

# Contribution to Protein/Energy Requirement and Metabolic Aspects for Two Carnivorous Species *Lates Calcarifer*, *Epinephelus Morio* Juveniles with Reference to Trout

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## Abstract

In nature, both species were identified as carnivorous and fed commonly on trash fish. These two marine fish were studied for optimum protein requirement and optimum energy content of compounded feeds. Those marine fish raised in tropical conditions showed some similarities in terms of protein requirement their metabolism differ en relation to swimming activity Swimming activity differed between the two species, active with *L.calcarifer* and quiet with *E. Morio* (200g av.wt or 86g MBW) that inferred on energy partitioning. Energy retention was high (40kJ/fish/d) for *E.morio* compared with other groupers or species such as *Cobia*, red drum. *L.calcarifer* data showed an optimum weight gain in tanks at 25mgDP/kJDE as reported previously.

From a 3D representation of weight gain at different DP and DE and DP/DE levels it was possible to identify such optima with possible extension for FCR and lipid accumulation. Such data helped to formulate feed for grow-out in floating cages. experimental diets for *E. morio* placed in running seawater tanks were formulated on a basis of digestible protein sources to measure its weight gain on a constant DP level and various sources of carbohydrates. Relative requirement was assess however the absolute requirement in terms of g protein per 100g biomass per day was 1.5g protein for 50kJ DE to reach a specific growth rate of 2.4% more useful from a practical point of view. In final all those values need each time to be reported to a well studied species such as salmonids RT (20g av. weight).

*Key words: seabass; grouper, energy budget, protein/energy ratio; RT.*

## Introduction

There is a considerable amount of data on fish bioenergetics, (Kleiber, 1975; Jobling, 1994); bioenergetics concerned with the energy transformation in living organisms is part of energetics generalized by the first and second law of thermodynamics (Brody, 1945). Main reactions for energy transfer in an organism imply that nutrient and oxygen remain available. The three phases of energy extraction from foods were described (Stryer, 1988) with  $\Delta H$ , enthalpia variation of interne energy of the system and  $\Delta G$ , free energy variation corresponding to energy utilized by fish. The transformation of energy substrates such as glucose or palmitate and indirect calorimetry was set with respirometer of different conception. The utilization of energy is produced under different levels of precision and according to intake, according to tropical or temperate origin, coefficients of Atwater and Brody, oxidation of protein and energy loss by excreta, the estimate of ME, energy value for nutrients, SDA (Beamish, 1975), NE (Smith, 1971) and input and output of energy metabolism according to Brett and Groves (1979), influence of nycthemeral rhythm, cost of swimming, abiotic factors, efficacy of utilization of energy for synthesis (Kaushik and Olive-Teles, 1986) and as noticed by Guillaume (1999), the main laws are described (Cho, 1992; Cho and Bureau *et al*, 2001; Lupatsch and Kissil, 2005) but lot of work remains to be done with candidates species for aquaculture such as seabass (Lee *et al.*, 1995; Guillaume, 1999).

Marine fish in tropical conditions used to be raised on trash fish. In case of seabass, it was not an adequate way to produce on a routine basis. It was then ultimately necessary to bring data part on bioenergetics with an emphasis on digestibility in case of seabass and heat production for grouper.

The plane of nutrition and energy losses in forms of feces, urine, SDA were described in cattle (Brody, 1945) and gave the lead for applying it to marine tropical fishes.

The purpose of this study was to compare two carnivorous species, for their ability to digest compounded feeds and calculate the amount of recovered energy whether by indirect calorimetry or carcass analysis in order to set some specifications for adequate feeds for grow out and limit wastes.

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## Material and methods

In the expression of the results, energy will be reported in kJ/kg fish/day or in kJ/kgMBW/day when metabolic weight is concerned (calculated with the coefficient  $b=0.84$ ).

For seabass ((a protandrous fish) research work conducted in Singapore all the methodology was previously described (Chou *et al.*, 1992, 1993); a whole installation was designed to run digestibility tests with conical bottom tanks of 200l and rectangular tanks for the comparison of diets on juveniles with a set of formulations including various protein and energy levels (Lee *et al.*, 1995).

