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Do dietary amino acid profiles affect performance of larval gilthead seabream?

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Abstract – Live preys commonly used in fish larval rearing seem to be imbalanced in terms of amino acids. Manipulation of their amino acid composition is difficult, but the use of microencapsulated diets allows this manipulation. This study analysed the effect of amino acid supplementation, in order to compensate for dietary amino acid imbalances, on growth and survival of gilthead seabream (*Sparus aurata*) larvae. Larvae were reared until 32 days after hatching, in a closed recirculating water system (19 °C), using only live food (rotifers and *Artemia*). Thereafter, larvae were fed *Artemia* or one of the experimental microencapsulated diets: supplemented with indispensable amino acids (IAAsup diet), or supplemented with dispensable amino acids (DAAsup diet). Experiment lasted for 10 days. Dietary supplementation with indispensable amino acids resulted in A/E ratios [(each indispensable amino acid) × (total indispensable amino acids)$^{-1}$ × 1000] more similar to the ones of larval seabream and in higher IAA:DAA ratios than in the DAAsup diet. Survival was similar in larvae fed the IAAsup diet (75%) or *Artemia* (87%), but was significantly lower in larvae fed the DAAsup diet (52%). Larvae from all treatments more than doubled their average dry weight during the experimental period. Final dry weight was similar in larvae fed both microcapsules, but these were lighter than larvae fed with *Artemia*. Relative growth rate (RGR) and total biomass production tended to be higher in larvae fed the IAAsup (RGR = 9% day^{-1}) than the DAAsup diet (RGR = 7.5% day^{-1}) and only in this last treatment these parameters were significantly lower than in larvae fed with *Artemia*. Therefore, dietary supplementation with indispensable amino acids resulted in a more balanced dietary amino acid profile, which significantly increased survival. Further studies introducing microdiets earlier in the development seem necessary in order to optimise growth.

Key words: Amino acids / Growth, Fish Larvae / Microencapsulated diets / *Sparus aurata* / Survival

Résumé – La composition en acides aminés, dans le régime alimentaire des larves de daurade royale, affecte-t-elle leur performance ? Les proies vivantes utilisées dans les élevages larvaires semblent déséquilibrées en terme d'acides aminés. Manipuler la composition en acides aminés est difficile mais l'usage d'aliments encapsulés le permet. Cette étude analyse l'effet d'une supplémentation en acides aminés sur la croissance et la survie des larves de daurade royale (*Sparus aurata*) afin de compenser un déséquilibre. Les larves sont élevées jusqu'à 32 jours après éclosion en circuit fermé (eau à 19 °C), en utilisant des aliments vivants uniquement (rotifères et *Artemia*). Les larves sont ensuite nourries avec des *Artemia* ou bien avec des aliments microencapsulés expérimentaux, supplémentés en acides aminés soit indispensables (régime IAAsup) soit non-indispensables (régime DAAsup). La durée de l'expérience est de 10 jours. La supplémentation en acides aminés indispensables conduit à des ratios A/E [(chaque AA indispensable) × (total en AA indispensables)$^{-1}$ × 1000] plus proches de ceux de la larve de dorade et des ratios IAA/DHA plus élevés que ne le fait la supplémentation en acides aminés non-indispensables. Le taux de survie est similaire chez les larves nourries avec le régime IAAsup (75 %) ou *Artemia* (87 %) mais inférieur significativement chez les larves soumises au régime DAAsup (52 %). Le poids sec moyen de ces larves, quel que soit leur régime alimentaire, a doublé durant la période expérimentale. Le poids sec est similaire chez les larves nourries de microcapsules mais moins élevé chez celles nourries d'*Artemia*. Le taux de croissance relative (RGR) et la production de biomasse totale tendent à être plus élevés chez les larves nourries en IAAsup (RGR = 9 % jour^{-1}) que celles nourries en DAAsup (RGR = 7,5 % jour^{-1}). C'est seulement dans ce dernier traitement que les paramètres sont plus faibles significativement que chez les larves nourries d'*Artemia*. Ainsi, les supplémentations alimentaires en acides aminés indispensables peuvent augmenter la survie. D'autres études, introduisant des micro-aliments de façon plus précoce dans le développement, semblent nécessaires afin d'optimiser la croissance.

