Tolo 3

UTILIZATION OF THE PRINCIPAL NUTRIENTS FOR ROUTINE METABOLISM IN ATIPA (*HOPLOSTERNUM LITTORALE*) ON STARVATION

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ABSTRACT

Atipa (*Hoplosternum littorale*), a tropical fish from South America, was starved for 50 days. Changes in whole body composition (dry matter, proteins, amino acids, minerals, fats, and energy) were studied in order to assess the utilization of such nutrients for routine metabolism.

The intensity of catabolism decreased with the duration of the fast. During the first 10 days the daily losses amounted to 0.55 g, 0.29 g, and 0.14 g, respectively for fresh body weight, dry matter, and proteins per 100 g initial body weight. During the following 40 days, daily losses were on average half of the previous values. There was remarkable conservation of certain nutrients including mineral and lipid reserves and amino acids.

There were two very distinct phases in energy utilization. Lipids played an important role in energy supply during the first phase but, in the long term, routine energy requirements are met mainly by protein mobilization.

Hoplosternum littorale (vernacular French name: Atipa) is an armoured catfish of the family Callichtyidae, of wide distribution in marshes and flood plains of the northern part of South America.

Although information is available on the behaviour of this species, data on its biology are very scarce (Sing, 1977; Machado-Allison, 1987; Winemiller, 1987; Gautier *et al.*, 1988), and nothing is known about its nutrition and feeding.

The present paper considers some aspects of the fasting metabolism in this fish. Studies on endogenous losses of nitrogenous compounds, fats, and energy by carcass analyses, besides being easy to implement, are of practical value for obtaining data on routine requirements for metabolism, and on the contribution of body stores to energy supply (Kaushik and Luquet, 1977).

The present work deals with the changes in Atipa body proteins, amino acids, fats, energy, and minerals during 50 days of starvation.

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MATERIALS AND METHODS

Immature, six-month-old Atipa were obtained from our experimental hatchery at Kourou (French Guiana). Fish were reared in ponds at low densities and fed dry pellets (45% crude protein). Fish were kept fasting a day prior to pond drainage, and thereafter were immediately transferred to the laboratory, hand sized, weighed, randomly sorted, and assigned to. indoor concrete 1 m^3 tanks. During the experiment, the temperature of the recirculating flowing water (25 m^3/h for the total experimental facilities) ranged between 28 and 30°C.

Samples

Initially, and then on the 5th, 10th, and 50th days, 10 fish were collected at 17:00 hours, drained, weighed, pooled, and immediately grounded with a meat grinder. The lateral armour bony plates required a further grinding with an Ultra Turrax homogenizer. After mixing and sampling for dry matter determinations, the product was freeze-dried.

Analytical determinations

Dry matter: in an oven at 107°C for 24 h.
Ash: in a muffle furnace at 550°C for 18 h.
Crude lipids: ether extraction (soxhlet).
Minerals: using plasma torch after mineralization at 550°C.
Proteins: as Kjeldahl N after acid digestion.
Amino acids: after acid hydrolysis (6N HCl; 24 h; 110°C) using a Varian amino acid analyser HPLC, according to the manufacturer's recommendations for protein hydrolysates. Tryptophan could not be analysed as it was destroyed by acid hydrolysis.
Energy: using an adiabatic bomb calorimeter (Gallenkamp).

No statistical treatment was done as all determinations were performed on pooled samples.

RESULTS

Weight and gross body composition changes

Body weight variations and proximate composition changes are given in Table 1. Recalculations were made assuming an initial wet body weight of 100 g in order to follow the changes of each body nutrient content. These data are reported in Table 2.

As illustrated in Fig. 1, Atipa lost very little fresh weight during starvation. For the whole period (50 days) a 100 g fish lost, on an average, 0.3 g/day. The body weight decrease was higher during the first 10 days (0.5 to 0.6 g/day) than in the following 40 days (0.25 g/day).

The water content increased only very slighty during the first five days, and then the dry matter proportion remained remarkably constant (37.3 to 35.5%). After an initial decrease of the dry matter during the first five days (0.36 g/day), daily losses decreased to only 0.09 g per day during the last period.

Table 1. Changes in body weight and proximate body composition of *H. littorale* during starvation

	Days of fasting			
	0	5	10	50
Body weight (g)				
initial	73.3	74.8	73.5	75.7
final	73.3	72.9	69.4	64.1
Dry matter (%)	38.1	37.3	37.3	37.5
Protein (N × 6.25) (% DM)	51.2	51.6	51.3	48.4
Fat (% DM)	19.9	18.2	17.9	20.4
Ash (% DM)	24.1	25.3	25.2	28.3
Energy (kJ/g DM)	20.3	20.1	19.5	19.1

Chemical determinations were performed in triplicate on pooled samples of 10 fish.

