Changes in Biochemical Composition in Starving Catfish *Heteropneustes fossilis*

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Abstract A prolonged deprivation of catfish *Heteropneustes fossilis* from food resulted in a state of physiological emergency during which proteins and fats were hydrolysed to meet the energy requirements of metabolism. The excretion of nitrogenous products formed at the expense of sarcoplasmic proteins caused negative nitrogen balance inasmuch as the replenishment from exogenous sources remained withheld. The consequences of the loss of endogenous chemical constituents and energy value together with a relative increase in the hydration of the tissues and a fall in the level of inorganic substances have been discussed.

A unique feature of fishes is their ability to withstand periods of prolonged starvation through physiological and biochemical changes in their body. Besides the fact that their basal energy consumption is generally lower compared to terrestrial vertebrates, their metabolic demands further decline during starvation (Love, 1970). Added to this is their adaptation to mobilize their body constituents as fuel for survival through the activity of autolytic enzymes which remain in readiness for such periods of total abstention from food (Siebert et al., 1964). The present study was undertaken to investigate the changes in the principal body composition, energy content, nitrogen balance and the inter-relationships, if any, of the chemical constituents during starvation of a freshwater catfish *Heteropneustes fossilis* (Bloch). A record was also made of the dynamics of the 'fillet condition factor' inasmuch as it can reflect the declining levels of body constituents and from a physiological viewpoint can be used as an index of the living state of the fish in the course of food deprivation.

Materials and methods

Specimens of the catfish forming the basis of present investigations were captured by cast nets from local ponds at Aligarh, India, and transported to the laboratory aquaria. The aquaria were supplied with water at a temperature of 14±2°C. The dissolved oxygen concentration of the water varied from 8.5 to 11 ppm. Body length of test specimens varied from 17 to 24.7 cm, although the majority of the individuals were close to the average of 20 cm. Likewise, the actual body weight of specimens varied from 30 to 92 g but their number was restricted to 2 for the two limits (minimum and maximum) of weight. An overwhelming number of specimens, however, were of a body weight closer to 50 g.

Fifty individuals were separately tagged for recording the decline in the body weight during successive periods of starvation. The modified collar tag devised by the author for this study consisted of a rectangular plate of ivory paper (9×6 mm) covered over by waterproof, transparent self-adhesive tape. The plate was pierced by a filamentous wire which formed a loop around the trunk region of the fish adjacent to the dorsal fin.

After making observations on 10 fresh and unstarved fish, subsequent analyses were made on an equal number of specimens (10 at a time) taken out after 10, 20, 30, 40 and 50 days of starvation. At the time of investigations the specimens were removed from the aquaria, weighed, measured and killed by a sharp blow on the head. Fillets were excised from the point of severance of head to the caudal peduncle, freed from the skin and bones, and recorded for their length and weight (noted on a sensitive electric balance). Fillet condition factor (F_k) was determined according to the equation adopted by Mustafa (1979):

$$F_k = W/L^3 \times 1000$$

where 'W' and 'L' were the weight (g) and length (cm) of the fillet. Each fillet was separately chopped and macerated by an electrically driven macerator. The resulting tissue lump was processed for the various biochemical assays. Methodology used for the determination of energy contents and principal chemical constituents (protein, fat, water, inorganic ash) was the same as referred to in earlier work (Jafri et al., 1964; Mustafa and Jafri, 1978; Shams, 1980). The photometric estimations were carried out on a Bausch and Lomb spectronic 20 spectrophotometer. Results were expressed on a fresh weight basis. For the evaluation of nitrogen excretion the method suggested by Niimi (1972) was followed, wherein the protein losses were divided by a factor of 6.25, yielding the nitrogen loss from the body. It was assumed that the entire nitrogenous fraction of the catabolized protein was excreted.

Although attempts were made to select fish specimens of identical size and weight, variations did occur and warranted a mathematical adjustment. Likewise, the loss in weight, principal chemical constituents and energy was made to correspond to that of a fish of 50 g initial weight. The data were processed statistically using the formulae outlined by Snedecor (1959).

Results

Data pertaining to changes in weight, fillet condition, chemical composition of the body and energy content are tabulated (Tables 1, 2). An elaborated account is presented below.

Changes in weight and fillet condition. A steady decline in the weight occurred with starvation. For fish of 50 g initial weight the decline in weight for each 10-day interval up to 50 days of food deprivation was of the order of 5.855 g, 6.599 g, 8.123 g, 9.604 g and 10.487 g, respectively. On the average, the loss in body weight was 209.7 mg/day. The regression analysis of the logarithmically transformed data of weight corresponding to the period of starvation gave the equation:

$$\text{Log } W = 1.7207 - 0.0701 \log D$$

where, 'W'=weight (g) and 'D'=number of days of starvation. The correlation coefficient, 'r' (-0.954) was found to be significant at 0.001 level of probability.

In the catfish the fillet, which constituted the major portion of the total body weight, seemed to be highly affected by the stress of starvation. This was evident from the changes in the fillet weight (indicated by fillet condition factor) in the direction of a progressive decline.