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1 Introduction

Fish larvae have higher growth rates and energy requirements than juvenile and adult fish. Growth is essentially protein deposition (Carter and Houlihan 2001) and amino acids are the building blocks. Protein synthesis requires that all indispensable amino acids are present at an optimum ratio to each other (Carter and Houlihan 2001). If one indispensable amino acid is found to be deficient, then the other indispensable amino acid will be considered in excess and will be deaminated and used for other purposes than protein synthesis, such as energy production, lipogenesis, or gluconeogenesis (Ballantyne 2001). Since proteins are the only form of amino acid storage, dietary amino acid imbalances will result in unavoidable amino acid losses. Still, fish larvae have high-energy requirements and a large portion of amino acids is used as metabolic fuel (Rønnestad and Conceição 2005). Therefore, part of the dietary amino acids is always used as energy source and there is always an obligatory amino acid loss. Considering the high potential for growth and the high food intake of fish larvae, the amino acid losses due to dietary deficiencies may have a much larger negative impact on growth and food conversion efficiencies than in older fish (Conceição et al. 2003a). Therefore, dietary protein should be supplied in the right quantity and quality to sustain optimal larval growth and development.

The larval rearing of most marine fish species still relies on the use of live preys, at least during their early stages. Poor results in terms of growth and survival have been obtained in several studies when using a microdiet in the absence of live food during these early stages (Tandler and Kolkovski 1991; Fernández-Díaz and Yúfera 1997; Kolkovski et al. 1997a; Takeuchi et al. 2003). Rotifers and *Artemia* are the cultured zooplankters widely used in larviculture, but these preys are imbalanced in terms of amino acids as a diet for fish larvae (Conceição et al. 2003a; Aragão et al. 2004a). The manipulation of the amino acid profile of live prey is not easily achieved and the possible changes rely essentially on the modification of the free amino acid pool, which represents less than 10% of the total amino acid pool (Aragão et al. 2004b). In recent years, a protein-walled microencapsulated diet has been developed and successfully used in the rearing of larval gilthead seabream (*Sparus aurata,* Yúfera et al. 2000). One of the advantages on the use of microdiets is the possibility to manipulate its composition. This encapsulation method has been proved to successfully deliver bioactive compounds such as free amino acids, hormones, and vitamins to fish larvae (Yúfera et al. 2003).

The indispensable amino acid profile (or the A/E ratios, i.e. ((IAA/total IAA) × 1000) of fish carcass or muscle has been proposed as a good index of the indispensable amino acid requirements of larval (Watanabe and Kiron, 1994), juvenile, and adult fish (Wilson 1994; Mambrini and Kaushik 1995). However, a more precise estimate of the ideal dietary amino acid profile requires information on the relative bioavailabilities of the individual amino acids, in particular in what refers to possible differential rates of absorption and catabolism (Conceição et al. 2003b). This information together with the A/E ratios of the larval protein should allow defining the ideal dietary amino profile for a given species (Conceição et al. 2003b).

Table 1. Ingredients used in the preparation of the experimental microcapsules (g kg^{-1} dry diet).

