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Amino acid requirements of Mediterranean fish species

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SUMMARY – The purpose of this paper is to review the available information on dietary amino acid requirements of Mediterranean fish species. Currently available information, although limited, shows considerable similarities between species in terms of essential dietary amino acid profiles. Data on quantitative requirements for maintenance and for unit protein growth show some degree of inter-species differences. We stress the need for using standardised methodologies in the determination of requirements. Given the importance of proteins and amino acids in fish nutrition and given the context of research on alternatives to fishmeal, much more concerted research on amino acid utilisation and metabolism is required, considering major critical aspects in their determination and suggesting topics of research in this field, to be addressed in the future.

Keywords: Mediterranean fish species, nutrition, amino acid, requirements.

RESUMÉ *– "Besoins en acides aminés des espèces méditeranéennes de poissons". L'objectif de cette présentation est de faire une synthèse de nos connaissances sur les besoins en acides aminés indispensables des poissons d'intérêt aquacole en Méditerranée. Les données disponibles montrent une grande similitude entre espèces dans les profils en acides aminés. En termes quantitatifs, des différences entre espèces existent quant aux besoins pour l'entretien ou pour l'accrétion protéique journalière. Compte tenu de l'importance de l'apport azoté en l'alimentation des poissons, et des enjeux liés à la substitution des farines de poissons par d'autres ingrédients, une meilleure connaissance des besoins et de l'utilisation métabolique des acides aminés est indispensable.*

Mots-clés : *Espèces méditeranéennes de poissons, nutrition, acides aminés, besoin, poissons.*

European seabass, gilthead seabream and a few other teleosts requiring high protein diets, make the bulk of intensive fish farming in the Mediterranean basin. Their production has steadily grown over the last decade to around 160,000 t, roughly requiring 360,000 t of aquafeeds per year, nearly half of which is the protein component. Even when fed nutrient-energy dense diets, based on high quality protein sources, under culture conditions which enables optimal growth performance to be achieved, gross protein retention of seabass and bream usually ranges between 25-35% making them poor protein converters in comparison to salmonids. As this could have a nutritional basis with obvious implications in terms of protein economy and environmental sustainability there is a need for strong efforts to improve our knowledge on nitrogen metabolism of Mediterranean fish species and particularly on amino acid (AA) nutrition; a research field which has deserved little attention to date.

The purpose of this paper is to review the available information on dietary amino acid allowances of Mediterranean fish species considering major critical aspects in their determination and to suggest topics of research in this field, to be addressed in the future.

As shown in Table 1, only a limited amount of information based on reliable but conventional dose-response studies, is currently available on quantitative indispensable amino acid (IAA) requirements for optimal growth of Mediterranean fish species and other marine teleosts.

Comparing values for the different teleosts, expressed as percent protein, reveals some similarities but even wide variation for single IAA. The data set is indeed too meagre to establish reliability of the apparent analogy/difference among fish species, given that experimental designs and conditions such as fish size, culture protocols, basal diet composition, adopted in different laboratories/experiments are quite different. Laboratory variance, with particular emphasis to composition of basal diets used in such experiments, has frequently been claimed as a major issue affecting reliability of IAA requirement estimates in fish and the need for standardization of methods has often been stressed (EIFAC, 1994; Cowey, 1995).

Table 1. Available values of dose-response IAA requirement estimates (g/16gN) of Mediterranean fish species and other marine teleosts

	Gilthead seabream	European seabass	*Pagrus major*	Asian seabass	Red drum	Yellowtail	References†
Arginine	<4.7	3.9-4.6			5.0	3.4	*14,25,27,3,22*
Lysine	5.0	4.8	4.4		4.4	4.1	*14,24,10,7,21*
Met + Cys	4.0	4.0		2.9	3.0	3.3	*14,23,5,17,20*
Threonine		2.6			2.3		*29,4*
Tryptophan	0.6	0.5-0.7					*14,26,30*

†: [3]Barziza *et al*., 2000; [4]Boren and Gatling III, 1995; [5]Coloso *et al*.,1999; [7]Craig and Gatling III, 1992; [10]Forster and Ogata, 1998; [14]Luquet and Sabaut, 1974; [17]Moon and Gatlin III, 1991; [20]Ruchimat *et al*.,1997a; [21]Ruchimat *et al*., 1997b; [22]Ruchimat *et al*., 1998; [23]Thebault *et al*., 1985; [24]Tibaldi and Lanari, 1991; [25]Tibaldi *et al*., 1993a; [26]Tibaldi *et al*., 1993b; [27]Tibaldi *et al*., 1994; [29]Tibaldi and Tulli, 1999; [30]Tulli *et al*., 1997.