Changes in principal biochemical constituents. The cumulative weight loss of the fish during starvation appeared to be the result of fall in the absolute concentration of each of the principal body constituents. However, when the values of these constituents were expressed through their percentages, a decline in the fat, protein and ash while a relative increase in water was observed.

Fat content: While a sharp decline in the fat content was found to occur from the very beginning of starvation, the rate of its fall seemed to have declined after the onset of 40 days of starvation. By the end of 50 days, a significant ($P<0.001$) fall of 81.3% in the fat was observed.

Moisture content: The percentage of moisture which increased during starvation maintained an inverse relationship with the percentage of fat. This relationship was expressed through the equation:

$$\text{Log } F = 9.8200 - 5.1625 \log M$$

where, F=percentage of fat and M=percentage of moisture (water); 'r' was found to be -0.980 ($P<0.001$).

The sum of fat plus water varied from 80.580% to 80.839% during the first 40 days of starvation. This value rose to 82.021% when the period of starvation extended to 50 days.

Protein content: Changes in the protein content during first 10 days of starvation remained relatively less pronounced. The mobilization of protein, however, appeared to have been triggered by the 20th day of food deprivation. When the fish was starved for 50 days, a fall of 30.590% was noted. A comparison of the prestarvation and poststarvation values of protein revealed significant difference ($P<0.001$).

Ash content: The percentage of ash declined with the period of starvation, till the end of 50 days when a fall of about 34.5% was observed. This decline in the ash level as assessed statistically was significant at $P<0.02$. A reciprocal relation also seemed to exist between ash and water contents. The regression analysis of this relation gave the logarithmic equation:

$$\text{Log } A = 2.5900 - 1.3605 \log M$$

where, A=ash %, and M=moisture %; 'r'=-0.970 ($P<0.001$).

Nitrogen equilibrium. A progressive decline in the nitrogen level was associated with the period of starvation. As the replenishment of nitrogen from the exogenous sources was cut off, the amount excreted obviously became an index of the quantity of the tissue protein catabolized. During the present study, the amount of nitrogen excreted at each 10-day interval up to a total of 50 days of starvation was 82.2 mg, 203.6 mg, 371.6 mg, 426.0 mg and 485.2 mg, corresponding to a weight loss of 5.8 g, 6.5 g, 8.1 g, 9.6 g and 10.4 g. This gave an average value of 9.7 mg nitrogen excreted per day and a weight loss of 209.7 mg in one day. This means an excretion of 9.7 mg endogenous nitrogen indicates a loss of 209.7 mg of indispensable body tissue (ratio=1:0.05). A logarithmic transformation of the amount of nitrogen excreted and decline in terms of actual loss of body weight gave the equation:

$$\text{Log } N = -0.0917 + 2.7926 \log W$$

where, 'N'=amount of excreted nitrogen (mg) and 'W'=weight loss (g); 'r'=0.933 ($P<0.001$).

Energy losses. The energy losses corresponding to the mobilization of protein and fat were estimated in specimens adjusted to 50 g initial body weight. A decline in the energy content with the period of starvation was evident. Regression analysis of this energy loss could be expressed by the equation:

$$\text{Log } E = 1.9480 - 0.3610 \log D$$

where, 'E'=energy (calories) and 'D'=days of starvation; 'r'=-0.985 ($P<0.001$).

Discussion

Although decline in the total body weight was one of the basic manifestations of starvation, this was due mainly to the loss in the weight of the fillet which formed the bulk of the fish body and could be considered as the major site of accumulation of principal biochemical constituents. Under prolonged food deprivation changes in the fillet weight as expressed through 'fillet condition factor' indicated more accurately the processes of depletion going on in the body than the changes in the total body weight (Wilkins, 1967). In general, the

Table 1. Fillet condition and principal body constituents of *Heteropneustes fossilis* starved for various durations. Values are mean ± standard error.

Days of food deprivation	Number of observations	Fillet condition factor	Loss in body weight %	Protein %	Fat %	Moisture %	Ash %
0	10	3.682±0.088	—	16.958±0.115	2.787±0.029	77.833±0.526	1.350±0.076
10	10	2.771±0.366	11.710	16.249±0.476	2.014±0.121	78.800±0.076	1.250±0.028
20	10	2.471±0.211	13.199	14.791±0.275	1.334±0.065	79.466±0.044	1.166±0.044
30	10	2.242±0.198	16.246	12.812±0.737	1.014±0.042	79.566±0.072	1.083±0.045
40	10	1.957±0.180	19.208	12.447±0.376	0.848±0.124	79.991±0.247	0.966±0.061
50	10	1.749±0.143	20.975	11.770±0.375	0.521±0.096	81.500±1.395	0.883±0.033

Table 2. Absolute concentrations (g) of principal chemical constituents and the energy content (calories/specimen) in *Heteropneustes fossilis* (model of 50 g initial weight) during starvation.