Grouper (a gynandrous fish) maintained in Sisal facilities allowed to run each experiment to determine oxygen consumption in routine with 3 organisms taken in random for each treatment. Indirect calorimetry was indicated with 6 respirometry chambers set and one without animals chosen as a control. It avoided to include side effect due to bacteria or algae present on the walls or inside tubes.

$$VO_2 = \{([O_2]_{inlet} - [O_2]_{outlet}) * F\} - \{([O_2]_{inlet} - [O_2]_{outlet})_{control} * Flow\ rate\}$$

where  $VO_2$  gave oxygen consumption in  $mg\ O_2\ h^{-1}\ animal^{-1}$ ,  $[O_2]$  at entrance is concentration ( $mg\ O_2\ L^{-1}$ ) in water entering the chamber,  $[O_2]$  at outlet is concentration ( $mg\ O_2\ L^{-1}$ ) in water leaving the chamber,  $F$  is the flux of water ( $L\ h^{-1}$ ) through the chamber. N-ammonia produced by organisms were necessary was collected from water samples from each chamber and placed in Ependorff microtubes each hour, samples freeze-dried at  $-80^{\circ}C$  sealed until analysis.  $N-NH_3 = (N-NH_3(in) - (N-NH_3(out) * Flow\ rate)$ . The excretion can range from 20 to  $60mg\ g^{-1}\ h^{-1}$  according to the level of feeding, 60mg corresponding to a postprandial peak of excretion. After measuring  $O_2$  consumption and ammonia production, organisms were sacrificed, weighed to get their energy content in each triplicate.

## Results

P/E for seabass (Chou *et al.*, 1992)

The first step of energy partition and carcass analysis were the main parameters controlled to set the experiments leading to experiments for identification of P/E ratio for specific growth rate (SGR) expressed in g digestible protein per kJ digestible energy and then convert the amount of dietary DE per g digestible protein per 100g fish per day. This last expression gave a characteristic of the absolute requirement for seabass juveniles.

Seabass juveniles were pooled in conical bottom tanks to collect the feces with an automatic system (St Pée, INRA). This system allowed getting consistent results for the digestibility of ingredients such as soya bean meal and fishmeal.

Table 1. Results of digestibility of ingredients to formulate practical feed for seabass P/E determination (Chou *et al.*, 1993).

	ADC <sub>DM</sub>	ADC <sub>protein</sub>	ADC <sub>lipid</sub>	ADC <sub>energy</sub>
ref. diet	0.69	0.943	0.954	0.813
DK fishmeal		0.947	0.962	0.942
CPSP <sup>80</sup>		0.937	0.999	0.813
soybean meal		0.937	0.846	0.818

Protein and energy budgets in fish lead to an approach with carcass analysis that with 8MJ/kg(4 from protein and 4 from fat) is added to no fecal energy (0.6MJ to get the requirement in digestible nutrients (13.3MJ) with a DP/DE~24g/MJ, added to feces (1.2MJ). in final the summation produce a conceptual diet in a range of 19 to 14MJ according to nutrient density of the feed.

Energy partitioning: energy partition was calculated with the abbreviations of the international nomenclature and expression on metabolic weight (MBW) took into account the incidence of the law on metabolic mass for fishes. It allowed comparison of maintenance of weight or daily growth among species (Nijkamp *et al.*, 1974). For interspecific relations Feldman and Mc Mahon

(1983) would coincide with a coefficient  $b=3/4$ . However in practice its incidence is not so great. For fish juveniles the value  $b\sim 0.8$  is acceptable in the range of size considered.

## DE

Digestibility of energy is one of the first steps to define practical feed to run experiments and determine an optimum P/E of the feed to be prepared for an optimum SGR or an optimum FCR or a minimum of body lipid deposition after a 60d trial for example. For seabass two main available ingredients were selected such as quality fishmeal and soybean meal to stay with conventional approach in a first attempt and concentrate on the definition of some parameters such as protein and energy in the diet.

Table 2- ADC results % for ingredients and reference diet for *Epinephelus morio*.

	ADC <sub>protein</sub>		ADC <sub>energy</sub>	
	diet	ingredient	diet	ingredient
Reference diet	44		67	
Menhaden fishmeal	56	86	67	68
soyabean meal	48	57	70	77

Digestibility values for protein and energy were higher for fishmeal than soybean meal.