Near optimal fish growth performance is a prerequisite to avoid biased estimates of the requirement to be made and this condition had not always been met in certain AA studies with Mediterranean fish species. Using diets supplying large amounts of crystalline AA without precautions give usually rise to growth rates that are lower than that which occurs when the dietary protein consists of whole protein, possibly reflecting impaired protein synthesis and deposition due to a much faster absorptive kinetics of pure AA relative to protein-bound ones (Cowey, 1995). This could explain low fish growth (SGR = 1.2 in 5 g fish after 12 weeks) in early studies by Luquet and Sabaut (1974) aimed at assessing Arg, Lys, SAA and Trp requirements of gilthead seabream. A separate approach, which avoids the need to include large quantities of crystalline AA in the diet, has been to use semi-purified preparations containing protein sources deficient in one or more IAA. Reference diets largely based on soy protein, maize gluten or gelatin and pure L-AA with a minimal supply of fish meal to simulate the AA pattern of fish muscle protein, gave rise to growth rates which were similar to those attained by feeding fish meal-based diets in the experiments carried out to assess SAA, Lys, Arg, Trp requirements of European seabass (Thebault *et al*., 1985; Tibaldi and Lanari, 1991; Tibaldi *et al*., 1993a; Tibaldi *et al*., 1993b).

Increasing the feeding frequency in some of these experiments (Fig. 1) has been shown to improve growth possibly by modulating wide variations in post-absorptive levels of plasma AA with diets supplying substantial amounts of AA. The same positive effect has recently been achieved by protecting with agar a casein+free AA diet offered to seabream, bass or turbot and designed to study their IAA requirements (Fournier *et al*., 2002). This latter approach to basal diet formulation and preparation is strongly recommended in view to standardize methodologies.

Besides the form and pattern of dietary AA, other aspects of the diet composition, which may affect requirement estimates and fish response to dietary AA, have not been thoroughly looked into in dose-response studies carried out so far with Mediterranean fish species.

Since digestible energy (DE) levels may actually vary considerably in marine aquafeeds, proper evaluation of requirements in terms of IAA to DE ratio is necessary.

Practical diets for marine fish are also developing through a large use of plant proteins where limiting supplies, excess or imbalances of dietary AA might occur leading to depressed feed intake, growth retardation and nutritional pathologies. Changes in feed consumption may be regarded as the primary response to dietary AA disproportions in several animal models (D'Mello, 1994) and, although no systematic work has been done so far in the fish species studied to date, there is evidence that voluntary feed intake in young sea bass may be affected to some extent by limiting or excessive levels of certain IAA in the diet.

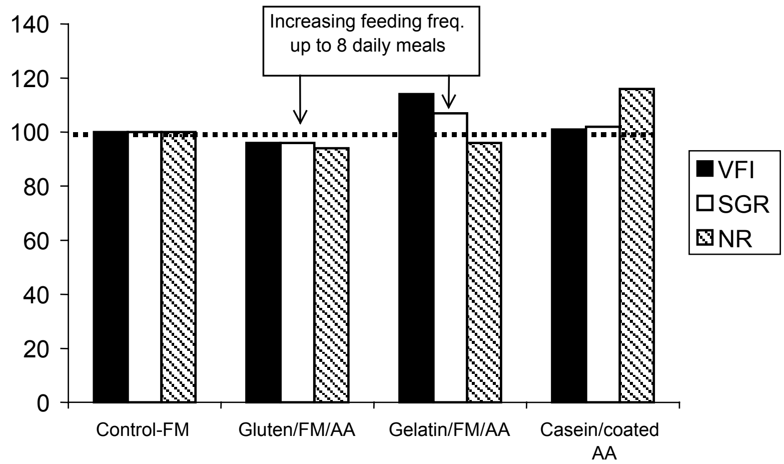

Fig. 1. Reference diets resulting in good performance in certain IAA requirement studies with European sea bass and gilthead sea bream (Tibaldi *et al*., 1991, 1993; Fournier *et al*., 2002). VFI, voluntary feed intake; SGR, specific growth rate; NR, nitrogen retention as % of nitrogen intake, are reported as ratios relative to control groups fed FM (fish meal)-based diets = 100.

