POSTPRANDIAL UTILIZATION OF ENERGY SUBSTRATES BY A TROPICAL CATFISH, *HOPLOSTERNUM LITTORALE*: INDIRECT CALORIMETRY ANALYSIS

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ABSTRACT

Indirect calorimetry method was used to measure postprandial utilization of energy substrates by atipa, *Hoplosternum littorale* (Callichthyidae, Siluriform). A model was adjusted to the data on respiratory exchanges and nitrogen excretion for this purpose. As for other fish species, protein was actively catabolized after feeding during the 60 hr measurement time. As long as carbohydrates were available, atipa was also able to use these substrates for its energy metabolism. A gradual increase of contribution of lipids was then noticed. Over a 24 hr period following the food intake, a 30 g fish catabolized 90 mg of proteins and 43 mg of carbohydrates. At the same time, the results led to a synthesis of 3.7 mg of fat. An estimation of the increase of the global metabolism associated to food intake was obtained from integration of the exponential terms of the model.

In fish, as in other animals, necessary nutrients for growth are provided by food. But food also provided fuels required by the energy metabolism. Among the three different energy substrates (proteins, lipids, and carbohydrates) which could be used by living beings, fish are reported to mainly use proteins even if they can burn fat too.

Referring to their final destiny, the best energy substrates must be those which are not concrete parts of growth products. In this way, non-protein energy substrate, such as fat and carbohydrates, should be more adequate. The apparent poor retention of non-protein substrates, observed among tropical fish, indicates their better utilization for energy needs (Luquet and Moreau, 1989). As they disappear, they are not misused by transformation into body fat for example.

To obtain both qualitative and quantitative evaluations of energy substrates utilization by fed animals, indirect calorimetry is the only available method. This method was already applied to several fish species (Gnaiger, 1983; Van Waversveld *et al.*, 1988; Kaushik *et al.*, 1989; Médale and Kaushik, 1991). It is based

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on respiratory gas exchanges which reflect cellular oxidation of energy-supplying substrates, and on nitrogen excretion arising from catabolism of nitrogen compounds. One of the first trials in this line was made by Kutty (1972) to study the metabolic rate associated with swimming activity in *Oreochromis mossambicus.* The attractions of this method in evaluating the standard metabolic rate of fish have been underlined by Brafield (1985) and compared to direct calorimetry by Van Waversveld *et al.* (1989).

Atipa (*Hoplosternum littorale,* Hancock 1828) is an air-breathing siluriform fish of the Callichthyidae family living in the marshy areas of northern South America. So far, the data on this species mainly dealt with its respiratory system (Carter and Beadle, 1931; Gee and Graham, 1978), its behaviour (Winemiller, 1987; Gautier *et al.*, 1988; Boujard *et al.*, 1990, Boujard and Meunier, 1991), its rearing, and its feeding (Luquet *et al.*, 1989; Moreau and Luquet, 1991). In earlier studies, the utilization of endogenous substrates was observed following body losses during starvation (Luquet *et al.*, 1991) or using the indirect calorimetry method (Moreau *et al.*, 1991). The aim of the present work is to identify and quantify the energy substrates supplied by food and used by this tropical catfish.

MATERIALS AND METHODS

One month before trials, fish reared in our experimental fish farm (Kourou, French Guiana) were transferred to the laboratory in a close circuit system. Trials were performed on five batches of five fish (31.8 g mean weight). Each batch was kept in 200 l tanks and submitted to natural photoperiod. Fish were fed daily with commercial food pellets at 3% of their body weight (Aqualim, 45% crude protein). The food was given at the end of the night, in accordance with their natural rhythm of food intake (Boujard *et al.*, 1990). To be sure of a good food consumption, the fish were kept for at least 1 hr in contact with food. They were then carefully transferred into a respirometer which allowed the measurement of exchanges occurring in water and in air, as previously described by Moreau *et al.* (1991). Ammonia excretion was used to determine total nitrogen excretion since urea excretion was found negligible. Oxygen uptake (from air and water), carbon dioxide, and ammonia excretion were measured, every 2 hr for 48 hr. The postprandial 60 hr period was covered by selecting the time of transfer for each fish group (Feeding time + 1 hr, batch 1; FT + 3 hr, batch 2 and 3; FT + 12 hr, batch 4 and 5).