Days of food deprivation	Number of observations	Principal chemical constituents				Energy content
		Protein	Fat	Moisture	Ash	
0	10	8.124	1.007	39.400	0.625	42.673
10	10	7.172	0.889	34.786	0.551	37.672
20	10	6.419	0.578	34.489	0.506	31.692
30	10	5.365	0.424	33.319	0.453	25.260
40	10	5.027	0.342	32.312	0.390	24.176
50	10	4.650	0.205	32.163	0.348	20.971

decline in this factor during starvation was characterized by a cumulative loss in the weight indicative of fall in the absolute levels of solid principal constituents of the body. A sharp depletion of fat from the very start of starvation revealed the preference with which this high energy constituent was drawn upon to meet the metabolic requirements of the fish. The declining values of fat were accompanied by a reciprocal increase in the degree of hydration of the tissues. Such an inverse relationship between fat and water during starvation has also been reported by Love (1958), Wilkins (1967) and Niimi (1972). The assumption that by increase in the proportion of one of these constituents with a simultaneous decrease in other, the sum of the two remained relatively constant (Black and Schwartz, 1950; Idler and Bitners, 1959; Coppini, 1967; Love, 1970) was found to be true during the period when much of the calorific requirements were maintained at the expense of endogenous fat. In the succeeding stage when the fat percentage was reduced to as low as 0.5% the constancy in the sum of fat plus water was altered. This could be due to the mobilization of protein and hence to protein-water effect. But as this difference in the sum of fat+water was only 1.128%, the fat/water relationship did not deviate to any great extent from the straight line obtained by the logarithmically transformed data.

Inasmuch as the fat percentage was only 2.787% on a fresh weight basis, protein also seemed to be mobilized. Changes in protein content, though little, started from the commencement of starvation while a sharp decline during the later stages indicated that the declining fat reserves could not sufficiently provide energy for the metabolic activity, and perhaps this might have stimulated the enhancement of muscle protein mobilization before the tissues were completely depleted of their fat content. Reduction in the endogenous protein before complete utilization of fat has also been reported by Templeman and Andrews (1956). Metabolism of protein during starvation thus appeared to be a requisite for sustaining the life of the fish particularly when concentration of this constituent was considerably higher than that of the fat.

Since exogenous sources of the energy food stuffs were cut off, the depletion of both protein and fat during starvation indicated the proteolysis and lipolysis of the essential endogenous protein and fat, with a resultant disruption of cellular metabolism (Wilkins, 1967). Steady decline of fillet condition factor during starvation was a reflection of this loss in tissue weight.

The protein breakdown resulted in a fall of nitrogen level in the body. This decline not only represented an alteration in the proportion or percentage of total nitrogen, but an actual decrease in its amount. Concordant to this observation was the finding of Wilkins (1967) on the herring *Clupea harengus*, which was even more fatty than the fish species studied here. Since nitrogen is regarded as the most characteristic and constant element of protein and of the products of protein metabolism, it was possible to determine the overall metabolism of protein by determining the so-called nitrogen balance. This nitrogen balance could be maintained through intake of proteinaceous food (Stanley, 1974). During food deprivation, however, the nitrogen loss could not be replenished and the conditions of negative nitrogen equilibrium were bound to prevail.

In fish of 50 g initial weight the rate of endogenous nitrogen excretion during 50 days of starvation averaged 9.7 mg N/day. As this excretion was exclusively at the expense of structural proteins which in the investigated fish contributed more to the total weight than the fat and ash combined, the quantity of nitrogen lost could be used as an index of the tissue loss.

The energy losses which run parallel with the period of starvation could be equated to the total energy equivalents derived from the amounts of indispensable tissue protein and fat utilized by the fish.

It was clear from the present investigations that the percentage of ash declined with the duration of starvation. This was so consistent with increase in the percentage of water that the dynamics of the change in one of these constituents reflected an inverse sequence of variation in the other. Divergent views have, however, been expressed as to the changes in ash content during starvation. While some workers (Phillips et al., 1960; Wilkins, 1967; Niimi, 1972) observed increase in the amount of ash during starvation, the others (Kordyl, 1951; Love, 1958; Love et al., 1968) reported decline in the ash content of the body. It was rather striking that ash was reported to increase in cases where whole fish including the bones and the skin was analysed, while in specimens where bone- and skin-free muscle was examined the ash was seen to decrease with starvation. It was, therefore, evident that the bones and skin known to be rich in ash (Young and Lorimer, 1960; Love, 1970) increase in relative proportion and become sources of increase in the whole body ash content. However, in the fillets cleared of bones and skin the fall in the percentage of ash was an invariable accompaniment of starvation.

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Heteropneustes fossilis の飢餓状態下の主要体成分の変動

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14±2°C で飢餓におき，10 日毎に 50 日まで調べた．体重の減少と共に，筋肉の蛋白質と脂質は水解されてエネルギーとして消費され，生理的危機状態を来した．餌料の補給が行われないと，筋肉蛋白質が消費されて形成された窒素代謝物は排泄され，負の窒素出納を来した．この時にみられた体内化学成分量とエネルギーの喪失および組織水分量の増加と無機物量の減少について述べた．