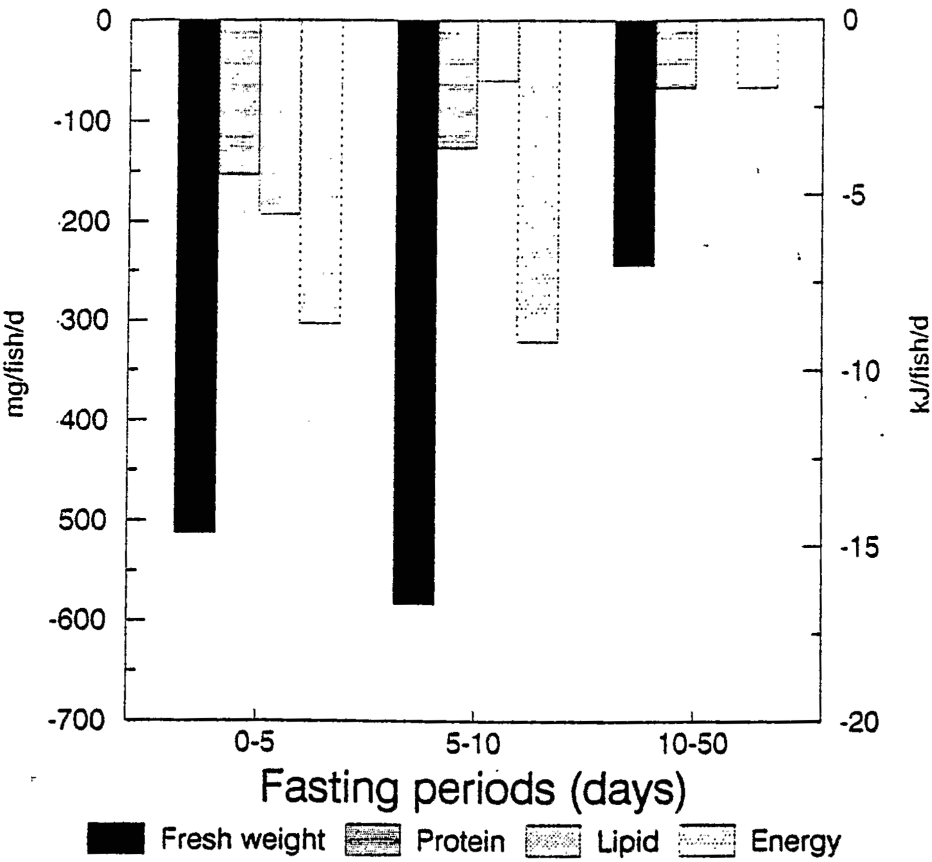

Fig. 1. Daily losses for fresh weight, proteins, lipids and energy, occurring during the different fasting periods (in mg or kJ/fish/day)

Table 2. Changes in body weight and composition of a standard fish of 100 g initial body weight

	Days of fasting			
	0	5	10	50
Fresh body weight (g)	100	97.4	94.5	84.7
Dry weight	38.1	36.3	35.3	31.8
Total protein (g)	19.5	18.7	18.1	15.4
Total fat (g).	7.6	6.6	6.3	6.5
Total minerals (g)	9.2	9.2	8.9	9.0
Total energy* (kJ)	775.2	731.7	685.7	607.5

*Measured energy.

Proteins

Proteins represented around 50% of the dry matter. The daily losses decreased with fasting from 0.16 g/fish/day during the first five days, to 0.07 g/fish/day for the last period.

Amino acids

Changes in amino acid composition are shown in Table 3. Essential amino acids (EAA) represented 45% of total amino acids (TAA) in both initial and 50th-day batches. Percentages were slightly higher (46 to 49%) in the other samples. Four EAA were prevailing: lysine, arginine, leucine, and the complex phenylalanine + tyrosine. Each one represented 15 to 18% of EAA. Arginine appeared to be the more consistent if expressed as per cent of the sum of EAA. No trend of variation in the relative proportions of EAA was observed during fasting, except for leucine, of which the proportion decreased evenly. At the end of the 50 days of fasting, Atipa lost 28% of leucine, whereas the final amount of the other individual amino acids represented from 80 to 90% of initial values.

Lipids

Fat content was very high (20% of the dry matter). Fat depletion was observed only during the first five days (0.19 g/day/fish); subsequently, total body fats remained more or less constant.

Minerals

Carcass mineral concentrations were very high and increased during fasting from 24 to 28% of dry body weight.

