# Amino acid nutrition of gilthead seabream Sparus aurata juveniles: Preliminary results on dietary lysine and methionine requirements

Marcouli P., Alexis M.N., Illiopoulou-Georgudaki J.

*in*

Montero D. (ed.), Basurco B. (ed.), Nengas I. (ed.), Alexis M. (ed.), Izquierdo M. (ed.).
Mediterranean fish nutrition

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 63

2005
pages 67-71

# Amino acid nutrition of gilthead seabream *Sparus aurata* juveniles: Preliminary results on dietary lysine and methionine requirements

**P. Marcouli*, M.N. Alexis**, A. Andriopoulou** and J. Iliopoulou-Georgudaki***
*Department of Biology, University of Patras, GR 26 500 Rion, Patras, Greece
**National Center for Marine Research, Aghios Kosmas, Helliniko, Athens, Greece

**SUMMARY –** A series of feeding experiments was conducted to determine indispensable amino acid (IAA) requirements of gilthead seabream juveniles. The development of a balanced diet that fosters good fish growth to be used as a reference diet was the objective of the first trial. The diet selected, based on growth parameters and nutrient utilization efficiencies, resembled the whole body IAA profile of wild seabream having 55% of the protein component provided by crystalline amino acids. In subsequent trials the dietary lysine and methionine requirements were determined by the growth response of the fish fed on six isonitrogenous diets containing graded levels of crystalline L-lysine.HCl and L-methionine. By analysis of the dose-response relationship based on weight gain % of the fish, to increasing levels of the test amino acid, the dietary requirements of lysine and methionine, estimated from a broken line regression, were calculated at 4.88 g/16 g N and 2.77g/16g N.

**Key words:** *Sparus aurata*, indispensable amino acids, lysine, methionine.

***RESUMÉ –** "Nutrition en acides aminés des juvéniles de daurade royale (*Sparus aurata *L.) : Résultats préliminaires sur les besoins en lysine et méthionine dans l'alimentation". Une série d'expériences de nutrition a été menée afin de déterminer les besoins en acides aminés indispensables (IAA) pour les juvéniles de daurade royale. L'objectif du premier essai était la mise au point d'une alimentation équilibrée permettant une bonne croissance des poissons et pouvant être utilisée comme aliment de référence. L'aliment sélectionné, basé sur les paramètres de croissance et l'efficacité d'utilisation des nutriments, ressemblait au profil global en IAA de la daurade sauvage où 55% de la composante protéique est fournie par des acides aminés cristallins. Dans les essais suivants, les besoins en lysine et méthionine alimentaires étaient déterminés par la réponse de croissance des poissons recevant six régimes à même niveau d'azote, et contenant des niveaux progressifs de L-lysine.HCl et L-méthionine cristallines. Par analyse du rapport dose-réponse en se basant sur le pourcentage de gain de poids des poissons, avec des niveaux croissants d'acides aminés testés, les besoins alimentaires en lysine et méthionine estimés à partir d'une ligne de régression brisée, étaient respectivement de 4,88 g/16 g N et 2,77 g/16 g N.*

***Mots-clés :*** Sparus aurata*, acides aminés indispensables, lysine, méthionine.*

## Introduction

Knowledge of the dietary indispensable amino acid (IAA) requirements of a fish is very important in evaluating the nutritive value of a protein source and generally in formulating balanced, cost effective fish feeds through optimization of the protein utilization. Although *Sparus aurata* is one of the most extensively cultured species in Mediterranean region, its IAA requirements based on growth criteria were not thoroughly investigated. Data available on the IAA requirement profile include determinations based on growth parameters (Luquet and Sabaut, 1974; Fournier *et al*., 2002) and estimations based on whole body IAA pattern (Vergara, 1992) and the A/E ratios of whole body IAA (Kaushik, 1998).

Indispensable amino acids requirements are mainly determined by the growth response of the fish fed on a number of test diets containing graded levels of the test amino acid. In these diets a large part of the protein component is usually derived by a mixture of crystalline amino acids that enables incremental doses of the test amino acid to be provided. However, growth rates obtained when large amounts of crystalline amino acids are used, are very often much lower than those achieved with a good practical diet, so the accuracy of the requirement values is uncertain (Wilson, 1994; Cowey, 1995). The good growth performance of the fish is a principal condition that should be fulfilled towards the accurate and reliable determination of the IAA requirements. The formulation of a reference diet that gives a good fish performance is therefore a prerequisite for this type of work.

The work presented, concerns the preliminary results of a series of experiments performed to determine IAA requirements of seabream juveniles. For this purpose a reference diet was first developed, in which approximately about 45% of the protein component was provided by fish meal and the rest by a mixture of crystalline IAA and DAA, so that its final IAA composition simulated that of wild sea bream whole body (Mambrini and Kaushik, 1995). This diet was subsequently used as a reference in dietary lysine and methionine requirement determination.