Ingredients	IAAsup diet	DAAsup diet
Casein [1]	400	400
Squid meal [2]	62	61
Fish meal [3]	72	63
Fish protein hydrolysate [4]	62	61
L-Histidine [5]	19	-
L-Threonine [6]	90	-
L-Arginine [7]	28	-
L-Methionine [8]	34	-
L-Cysteine [9]	16	-
L-Serine [10]	-	88
L-Alanine [11]	-	27
L-Glutamic acid [12]	-	52
L-Proline [13]	-	34
Dextrin (type I) [14]	37	37
Fish oil [15]	93	91
Soy lecithin [16]	47	46
Vitamin C [17]	30	30
Vitamin E [18]	10	10

[1] ICN 901633; [2] Rieber & Sons, Norway; [3] Aglonorse, Norway; [4] CPSP 90, Sopropêche, France; [5] Sigma H-8000; [6] SigmaT-8625; [7] Aldrich A-92406; [8] Sigma M-9625; [9] Aldrich 168149; [10] Fluka 84960; [11] Fluka 05129; [12] Sigma G-1251; [13] Sigma P-0380; [14] ICN 101517; [15] A1 DHA Selco; [16] ICN 102147; [17] Phospitan C, Showa Denko, Japan; [18] DL-alpha-Tocopherol, ICN 100555.

The aim of this study was to analyse the effect of amino acid supplementation, in order to compensate for eventual dietary amino acid imbalances, on growth and survival of gilthead seabream larvae, using microencapsulated diets.

2 Material and methods

2.1 Experimental diets

Two experimental microencapsulated diets were formulated using the A/E ratios (Arai 1981) of larval seabream obtained by Aragão et al. (2004a) as a guideline. These values were corrected with the relative bioavailabilities of each amino acid, according to the results obtained by Conceição et al. (2003b). The basal formulation was identical for both microcapsules, but different crystalline L-amino acids (from Sigma-Aldrich, Germany) were included in order to obtain two different dietary amino acid profiles. One of the microdiets (IAAsup) was supplemented with indispensable amino acids in order to obtain dietary A/E ratios similar to the A/E ratios of larval seabream [accordingly to the results of Aragão et al. (2004a) and taking into account the relative bioavailabilities of each amino acid (Conceição et al. 2003b)]. The other microdiet (DAAsup) was supplemented with dispensable amino acids.

The microencapsulated diets were prepared by interfacial polymerisation of the dietary protein. The dietary ingredients are shown in Table 1. The process is described in detail by Yúfera et al. (2000). The microcapsules were freeze-dried

and then sieved into the range between 200 and 400 μm. Dietary samples were analysed for free and protein-bound amino acids.

Leaching tests from microencapsulated diets were carried out according to Yúfera et al. (2002). At time zero, 56 mg of microcapsules were added to 25 ml of distilled water. Water samples of 5 ml were taken at 5 and 60 min, using a 10 ml syringe. These samples were filtered through a 0.45 μm membrane and further analysed for free amino acid content.

2.2 Fish rearing and sampling

Eggs were obtained from natural spawning of seabream broodstock maintained at the IPIMAR research station (Olhão, Portugal) and larval rearing was carried out at the CCMAR experimental station (Faro, Portugal). Seabream were reared in a recirculating system comprising 100 L conical-cylindrical fibreglass white tanks, according to standard rearing procedures (Moretti et al. 1999). During this initial rearing period larvae were fed exclusively on live prey (rotifers and *Artemia*) enriched with commercial products.

At 28 days after hatching (DAH) larvae were counted and distributed into nine tanks, identical to the ones used for larval rearing, at a density of 6.5 larvae L^{-1}. A group of 20 larvae from the initial stock was measured, weighed, and frozen for posterior analysis of dry weight and amino acid content. The experiment started at 33 DAH and lasted 10 days. During the experiment water flow rate was 16.7 L h^{-1} and temperature (19.3 ± 0.2 °C), salinity (36 ± 1 ppt), and dissolved oxygen (97 ± 2% saturation) were measured daily. A 24 h light cycle was adopted, although from 05h00 to 09h00 the light intensity was reduced.

Each experimental microdiet was randomly assigned to triplicate tanks. The microcapsules were distributed by automatic feeding devices from 10h00 to 04h00, in six meals per day (2 h each), with 1h interval between each meal. In other three tanks (reference treatment) larvae were fed with *Artemia* metanauplii enriched for 24-h with Ratio HUFA (Salt Creek Inc., Utah, USA), three times a day, during the whole experimental period. Microcapsules and *Artemia* were provided in excess.