The reference diet was used to determine ADC protein for each major ingredient when incorporated at 30% in a test diet (70% ref. diet+30%ingredient) to find a significant difference between fishmeal and soybean meal, difference that disappeared for energy.

Comparative values were reported for the two species aside from data obtained with trout (table 3).

Table.3.Comparative values for diet, intake and digestibility

	<i>L.calcarifer</i>	<i>E.morio</i>	RT
av. initial weight g	100	200	20
rearing T°C	28	28	15
optimum DP %	50	45	42
optimum DE kJ/g	17	19	19
GE intake kJ/kg MBW/d	239	76	100
ADC <sub>energia</sub> %	80-86		85
DE intake	201	62	85

Initial weight differed from one species to another, however indications on diet composition were not so different in terms of protein and energy, and intake also, in order to be able to calculate DE intake and report in percentage the proportion of each component of energy budget.

#### Excretion (UE+ZE)

Excretion was measured in postprandial conditions (Fig. 1) and the production of N-NH<sub>3</sub> followed the dietary protein content. This peak of N-ammonia production followed the peak of oxygen consumption in postprandial conditions.

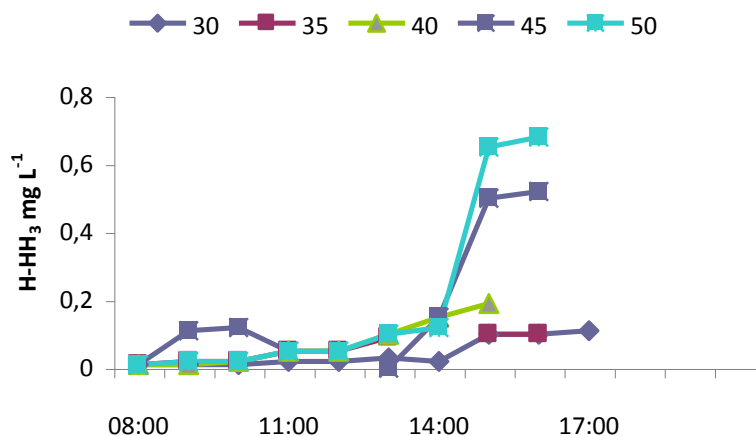


Fig 1-Production of N-ammonia (in mg N-NH<sub>3</sub> /L) by *E. morio*.in postprandial status fed 30, 35, 40, 45 and 50% CP experimental diets.

Postprandial excretion appeared to be related to dietary protein concentration, and animals fed at 2pm presented an increase in N-ammonia excretion following the peak of heat increment of

feeding and much clearly evidenced with the two most concentrated compounded feeds in protein (45 and 50CP). However, the trend of curves did not follow the general trend with a peak instead of a plateau. There appeared that only fish fed protein dense diets produced an increment in excretion, at the difference with fish fed all other treatments.

## ME

Metabolizable energy calculation was possible with N-ammonia excretion that changed with protein level and represented as with other species a low percentage of digestible energy. As in the above table the level of excretion appeared similar between marine species especially with seabass and RT.

Table 4. Comparative values for excretion

	<i>L.calcarifer</i>	<i>E.morio</i>	RT
(UE+ZE) kJ/fish	12	7	6

By and large, excretion in marine fish will not represent more than 6-8% of DE intake and it is verified for the two species and trout as a reference.

## HeE

Maintenance was addressed for *E.morio* with several fish because of the difficulty to feed a fish alone in the respirometric chamber and so results were given only under the form of heat production (HP) that indicate a relative higher level compared to seabass or trout and this measured need further refinement.

Energy spent in starvation was lower for seabass compared to trout and probably higher than for grouper.

## HiE

Heat increment of feeding was determined experimentally for *E.morio* and trout and by calculation for seabass. The expression was given in kg biomass or kg MBW and the biomass allowed maintain several fish especially for estimating the feed intake in the metabolic chamber.

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*E.morio* did not permit to separate between HiE and HeE. By and large the values for heat production were in the same order of magnitude with diets containing a level of protein that fits for carnivorous species.

Table 5. Ccomparative values for heat production.