In spite of the body weight decrease, a relatively constant value of 9 g of total minerals per fish (of 100 g initial body weight) was noticed. Analytical composition of ashes for major minerals is given in Table 4.

Calcium accounted for 8 to 10% of dry body weight, and phosphorus for 4 to 5%. Calcium to phosphorus ratio showed a remarkably constant value of 2 irrespective of the duration of the fast.

Energy

Whole body energy, when measured by bomb calorimeter, represented 19 to 20 kJ/g DM. During starvation, average daily losses amounted to 3.3 kJ/day in a 100 g initial body

Table 3. Amino acid composition of the whole body of *H. littorale* during fasting

	Initial			Days of fasting									Final/initial[4] × 100
				5			10			50			
	g/16 g N[1]	%EAA[2]	%ARG[3]	g/16 g N	%EAA	%ARG	g/16 g N	%EAA	%ARG	g/16 g N	%EAA	%ARG	
Lysine	6.5	15.5	94	6.4	13.9	84	8.2	18.1	116	7.5	17.0	100	92
Arginine	6.9	16.5	100	7.6	16.5	100	7.1	15.6	100	7.5	16.9	100	85
Histidine	1.8	4.4	27	3.1	6.7	41	2.2	4.9	31	2.3	5.2	31	85
Isoleucine	2.9	7.1	43	4.0	8.8	53	3.8	8.3	53	3.0	6.8	40	81
Leucine	6.9	16.6	100	7.3	15.9	96	6.6	14.5	93	6.3	14.3	85	72
Valine	3.6	8.5	52	4.7	10.4	63	4.5	9.9	63	3.7	8.5	50	83
Methionine	2.4	5.8	35	–	–	–	2.3	5.1	33	2.7	6.1	36	87
Phenylalanine + Tyrosine	6.5	15.6	95	7.0	15.2	92	6.4	14.0	90	6.6	14.9	88	79
Threonine	4.1	9.9	60	4.7	10.3	62	4.3	9.4	60	4.5	6.7	40	86
Aspartic acid	11.8			12.0			10.4			10.9			
Glutamic acid	14.9			15.1			13.2			17.1			
Serine	5.6			5.7			4.7			5.2			
Glycine	10.9			11.6			11.5			13.5			
Alanine	7.9			8.4			7.6			8.2			
EAA/TAA	44.9			46.3			48.9			44.5			

[1]amino acid/100 g protein.
[2]amino acid % sum of EAA.
[3]amino acid relative to arginine content.
[4]final amino acid content relative to initial content in a 100 g initial body weight fish.

Table 4. Mineral composition of *H. littorale* during experimental period (% DM)

	Days of fasting			
	0	5	10	50
Phosphorus	4.2	4.4	4.5	4.9
Calcium	8.4	8.9	9.0	9.8
Magnesium	0.2	0.2	0.2	0.2
Potassium	0.7	0.7	0.7	0.7
Sodium	0.3	0.4	0.4	0.4

weight fish. Over the whole period, the decrease was 21%. Energy losses were higher during the first 10 days (8 to 9 kJ/day) than during the last 40 days (2 kJ/day).

DISCUSSION

Peculiarity of Atipa

Due to the presence of a dermal skeleton (armour), Atipa presents some very pronounced features. Bony parts (head and bony plates) account for 55 to 60% of fresh body weight, and the muscle represents no more than 30% of body weight.

It would be of value to study the phosphorus and calcium metabolism of such a fish which lives in fresh water in French Guiana where water conductivity is very low (only 20 to 50 μmhos, equivalent to 30 to 70 ppm mineral content: Gosset, personal communication). Given the high carcass mineral content, and the little contribution from dissolved mineral sources of surrounding waters, dietary requirements for calcium and phosphorus should be higher than those of other warm-water fish.

Protein and amino acids

Amino acid composition of Atipa does not vary greatly during starvation, even if the EAA/TAA ratio varies slightly within the samples. When comparing the mean amino acid composition (average of values, in percentage of their sum) of whole Atipa to values for rainbow trout and channel catfish (from data of Wilson and Cowey, 1985), there is a remarkable agreement (Table 5). The amino acid requirements of Atipa, when expressed as percentage of EAA, do not appear to present any peculiarities, if we accept the concept that the pattern of amino acid deposition in tissues should be a useful guide to the pattern of amino acid requirements (Cowey and Luquet, 1983; Wilson and Cowey, 1985).

Measurement of changes in the proximate composition of fish during fasting generally provides higher, but more reliable, values of endogenous nitrogen losses than direct measurement through balance studies (Luquet and Kaushik, 1981).