If calculations are made from a set of uptake or excretion data observed at the same time, some discrepancies can occur. As exchanges do not necessarily take place at the same time, a models was adjusted to the data to describe the general tendency of uptake and excretion during the whole measurement period. A delay between O_2 uptake and CO_2 excretion might result from an increase in fish activity (Wood and Perry, 1985, cit. in Playle *et al.*, 1990) as during bursts through the surface for air breathing or from the air breathing itself, some of the oxygen measured as uptake being trapped in air bubbles before their use.

Food intake induces a large income of metabolites, which are gradually absorbed by metabolism. An exponential model is often used to describe this kind of process involving a steady treatment (or utilization) of products. But, as this formulation tends towards zero, it does not take into account a routine level reached at the end of the process. The model designed to adjust the data was then as follows:

$$y = b_0 + b_1 e^{-rt},$$

where y is the expected value of oxygen uptake, carbon dioxide, or ammonia excretion, and t the time in hours. b_0 can be assimilated to the routine metabolism rate, b_1 to the income associated to feed intake, and r to the rate of the different metabolism processes.

The analytical methods were the same as those described in Moreau *et al.* (1991). Energy expenditure and quantity of oxidized substrates were calculated according to Van Waversveld *et al.* (1988). The SAS statistics software package has been used for statistical inference.

RESULTS AND DISCUSSION

Postprandial gas exchanges and ammonia excretion are plotted on Fig. 1. The postprandial increase of oxygen uptake and ammonia excretion usually described (Brett and Groves, 1979; Vellas, 1981) was not observed. This step should be missing as the first observations were made in fact 3 hr after feeding, i.e., sooner transfer time + 2 hr. All exchanges were only decreasing steadily over the measuring period to level off at the routine metabolism rate.

The equations obtained for the different models are reported in Table 1. From these three equations, the types and amounts of energy substrates used by fish have been calculated (Fig. 2). Just after feeding, proteins are actively catabolized and their utilization decreases during the entire period of measurement to reach a level of about 50 mg/kg/hr after 58 hr. During the first 14 hr after feeding, a large amount of carbohydrates is also catabolized. At the same time, the observed RQ values come to a negative value for lipids which could be interpreted as a synthesis. Afterwards the lipids begin to be involved in the energy metabolism and their contribution increases as the involvement of carbohydrates tends to disappear. Fifty-five hours later, the carbohydrates are no longer used and lipids contribution represents about 16 mg/kg/hr. The amounts of substrate oxidized by a fish during the post-feeding periods can be calculated from the integration of the equations obtained for O_2 uptake and CO_2 and NH_3 excretion (Table 2). Furthermore, the contribution of each substrate to the total energy expenditure is deduced using the heat of combustion given for them (Brafield, 1985).

Proteins are an important source of energy for atipa, as for other species. But this species is also able to burn carbohydrates. As carbohydrates are nearly absent from the whole body composition (Luquet *et al.*, 1991), carbohydrates utilization must be related to their availability from feed. Increase in the carbohydrates level of feed is generally associated with a high body fat deposition