## Materials and methods

Triplicate groups of 25 fish (average initial weight 3.7 g) were used for the evaluation of the reference diet (first experiment) and duplicate groups of 30 fish (average initial weight 3.5 g) were used in the subsequent feeding trials (second and third experiments). Fish were fed the test diets to satiation twice a day, 6 days per week for 6 weeks and individually weighted every fortnight.

For selecting a reference diet, 4 test diets (Table 1) containing on the average 43% protein, 13.5% lipid, 11% ash, 24.6% starch, and 20 MJ/kg (gross energy) were formulated. Fish meal LT was the only AA source in the control diet (C). A second diet (S) in which part of the protein component (approx. 45%) was provided by fish meal and the rest by a mixture of IAA and DAA so that the final IAA composition simulated that of wild gilthead seabream whole body, was formulated. The third diet (D) was supplemented only with DAA so as to keep all IAA in deficiency. Increasing the amount of crystalline IAA by 10% of that used in diet 2 with the appropriate reduction in DAA, a fourth diet (E) was formulated.

For determining the lysine requirement, six test diets were prepared containing graded levels of crystalline L-lysine.HCl at incremental doses of 0.43 g/100 g diet, with dietary lysine content ranging from 3.63 to 7.97 g/16 g N. For determining the methionine requirement, six test diets were also produced that contained graded levels of crystalline L-methionine at incremental doses of 0.29 g/100 g diet, so that dietary methionine was ranging from 1.26 to 5.08 g/16 g N. All cystine contained (0.30 g/16 g N) in this series of diets originated from the fish meal used and no additional cystine was added. The formulation and proximate composition (except of the amino acid under study) of the test diets used in both experiments was the same with that of diet S.

## Results and discussion

### First experiment

All performance parameters of diet D were inferior to that of the other diets. Growth parameters [final weight, weight gain % (WG%), specific growth rate (SGR)] and feed efficiency were similar among fish groups fed on diets C and S. Excessive amount of IAA in diet E has a negative effect on most of growth parameters compared to the control. These results showed that, since not significant differentiations were arisen by the presence of a large amount of crystalline AA in diet S regarding fish performance, this diet could be used as a reference in IAA requirement studies in this fish.

### Second and third experiments

Survival observed in both experiments was excellent in all dietary treatments. No outward pathological signs were noted in fish given low levels of either dietary lysine or methionine. WG%, SGR and feed efficiency increased with increasing levels of dietary lysine up to the level of 5.27% and remained nearly constant thereafter. The same trend was observed with dietary methionine up to the level of 3.51%.

By analysis of the dose-response relationship using the broken line model (Zeitoun *et al.*, 1976; Robbins *et al.*, 1979), based on WG%, the dietary requirement of lysine was found to be 4.88 g/16 g N (Fig. 1), while the value determined for dietary methionine requirement was 2.77 g/16 g N (Fig. 2).

Table 1. Ingredients and IAA supplementation (g/kg diet)of the test diets in the first experiment

| Diets | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Fish meal | 576.9 | 256.4 | 256.4 | 256.4 |
| Cod liver oil | 103.3 | 129.2 | 129.2 | 129.2 |
| Vitamin-mineral premix† | 20.0 | 20.0 | 20.0 | 20.0 |
| Pre-gelatinized starch | 243.4 | 235.0 | 235.0 | 235.0 |
| Dicalcium phosphate | 5.0 | 58.0 | 58.0 | 58.0 |
| a-Cellulose | 51.4 | 51.4 | 51.4 | 51.4 |
| Lys.HCl | ----- | 21.1 | ----- | 23.2 |
| Thr | ----- | 11.6 | ----- | 12.8 |
| Met | ----- | 10.3 | ----- | 11.3 |
| Cys | ----- | 6.5 | ----- | 7.1 |
| Trp | ----- | 2.8 | ----- | 3.1 |
| Arg | ----- | 18.0 | ----- | 19.8 |
| His | ----- | 10.5 | ----- | 11.6 |
| Ile | ----- | 11.2 | ----- | 12.3 |
| Leu | ----- | 16.6 | ----- | 18.3 |
| Phe | ----- | 9.9 | ----- | 10.9 |
| Tyr | ----- | 8.3 | ----- | 9.1 |
| Val | ----- | 13.1 | ----- | 14.4 |
| Glu. Na | ----- | 42.0 | 94.5 | 37.0 |
| Gly | ----- | 16.8 | 38.3 | 14.7 |
| Ala | ----- | 15.9 | 37.0 | 13.8 |
| Asp | ----- | 23.5 | 52.9 | 20.2 |
| Ser | ----- | 11.9 | 27.3 | 10.4 |

†Supplying mg/kg of diet: Vitamin A, 18.000 IU; vitamin D, 2.700 IU; vit C-stay, 500; vitamin E, 270; vitamin K, 15; vitamin $B_1$, 40; vitamin $B_2$, 40; vitamin $B_6$, 30; vitamin $B_{12}$, 0.07; niacin, 150; Ca-D-pantothenate, 65; folic acid, 6; d-biotin, 1.5; choline chloride, 3600; myo-inositol, 500; Co, 2.5; I, 4; Se, 0.1; Fe, 130; Mn, 100; Cu, 5; Zn, 130.