	<i>L.calcarifer</i>	<i>E.morio</i>	RT
O <sub>2</sub> uptake g/kg/d			3.4
HeE kJ/kg MBW/d	40	8	37
HiE	44	12	15
HP	88	20	52

Heat increment of feeding varied in large proportion with species and also temperature or feed composition, and for *E.morio* in experimental conditions, only HP could be determined.

## RE

Retained energy indicative of potential weight gain was similar between seabass and trout however, *E.morio* had unexpected low value because probably non optimal rearing conditions in clear water tanks. Such a quite fish should present a good potential for growth.

Table 6. Preliminary results on energy partitioning for carnivorous species.

	<i>L.calcarifer</i>	<i>E.morio</i>	RT
RE kJ/kg MBW/d	101	35	27
RE/DE %	50	58	30
wt gain g/wk	2	1.4	5
GE fish kJ			8.6
ration allowance			85
kJ/fish/wk			

Related to DE retained energy values were in a range of 30-40% with trout or seabass and could improve with high ADC or optimum intake.

## Discussion

The results evidenced a difference in basal metabolism with a seabass on constant physical activity and a grouper pretty quiet fish hiding all the time. A slow fish required 200mW for a

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velocity  $\mu=2$  (body length per second) while a normal fish (seabass) could reach 400mW for a  $\mu=3$  (Pierce, 1977). The cost of swimming (km) will vary with a velocity and size of fish (Dabrowski, 1986). According to Brett and Groves (1979) a metabolism in full activity of a salmon could be a multiple of 11 compared to standard metabolism.

Energy spent by fish under starvation (basal metabolism) was determined generally after a week starvation and provided values with RT of around 37kJ kgMBW<sup>-1</sup>day<sup>-1</sup>. such value is compared with other species and between sturgeon, trout or *Atipa* 39, 57 and 48kJ/kg/day respectively indicating a contribution for 20% approx. in the energy partition for fish with lipids contributing the most to the supply of energy and nitrogen expenditure was in a range of 1.5 to 4.5 mg N/kJ that is significantly higher than for mammals (Guillaume, 1999). Active metabolic rates of fish are a 5-fold difference between spp (Brett, 1972) with a max at 1000mgO<sub>2</sub>/kg/h or 14.7kJ/kg/h or 353kJ/kg/day or 138 kJ/kgMBW/day.

Heat increment of feeding is certainly the part to address that can change in the contribution of energy partition with the nature of the feed, and the relative proportion of lipid and protein as well as the digestibility of the protein source.

Retained energy by fish in production and not only weight gain is a linear function of ingested ME (Guillaume, pers. comm.). It depends on respective importance of apparent synthesis of protein and lipid and the nature of energetic nutriment. In trout for example, with 17% protein+10%lipid in tissue there was an energy cost (energy value in kcal)/energy yield of apparent synthesis ( $b=kf$ ) of 2.7kcal (11kJ) expressed in ME.

Aside from energy partition another approach with carcass analysis was used as another option to verify the amount of retained energy by fish fed on a given feed composition.

Similar to what was done on *L. calcarifer*, several authors reported optimum for protein on *E. coioides* (Luo *et al.*, 2004), *E. aeneus* (Lupatsch and Kissil, 2005) and P/E ratio with *E. salmonides* (Teng, 1978), *E. malabaricus* (Tsai, 1991; Shiau et Lan, 1996) but generally the requirement was not expressed in 100g biomass per day to reflect an absolute requirement.

In terms of wastes management, it seems that heat production of feeding is not a term of energy budget to impact positively compared to N-ammonia excretion of digestibility of the feed. The feed intake is another term in the budget that takes importance, as said before, feed pollutes, fish doesn't not pollute and then the energy density of the feed should fit with the optimum intake to reduce losses with uneaten feed. The P/E ratio at its optimum showed a good level of intake and afforded to reach an optimum SGR for example 2.4% with seabass and FCR or optimum lipid deposition in the body.

## Conclusion

Seabass and grouper are two candidates species for cage culture butt the first species is already at a level of commercial production at the difference with this grouper species coming just from the wild with little information on its potential and requirements. Thee comparison enlighten a difference in metabolism due to a difference in behavior with a seabass in constant movement and a grouper quiet and resting all the time in captivity. However such difference is difficult to characterize with the indirect calorimetry and so both species tend to follow energy partition as previously found for marine fish species and similar in many points with a carnivorous species such as the well-studied trout.

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