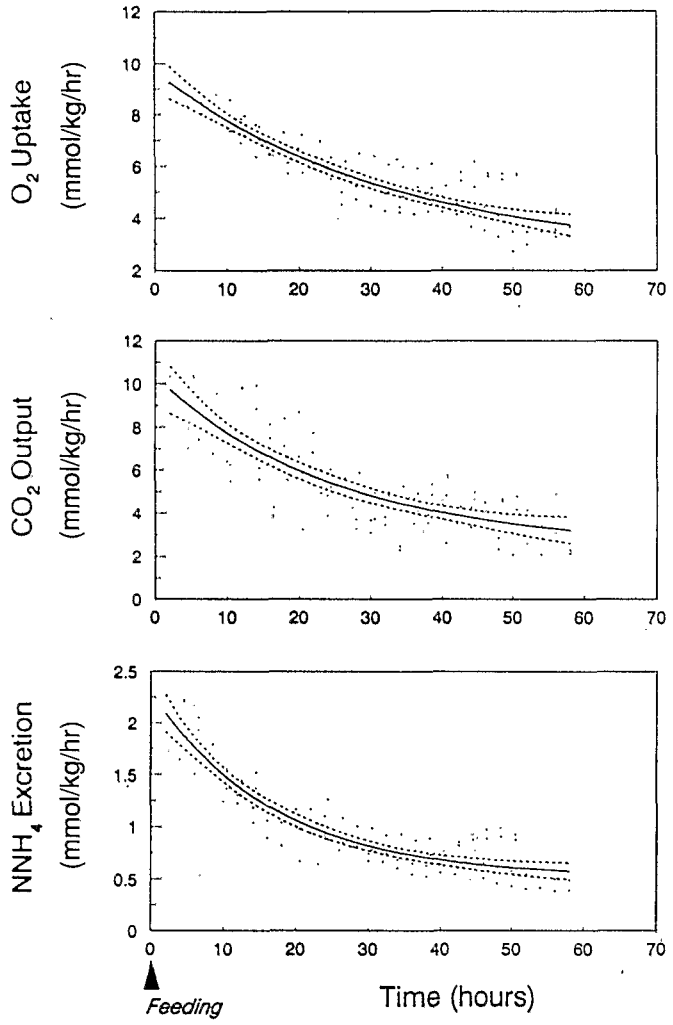

Fig. 1. Evolution of oxygen uptake, carbon dioxide and ammonia excretion in *H. littorale* during the hours following food intake. The solid lines represent the adjustment of the data to a model, $y = b_0 + b_1 e^{-rt}$, and the broken lines are the 95% confidence levels of the means.

Table 1. Values obtained after adjustment of gas exchange and nitrogen (in μmol/kg/hr) to a model, $y = b_0 + b_1 e^{-rt}$ (with t in hours). n is the number of observations. Significance is given by the probability of finding an F statistic greater to f calculated between mean squares explained by the model and residuals mean squares

	b_0	b_1	r	n	Prob ($F > f$)
O_2 uptake	2,591	7,114	0.03	100	< 0.001
CO_2 excretion	2,454	7,893	0.04	96	< 0.001
NH_3 excretion	524	1,772	0.06	97	< 0.001

(Steffens, 1989). In this way carbohydrates will not be consumed as a fuel but

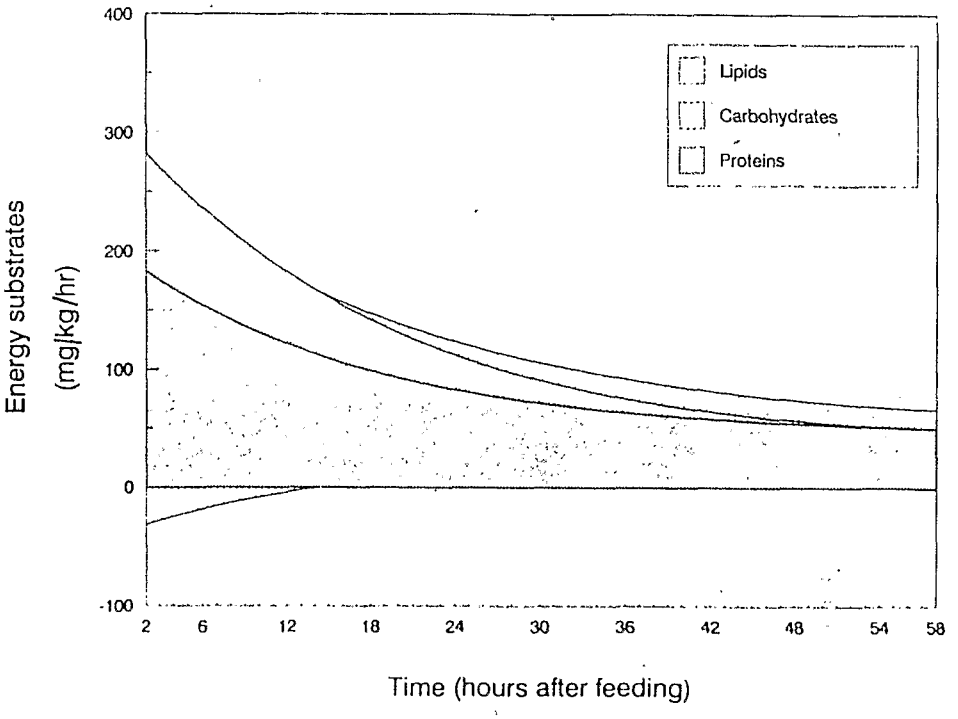

Fig. 2. Utilization of different energy substrates by *H. littorale* during the hours following food intake.

