1959), and Salam and Davies (1994). Powdered dry tissue (3 mg) was mixed into 10 mL solution of chloroform and methanol (in the ratio 1:2), and stirred with a glass rod. The resultant mixture was left over night and then centrifuged. After centrifugation, the clear supernatant was removed carefully into washed, dried and pre-weighed small bottles. These bottles were then put in an oven at 40-50°C to evaporate the solvent leaving the lipid fraction.

Total protein in dry mass was calculated by the difference method from the mass of other main constituents like ash, lipid and water (Ali et al., 2005, Ali et al., 2006). Carbohydrates do not form a major component of fish and thus are generally neglected due to their negligible contribution (Elliott, 1976; Caulton and Bursell, 1977; Salam and Davies, 1994). Data were analyzed statistically using analysis of variance.

RESULTS AND DISCUSSION

The comparison of control, 5D5F and 10D10F feed cycling of *L. rohita* shows that feeding cycle had highly significant (P<0.001) effect on specific growth rate (Table 1). There was a gradual decrease in specific growth rate in 5D5F and 10D10F feed cycling as compared to control while feed cycling had no significant effect on condition factor (Table 2). The comparison of RNA/DNA ratio of three feeding regimes indicates that feed cycling had a highly significant (P<0.001) effect on RNA/DNA ratio of *L. rohita* which was higher in 5D5F feed cycling than the control and 10D10F feed cycling (Table 2).

There was no significant effect of feed cycling on ash, fat, protein (all dry and wet body weight) and organic contents (dry body weight) while the effect on dry body

Table 2. Comparison of specific growth rate, condition factor and RNA/DNA ratio of *Labeo rohita* under different feed cycling regimes (mean \pm SD).

Observations	Control	5 days feed cycling	10 days feed cycling
Specific growth rate (% g day ⁻¹)	0.12731 \pm 0.8269 ^a	0.01476 \pm 0.0932 ^b	-0.10645 \pm 0.0818 ^c
RNA/DNA ratio	3.6745 \pm 0.2572 ^b	4.1107 \pm 0.0088 ^a	3.2400 \pm 0.0251 ^c
Condition factor	0.8168 \pm 0.0936	0.7819 \pm 0.1628	0.7466 \pm 0.0799

The lettering indicates that the values in each row are significantly different at P<0.001.

Table 3. Analysis of variance (ANOVA) of body composition (%) of *Labeo rohita* under different feed cycling regimes.

Source	df	SS	MS	F	P
Dry body mass	2,57	46.19	22.59	6.29	**
Water	2,57	46.68	23.34	6.63	**
Ash (dry wt.)	2,57	95.0	47.5	0.91	n.s
Ash (wet wt.)	2,57	3.484	1.742	1.77	n.s
Fat (dry wt.)	2,57	149.0	74.5	1.89	n.s
Fat (wet wt.)	2,57	11.11	5.55	2.69	n.s
Protein (dry wt.)	2,57	187.9	94.0	1.46	n.s
Protein (wet body weight)	2,57	10.47	5.23	2.17	n.s
Organic contents (dry wt.)	2,57	94.4	47.2	0.91	n.s
Organic contents (wet wt.)	2,57	33.68	16.84	3.76	*

Significance level: * P<0.05, ** P<0.01, n.s. = Non significant.

Table 4. Comparison of body constituents (%) of *Labeo rohita* under different feed cycling regimes (mean \pm SD).