As shown in Fig. 2, feeding diets limiting in tryptophan to juvenile seabass resulted in loss of appetite and reduced feed intake was noted by Thebault *et al*. (1985) in fish fed diets marginal in methionine. On the contrary a moderate deficiency of arginine and threonine had no detrimental effects on feed consumption while a compensatory effect on feed intake was noted in response to a diet marginal in lysine. In the same species, feeding excess of single IAA (Lys, Arg, Thr, Trp) in semi-purified diets presumed to be adequate for all IAA, was shown to have little or no effect on feed intake, growth or nitrogen balance and no apparent antagonism between Lys and Arg was found when moderate reciprocal disproportions of both IAA were used in the diet.

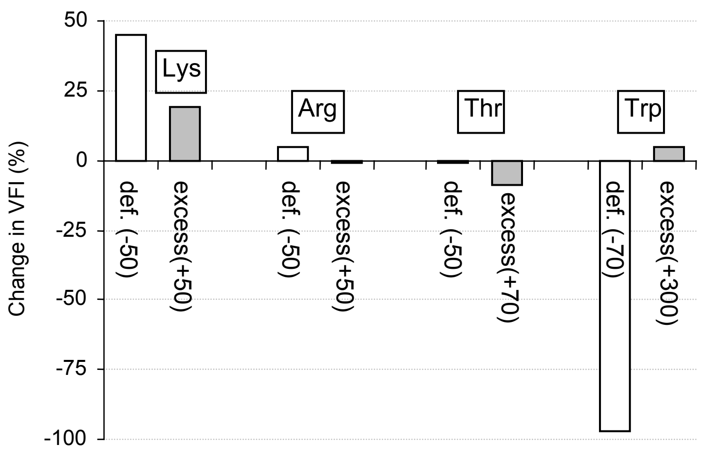

Fig. 2. Changes in voluntary feed intake of juvenile European sea bass fed diets limiting or excessive in single IAA (% change relative to control groups fed diets adequate for all IAA; Tibaldi and Lanari, 1991; Tibaldi *et al.,* 1993a,b; Tibaldi and Tulli, 1999).

Similarly to salmonids, spinal deformities, lens opacity and increased levels of Ca^{++} and Mg^{++} in liver developed in fingerling sea bass fed diets marginal in tryptophan (Tibaldi *et al*., 1993b;Tulli *et al*., 1997). As shown in Fig. 3, the deficiency signs were present even in some fish fed a diet providing a Trp level supporting optimal growth.

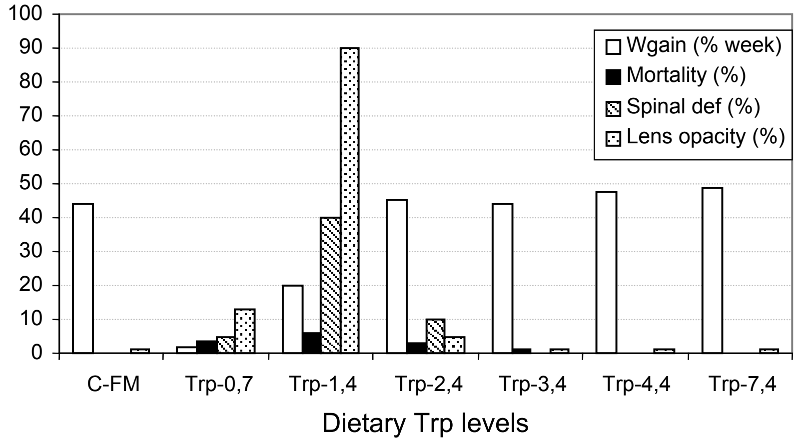

Fig. 3. Incidence (%) of tryptophan-deficiency signs in fingerling European sea bass fed graded levels of L-Trp (g/kg) in the diet relative to controls fed a fish meal-reference diet (Tibaldi *et al*., 1993b).

Further research in this field is strongly needed as various teleosts may respond differently to disproportionate levels of AA in the diet. Optimization of dietary ratio between indispensable and dispensable AA also warrant investigation in view of improving N efficiency.

Apart from dose-response experiments, estimation of IAA requirements in certain Mediterranean fish species had been done by alternate approaches based on the evidence that the IAA whole body (WB) protein profile strictly reflects the corresponding requirement profile in fast growing fish (Mambrini and Kaushik, 1995a).

The first section of Table 2 shows estimates of dietary requirements for all 10 IAA of juvenile seabass and dentex (Tibaldi *et al*.,1996; Kaushik and Tibaldi, 1998) as obtained with the method first proposed by Ogino (1980), i.e. by measuring daily increment of each IAA in the whole body (WB) of fish exhibiting optimal growth and fed diets where protein level and biological value were not limiting.