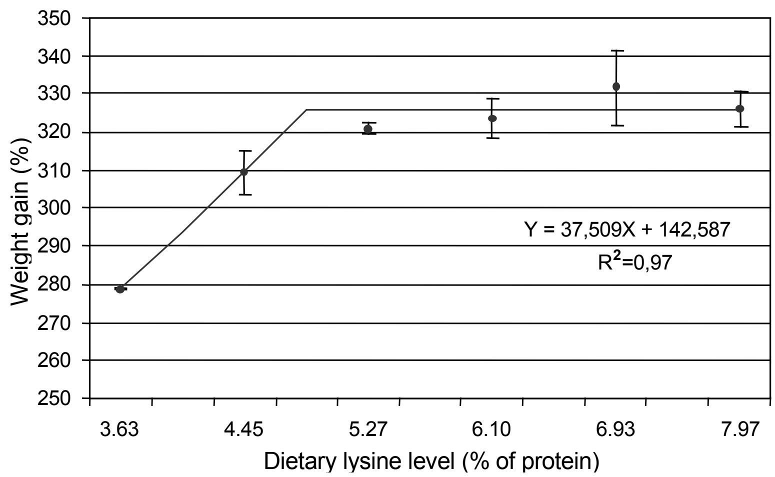


Fig. 1. Dietary lysine requirement estimated from a broken line regression.

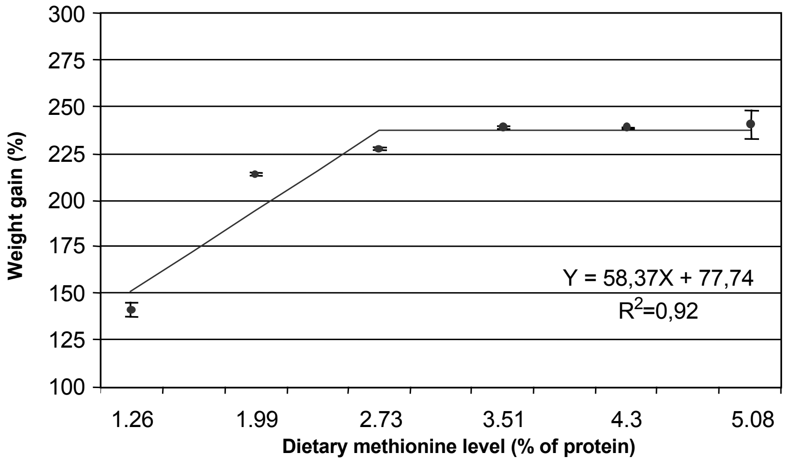


Fig. 2. Dietary methionine requirement estimated from a broken line regression.

Dietary lysine requirements of 13 fish species (% of protein) as presented by Akiyama *et al*. (1997) range from 3.8% to 6.2%. Concerning gilthead seabream requirement level, Luquet and Sabaut (1974) estimated a value of 5% which is in a close agreement with the value of 4.88% determined in the present study, while Vergara (1992) based on the IAA profile of seabream carcass, calculated a value of 5.49%. A similar value (4.8%) was determined by Tibaldi and Lanari (1991) for sea bass.

Regarding methionine+cystine requirement, values for several fish species as reviewed by Akiyama *et al*. (1997) vary from 2.2 to 4%. Seabream requirement level for (Met+Cys) as determined by Luquet and Sabaut (1974) is 4%, while according to the estimations of Vergara (1992) and Kaushik (1998) the Met+Cys requirement level of seabream is 2.7% and 2.4% respectively. A higher requirement level (4%) was determined by Thebault *et al*. (1985) for juvenile sea bass in comparison to the level of 3.07% determined for seabream in this study.

Although broken line is the most commonly used model in IAA requirement studies in fish, several recent publications have been focused on the influence that different mathematical models have on nutrient requirement estimations (Robbins *et al*., 1979; Rodehutscord and Pack, 1999; Shearer, 2000). Generally, non linear models are regarded as most appropriate for evaluating results from dose-response experiments (Mercer, 1989; Cowey, 1992; Fuller and Garthwaite, 1993; Schutte and Pack, 1995; Rodehutscord *et al*., 1995). Furthermore, the effect of the growth criteria used for the estimation of the requirement level, has been also discussed elsewhere (Rodehutscord *et al*., 1997; Forster and Ogata, 1998; Tibaldi and Tulli, 1999; Hauler and Carter, 2001). This work refers to the preliminary results derived from a study which is still under progress. Therefore, future thorough analysis of the dose-response data will include the application of different mathematical models and growth criteria in order for the most appropriate one to be chosen.