Observations	Control	5 days feed cycling	10 days feed cycling
Dry body mass	16.525 \pm 1.862	14.567 \pm 1.580	16.671 \pm 2.127
Water	83.470 \pm 1.854	85.459 \pm 1.510	83.320 \pm 2.126
Ash (dry wt.)	31.732 \pm 6.552	31.735 \pm 6.578	29.001 \pm 8.218
Ash (wet wt.)	5.1753 \pm 0.9268	4.5693 \pm 0.08493	4.7530 \pm 1.1432
Fat (dry wt.)	25.110 \pm 5.794	21.006 \pm 7.554	23.954 \pm 5.667
Fat (wet wt.)	4.200 \pm 1.344	3.081 \pm 1.149	3.892 \pm 1.694
Protein (dry wt.)	43.350 \pm 7.093	47.005 \pm 9.222	47.318 \pm 7.927
Protein (wet wt.)	7.153 \pm 1.476	6.810 \pm 1.574	7.868 \pm 1.610
Organic content (dry wt.)	68.268 \pm 6.552	68.266 \pm 6.578	70.990 \pm 8.221
Organic content (wet wt.)	11.344 \pm 2.156	9.919 \pm 1.635	11.864 \pm 2.376

mass and water content was highly significant at P<0.01 and on organic contents (wet body weight) was significant at P<0.05 (Table 3). The dry body mass and organic content (wet body weight) were higher in 10D10F feed cycling as compared to control and 5D5F feed cycling, while water content was highest in 5D5F group than the control and 10D10F group (Table 4). The non significant results of ash, fat, and protein (in dry and wet body weight) showed that starvation and re-feeding in 5D5F and 10D10F groups did not affect the body constituents.

Experiments performed on feed cycling have shown a

compensatory response depends on the length of the starvation and subsequent re-feeding periods. Compensatory or catch-up growth occurs mostly in cold-water species, whereas data on warm water species are not consistent. The occurrence of compensatory growth is species specific and depends upon the duration of the fasting period in warm water fish species (Xie et al., 2001). All the *L. rohita* were approximately of the same age and were collected from the same locality. Later they were exposed to identical experimental conditions. However, the response to 5 and 10 days cycle of food deprivation and re-feeding was different.

There was a decrease in specific growth rate in 5D5F and 10D10F feed cycling as compared to control, with 10D10F feed cycling producing a negative specific growth rate. This indicates that the length of starvation had a marked effect on specific growth rate. No effect of feed cycling was observed on condition factor of *L. rohita*. A similar weak compensatory response was observed by Wu et al. (2003) in European minnows *Phoxinus phoxinus* (Cyprinid). The mass trajectory of the minnows experiencing the cyclical regime fell below the trajectory of the control minnows. The growth rate of the deprived fish was also significantly greater than the control in second periods of re-feeding. Quinton and Blake (1990) kept a group of 50 rainbow trout, (*Oncorhynchus mykiss*) on a schedule of three weeks of deprivation and three weeks of feeding for 18 weeks. Although the rainbow trout showed compensatory growth in re-feeding periods, their mass trajectory progressively deviated from the control fish indicating a weak compensatory growth. Jobling et al. (1993) subjected groups of Arctic charr, (*Salvelinus alpinus*) to cycles of 1:1, 1.5:1.5 and 3:3 weeks of deprivation and re-feeding for 24 weeks. The growth trajectory of the Arctic charr experiencing the cycles deviated from the control fish, indicating that the fish were unable to compensate fully for the periods of deprivation. However, three spined sticklebacks (*Gasterosteus aculeatus*) was able to fully compensate when subjected to re-feeding after food deprivation periods (Ali, 1999). This weak compensatory response of *L. rohita* may be due to species difference, temperature effect, feeding habits and experimental design. *L. rohita* is herbivorous, warm water fish. Its main food is natural aquatic organisms (plankton) and its digestive physiology is different from a carnivorous fish. These factors may contribute to weak its growth compensation as compared to fish showing full compensatory response. Ali (1999) and Van Dijk et al. (2005) reported that fishes showing full compensatory response become hyperphagic (higher feed intake than the control) when transferred to re-feeding. However, this feature could not be observed in *L. rohita*.

Feed cycling did not have any detectable effect on ash, fat, protein (dry and wet body weight), and organic contents (dry body weight). However, dry body mass, water and organic content (wet body weight) were significantly different. There was partial decrease in dry body mass in 5D5F feed cycling while 10D10F feed cycling had higher dry body mass compared to the control. The amount of water was higher in 5D5F feed cycling than the control and 10D10F feed cycling. The protein content (dry and wet body weight) was higher in 10D10F feed cycling than the control and 5D5F feed cycling. There was a partial decline in organic content (wet body weight) in 5D5F feed cycling, while 10D10F feed cycling had higher organic contents (wet body weight) compared to control and 5D5F feed cycling (Table 3-

4).