The observed values for endogenous nitrogen losses range from 200 mg/kg/day for the first 10 days to 120 mg/kg/day during the whole experimental period. These values show a remarkable concordance with data previously compiled by Luquet and Kaushik (1981) for a large number of teleosts.

Table 5. Essential amino acid composition of the whole body of *H. littorale* compared to values for rainbow trout and channel catfish (in % of their sum).

	Atipa[1]	Rainbow trout[2]	Channel catfish[2]
Lysine	16.1	16.9	17.4
Arginine	16.4	12.7	13.6
Histidine	5.3	5.9	4.4
Isoleucine	7.7	8.6	8.8
Leucine	15.3	15.2	15.1
Valine	9.3	10.1	10.5
Methionine	5.6	5.7	6.0
Phenylalanine + Tyrosine	14.9	16.4	15.2
Threonine	9.1	9.5	9.0

[1]average of values from Table 3.
[2]from data of Wilson and Cowey, 1985.

Energy

Measurements made with a bomb calorimeter should provide more accurate values in comparison to those based on calculation of the energy values of lipids and proteins. Energy derived from carbohydrates is often neglected because the glycogen stores in fish are very small (Love, 1970). In the present study, the carbohydrate component was not taken into account though the sum of proteins, fats, and ashes does not give the exact amount of dry matter recorded. The calculated nitrogen-free extract (NFE) which represents carbohydrate accounts for 2.6 to 4.7% of body dry matter. Even though quite high, this undeterminated body compartment remains in the range of the errors of determinations.

To compute the energy content of body stores, the Atwater values for heat of combustion are used as 38.2 kJ/g for lipids and 19.8 kJ/g for proteins (Lloyd *et al.*, 1978). Differences between the measured values for total energy (TME = total measured energy) and that calculated by summing up energy from proteins and from fats (TCE = total calculated energy) represent here from 9 to 15%, which is not negligible. In addition to the possible error due to the neglected carbohydrate component, the discrepancy may also result from the assumption that all proteins contain 16% nitrogen. Consequently, in the following tables, results are discussed taking into account both calculated and measured total energy values.

Energy is stored mainly as proteins. Indeed, protein energy represents 50 to 52% of TCE or 55 to 60% of TME (Table 6).

During 50 days of starvation, Atipa with 100 g initial body weight lost 124.4 (TCM) or 167.7 (TME) kJ, which corresponds to between 18 and 21% of initial energy. The daily energy losses varied between 25 and 33 kJ, for the 50 days of fasting. In fact, as for all nutrients, daily energy loss rapidly falls from 105 to 12 kJ/kg/d. Basal or routine metabolism (Fry, 1957) of this tropical fish is in good agreement with the energy requirements (29 kJ/kg/d), derived from O_2 uptake, reported for temperate species by Brett and Groves (1979). However, these values are lower than the values (50 kJ/kg/d) given by these authors for tropical fish at 26°C. Atipa appears to have quite low routine energy requirement, and should not be considered as an active fish despite its frequent surfacing for air breathing (Boujard *et al.*, 1990).

Table 6. Energy schedule in fish

Days of starvation	0	5	10	50
Energy measured kJ/g DM	20.3	20.1	19.5	19.1
Energy calculated kJ/g DM	17.7	17.2	17.0	17.4
Measured energy	775.2	731.7	685.7	607.5
Calculated energy	675.9	623.5	599.3	551.7
Relative contribution of proteins and lipids to total energy content				
% of energy measured				
Proteins	50	51	52	50
Lipids	37	35	35	41
% of energy calculated				
Proteins	57	59	60	55
Lipids	43	41	40	45

(1) coefficients (Lloyd *et al.*, 1978)
Lipids: 38.2 kJ/g.
Proteins: 19.8 kJ/g.

Lipids seem to play an effective role as energy suppliers only during the early stages of fasting. They contribute 70 to 85% to total energy losses during the first five days, but their contribution falls to 25 to 50% during the five following days. Thereafter, body lipid content remains constant and all basal energy requirements are supplied by endogenous protein oxidation.

A question arises about the metabolic role of stored body lipids which represent 35 to 45% of total energy stores. It may be suggested that they have a major role in releasing energy for possible long fasting periods during the dry season, but the present data show that lipids play only a marginal role in such a function.

As for most fish, proteins are the most effective energy source for long-term starvation, during the slow phase of catabolism (Table 7).

Table 7. Percentage of total energy derived from proteins during starvation

Days	0–5	5–10	10–50	0–50
as % of measured energy	35	28	69	49
as % of calculated energy	29	53	100	66

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