## References

Akiyama, T., Oohara, I. and Yamamoto, T. (1997). Comparison of essential amino acid requirements with A/E ratio among fish species. *Fish. Sci*., 63: 963-970.

Cowey, C.B. (1992). Nutrition: Estimating requirements of rainbow trout. *Aquaculture,* 100: 177-189.

Cowey, C.B. (1995). Protein and amino acid requirements: A critique of methods. *J. Appl. Ichth*., 11: 199-204.

Forster, I. and Ogata, Y. (1998). Lysine requirement of juvenile japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. *Aquaculture*, 161: 131-142.

Fuller, M.F. and Garthwaite, P. (1993). The form of response of body protein accretion to dietary amino acid supply. *J. Nutr*., 123: 957-963.

Fournier, V., Gouillou-Coustans, M.F., Metailler, R., Vachot, C., Guedes, M.J., Tulli, F., Oliva-Teles, A., Tibaldi, E. and Kaushik, S.J. (2002). Protein and arginine requirements for maintenance and nitrogen gain in four teleosts. *Br. J. Nutr*., 87: 459-469.

Hauler, R.C. and Carter, C.G. (2001). Reevaluation of the quantitative dietary lysine requirements of fish. *Rev. Fish. Sci.*, 9(3): 133-163.

Kaushik, S.J. (1998). Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. *Aquat. Living Resour.*, 11: 355-358.

Luquet, P. and Sabaut, J.J. (1974). Nutrition azotée et croissance chez la daurade et la truite. In: *Actes Colloq. CNEXO*, 1: 243-253.

Mambrini, M. and Kaushik, S.J. (1995). Indispensable amino acid requirements of fish: correspondence between quantitative data and amino acid profiles of tissue proteins. *J. Appl. Ichthyol.*, 11: 240-247.

Mercer, L.P., May, H.E. and Dodds, S.J. (1989). The determination of nutritional requirements in rats: mathematical modelling of sigmoidal, inhibited nutrient-response curves. *J. Nutr.,* 119: 1465-1471.

Robbins, K.R., Norton, H.W. and Baker, D.H. (1979). Estimation of nutrient requirements from growth data. *J. Nutr.*, 109: 1710-1714.

Rodehutscord, M., Jacobs, S., Pack, M. and Pfeffer, E. (1995). Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 170 g to supplements of either L-arginine or L-threonine in a semipurified diet. *J. Nutr.,* 125: 970-975.

Rodehutscord, M., Becket, A., Pack, M. and Pfeffer, E. (1997). Response of rainbow trout (*Oncorhynchus mykiss*) to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. *J. Nutr.*, 126: 1166-1175.

Rodehutscord, M. and Pack, M. (1999). Estimates of essential amino acid requirements from dose response studies with rainbow trout and broiler ckicken: Effect of mathematical model. *Arch. Anim. Nutr.*, 52: 223-244.

Schutte, J.B. and Pack, M. (1995). Sulfur amino acid requirement of broiler chicks from fourteen to thirty-eight days of age. 1. Performance and carcass yield. *Poult. Sci.*, 74: 480-487.

Shearer, K.D. (2000). Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: A critical review. *Aquacult. Nutr.,* 6: 91-102.

Thebault, H., Alliot, E. and Pastoureaud, A. (1985). Quantitative methionine requirement of juvenile sea bass (*Dicentrarchus labrax*). *Aquaculture*, 50: 75-87.

Tibaldi, E. and Lanari, D. (1991). Optimal dietary lysine levels for growth and protein utilisation of fingerling sea bass (*Dicentrarchus labrax* L.) fed semipurified diets. *Aquaculture,* 95: 297-304.

Tibaldi, E. and Tulli, F. (1999). Dietary threonine requirement of juvenile european sea bass (*Dicentrarchus labrax* ). *Aquaculture,* 175: 155-166.

Vergara, J.M. (1992). *Studies on the utilization of dietary protein and energy by gilthead sea bream (*Sparus aurata*).* PhD thesis, University of Stirling, UK.

Wilson, R.P. (1994). Amino acid requirements of finfish. In: *Amino Acids in Farm Animal Nutrition*, D'Mello, J.P.F. (ed.). CAB International, Wallingford, UK, pp. 377-399.

Zeitoun, I.H., Ullrey, D.E., Magee, W.T., Gill, J.L. and Bergen, W.G. (1976). Quantifying nutrient requirements of fish. *J. Fish. Res. Board Can.*, 33: 167-172.