These results suggest that *L. rohita* was able to maintain its main body constituents such as fat, protein, organic and inorganic contents. This shows that repeated starvation followed by subsequent re-feeding did not affect the body composition of *L. rohita*. The occurrence of higher dry body mass, protein and organic contents in 10D10F feed cycling of *L. rohita* indicates that growth rate was faster in this group than the control and 5D5F feed cycling. This shows that long-term starvation cycle was better than short-term in relation to body composition compensation in *L. rohita*. These results are in agreement with previous studies carried out on a range of fish species with respect to effects of different feeding regimes on energy content and body composition. Compensatory growth of warm water species was reported in Channel Catfish, *Ictalurus punctatus* (Kim and Lovell, 1995), and hybrid sun fish, *Lepomis cyanellus* × *L. macrochirus* (Hayward et al., 1997), Gibel carp (Qian et al., 2000), but not in carp, *Cyprinus carpio* (Schwarz et al., 1985) and cold water fishes, *Phoxinus phoxinus* (Cui and Wootton, 1988), Juvenile Arctic charr, *Salvelinus alpinus*, (Miglav and Jobling 1989), three spined sticklebacks, *Gasterosteus aculeatus* (Ali and Wootton, 1998) and Juvenile roach (Van Dijk et al., 2005). The present study confirms the existence of compensatory growth in warm water carps, *L. rohita*.

Starvation or restricted feeding generally leads to a reduction in the percentage of lipid content and an increase in the water content of fish tissues. Although the main lipid storage organ varies amongst fishes (Black and Love, 1986; Elliott, 1976; Jobling, 1980; Love, 1980), the lipid store is labile since a variety of nutritional conditions influence body composition in fishes. It might be expected that a switch from a restricted to a satiation-feeding regime would affect the patterns of energy utilization, deposition and proximate body composition. Several mammalian species may respond to such a switch by showing improved food utilization efficiency (Ozelci et al., 1978; Szepesi and Epstein, 1976; Williams and Sheedy, 1987) and may also deposit greater quantities of lipid than animals reared continuously on a satiation and libitum regime (Greeff et al., 1986b; Mersmann et al., 1987; Ozelci et al., 1978; Szepesi and Epstein, 1976), but the effect appeared to be influenced by the degree of restriction and previous nutritional history (Greeff et al., 1986a,b; Mersmann et al., 1987; Ozelci et al., 1978). The capacity of restricted-satiation fed mammals to display complete growth compensation following the period of under-nutrition appears to depend upon factors such as severity and duration of the restriction and upon the ontogenetic stage or age of animals when the restriction is applied (Greeff et al., 1986a). The same may be true for different fish species.

Feed cycling had a marked effect on RNA/DNA ratio. There was a significant ($P < 0.001$) decrease in RNA/DNA

ratio in 10 days feed cycling as compared to control (Table 2). However, results of previous studies suggest that recovery may have occurred with in few days of transfer from restricted to satiation feeding (Bulow, 1970; Lied et al., 1983; Miglavs and Jobling, 1989; Steinhart and Eckmann, 1992; Wright and Martin, 1985). Decreased RNA/DNA ratio during feed cycling suggests that RNA/DNA attributes were most sensitive to the effect of feed cycling even though the fish were fed with double quantity of feed to compensate non-feeding days. This difference may be due to different species, the nature of experiment, feeding and environmental adaptations differences. The decreased RNA/DNA ratio could be due to the result of decrease in ribosomal activity, increase in protein synthesized per ribosome in these fishes. Compensatory growth in *L. rohita* was accompanied by improved efficiency and energy retention, but such improvements were not caused by a higher digestibility in this fish.

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