Table 2. Estimates of the dietary IAA profile (g/16gN) of certain Mediterranean fish species obtained by methods based on WB protein IAA composition

	Daily IAA increment		Ideal protein		
	Seabass	Dentex	Seabass	Seabream	Turbot
Arg	3.7	3.8	4.6	5.4	4.8
Lys	4.7	5.0	*4.8*	*5.0*	*5.0*
His	1.3	1.2	1.6	1.7	1.5
Ile	2.5	2.6	2.6	2.6	2.6
Leu	4.1	4.4	4.3	4.5	4.6
Val	2.8	2.8	2.9	3.0	2.9
Met+Cys	2.2	2.1	2.3	2.4	2.7
Phe+Tyr	4.1	4.0	2.6	2.9	5.3
Thr	2.4	2.6	2.7	2.8	2.9
Trp	0.5	0.6	0.6	0.6	0.6

The second section of Table 2 presents the dietary IAA requirement profile of seabass, seabream and turbot as obtained by Kaushik (1998) by applying the ideal protein concept (see Wilson, 1994) which derives IAA requirements from the A/E ratio of each IAA in the whole body protein of the fish (Arai, 1981) relative to the dose-response Lys requirement, if available.

The Table shows considerable homogeneity of ideal profiles in the diet for most IAA when estimated from the IAA body composition, nor were them expected to vary significantly among fish species given that in general there is little or no variation in the whole body protein IAA profile of various teleosts (Akiyama *et al*., 1997).

A major benefit of these alternate methods is that they provide estimates for all ten IAA in a single mass-balance experiment (Ogino, 1980) or by simple calculations once the dose-response lysine requirement value and the WB IAA composition are available (ideal protein concept).

They fail however to consider possible effects of the physiological status, diet composition and culture conditions on requirement estimates. Besides, reflecting in the most the IAA needs for growth, they apparently ignore maintenance requirements (ideal protein approach) or assume them to be similar in pattern irrespective of the two distinctive functions, i.e. maintenance and growth (Ogino's approach), a point that does not hold for certain IAA based on recent studies with different fish species.

Data from Rodehutscord *et al.* (1997) reported in Table 3, clearly show that the proportion of ingested individual IAA used to cover the maintenance need in well growing rainbow trout is quite different and could be as high as 17-32% of the total requirement for protein deposition for threonine, trypthophan or leucine, or less than 5% in the case of lysine or isoleucine. Furthermore the comparison between dietary amino acid patterns (relative to lysine) for maintenance and those of the whole body protein reveals marked differences for individual amino acids.

Table 3. Proportion of ingested IAA used to cover the maintenance requirement and comparison between maintenance and whole body amino acid profiles in well growing rainbow trout

	Maintenance % total req.	Maintenance profile	WB protein profile
Lys	4	100	100
Thr	17	92	55
Trp	30	25	12
His	8	27	46
Leu	32	189	90
Ile	4	78	52
Val	10	128	62

Very high maintenance requirement of sulphur AA have been estimated in rainbow trout and European catfish by Mambrini and Kaushik (1995b), the values found being two-fold higher than those of terrestrial omnivores such as the pig. On the contrary, recent results by Fournier *et al*. (2002) reported in Table 4, have shown juvenile seabass, seabream, turbot and rainbow trout, to have no or very low dietary Arg requirements for maintenance. Besides, the comparison of the Arg requirements per unit of protein accretion in the four teleosts indicated a variation of about 20%.

A general consequence of the studies summarized above is that alternate methods based on whole body protein amino acid composition, although useful as guidelines in establishing IAA patterns to formulate practical or experimental diets for fish species whose IAA are partially or totally unknown, should be applied with caution since they do not provide "true" amino acid requirement values.

This in turn recalls the need to undertake dose-response studies investigating a wide range of IAA intakes (well below and above the expected requirement value) as the proper approach enabling fish response to dietary AA to be clearly understood.

Table 4. Arginine requirements for maintenance and protein growth of seabass, seabream, turbot and rainbow trout

	Seabass	Seabream	Turbot	Rainbow trout
Size (g)	5.0±0.1	7.4±0.2	5.0±0.1	6.7±0.1
Temperature (°C)	24±1	24±1	17±1	16±1
Maintenance req. (mg/kg$^{0.75}$/d)	0	4.4	0	0
Req. protein gain (mg/g protein)	178	166	167	138

Given the quantitative importance of dietary proteins, of the ideal dietary AA supply in nitrogen utilisation affecting fish growth, its implications on nitrogenous losses and given the strong necessity for use of alternatives to fish meal in aquafeeds, understanding amino acid metabolism in fish from a comparative perspective using well defined methodologies is strongly warranted.

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