Table 2. Utilization of different substrates and their contributions to the total energy expenditure by *H. littorale*, after a meal. The values are given for 30 g fish. Energy equivalents are calculated from the heat of combustion values given by Brafield (1985): 19.67 kJ/g for proteins to ammonia, 39.5 kJ/g for lipids and 17.2 kJ/g for carbohydrates.

Hours after feeding	Substrates	Oxidized amount (mg)	Energy contribution (% of total)
1–14	Proteins	57.6	69
	Lipids	(6)	
	Carbohydrates	30	31
15–54	Proteins	84.6	62
	Lipids	16.8	25
	Carbohydrates	21	13
55–60	Proteins	7.5	61
	Lipids	2.4	39
	Carbohydrates	(.09)	

transformed as fat. Based on one meal per day, total amounts of involved carbohydrates and lipids can be evaluated from integration over 24 hr. For a 30 g fish, this will give 43 mg of catabolized carbohydrates and simultaneously 3.7 mg of

produced fat. If all food was ingested and fish fed twice daily, the values would correspond to the post-feeding period of 1–14 hr (Table 2).

The amount of carbohydrates needed to produce such an amount of fat can be estimated from metabolic fatty acid synthesis pathway. Three moles of carbohydrates carbon are necessary to obtain 2 mol of fatty acid carbon (Stryer, 1988). Using the global formulas given by Gnaiger (1983) for carbohydrates and fat, 1 g of carbohydrate is required to make 380 mg of lipids. Even if fish were becoming more fatty, this could not be a simple transfer from carbohydrates. If so, the amount of deposit would be much higher. Thus, carbohydrates are used as energy supply.

Over a 24 hr period, the amount of catabolized protein is near 90 mg for a 30 g fish. This value represents about 22% of the protein supplied in the food, which is much less than the value previously reported on rainbow trout (*Oncorhynchus mikyss*) fed at a lower rate (Kaushik *et al.*, 1989), or those given for eel (*Anguilla anguilla*) or catfish (*Clarias gariepinus*) fed at a close level but continuously (Heinsbroek *et al.*, 1989).

Integration of the model for nitrogen excretion gives an estimation of the amount of catabolized proteins. For five days, a 100 g fish would use approximately 800 mg of proteins. This is close to the values of endogenous losses reported for the same species (Luquet *et al.*, 1991). Conversely, estimates for lipids are not consistent. This could largely result from differences in the general state of the studied fish. For the former fasting experiment, fish were taken directly from the rearing pond, whereas those of the present study stayed one month in tanks. Nevertheless, the great similarity between the estimates of protein utilization seems to indicate that this is quite reliable.

Studies on fasted fish show that the nature and amount of catabolized substrates were not constant over the fasting period (Luquet *et al.*, 1991). So, the metabolism of starving fish changes as fasting time increases. Therefore, metabolism of starved fish could not be strictly taken as an estimate of standard or routine metabolism. Thus, specific dynamic action, or more precisely metabolism rate associated with feeding (including rise of activity), cannot be calculated by the difference between fed and unfed fish. In the model used for the present study, the exponential term corresponds to the overall process of ingested food, whereas the constant term could be assumed as the routine metabolism of fed fish. In fact these values are much higher than those found in fish fasted for 10 days, as routine metabolism of fish had certainly changed during the fasting period. Moreover, the increase in metabolism resulting from food intake can be evaluated by the integration of the exponential term of the adjusted models. This leads to an energy expenditure of 2.97 kJ for a 30 g fish provided by utilization of 77.8 mg of proteins (52% of supplied energy), 27.6 mg of lipids (37%), and 19.6 mg of carbohydrates (11%). At the same time, 80% of the energy expenditure for routine metabolism (0.034 J/hr) came from protein catabolism.

In summary, these results confirm the main role of protein as energy-yielding substrate for fed *Hoplosternum littorale*, as for starved ones. In this regard, atipa does not seem to be mainly different from other species (Cho and Kaushik, 1990).

Besides, the effective contribution of carbohydrates to energy metabolism indicates that the metabolism of carbohydrate is active in this species.

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