Survival was daily monitored. At the end of the experiment, the remaining fish in each tank was counted and 20 larvae from each tank were measured and frozen for subsequent determinations of individual dry weight after freeze-drying.

2.3 Amino acid analysis

Dietary samples were analysed for free and protein-bound amino acid content. Extraction of free amino acids and sample deproteinisation were carried out according to Cohen et al. (1989). Both larval and dietary samples were hydrolysed (6 M HCl at 106 °C over 24 h in nitrogen-flushed glass vials), after free amino acid extraction, for protein-bound amino acid analysis. Both free and protein-bound amino acids were analysed by High Pressure Liquid Chromatography (HPLC) in a Pico-Tag Amino Acid Analysis System (Waters, USA), using norleucine as internal standard and according to the procedures described by Cohen et al. (1989). The protein-bound tryptophan was not determined, since it is partially destroyed by acid hydrolysis. Asparagine is converted to aspartate and glutamine to glutamate during acid hydrolysis, so the reported values for these amino acids (Asx and Glx) represent the sum of the respective amine and amino acid in the proteins. Resulting peaks were analysed using the Breeze software (Waters).

2.4 Data analysis

The A/E ratios (Arai 1981) were calculated as: (each indispensable amino acid content) × (total indispensable amino acid content including cysteine and tyrosine)$^{-1}$ × 1000. A dietary imbalance for a given indispensable amino acid is assumed to occur when its A/E ratio in the fish is higher than the A/E ratio in the food item.

Relative growth rate (RGR, % dry weight day^{-1}) was calculated as: $RGR = (e^{g}-1)\times 100$, where $g = (\ln W_t - \ln W_0)\times t^{-1}$, W_t and W_0 are the final and initial dry weights, respectively, and t is the duration of the trial. Total biomass production has been estimated from the total increase in population dry weight per weight unit during the experimental time.

The results are expressed as means ± standard deviation (SD). Data were tested by one-way ANOVA followed by Bonferroni t-test. The significance level used was $p \leqslant 0.05$.

3 Results

The amino acid composition of the experimental microcapsules is given in Table 2. The IAAsup diet presented more 24% of histidine, 73% of threonine, 7% of arginine, 9% of methionine, and 2100% of cysteine than the DAAsup diet. On the contrary, the DAAsup diet had more 3% of glutamic acid, 8% of serine, and 7% of proline than the IAAsup diet. The IAAsup diet had significantly more amino acids in the free form than the DAAsup diet (13.3 ± 2.0 mg g^{-1} DW versus 6.9 ± 0.1 mg g^{-1} DW, respectively), corresponding to 3 and 1.5% of the total amino acids, respectively. The IAA:DAA ratio was significantly higher in IAAsup than in DAAsup diet (1.05 ± 0.02 versus 0.93 ± 0.02, respectively; $p < 0.01$).

Leaching from both microcapsules seemed to occur especially during the first 5 min of immersion in water (Table 3), with some exceptions. Regarding the loss of each specific free amino acid (FAA) supplemented to the diets [(micrograms of each FAA found in water) × (micrograms of each FAA in microdiet before immersion)$^{-1}$ × 100], in the IAAsup diet 60% of free histidine and 40% of free cysteine had leached into the water within 5 min of immersion, but these values remained stable until 1 h after immersion. However, 10% of free arginine and 40% of free methionine had leached into the water 5 min after immersion, but these values increased, respectively, to 33 and 95% after 1 h of immersion. The leaching of free threonine was very low, attaining 10% after 1 h of immersion. In the DAAsup diet, 40% of the free serine and 50% of free alanine had leached into the water after 5 min of immersion and these values increased up to more or less 90% after 1 h. All the free proline and glutamic acid in the DAAsup diet had leached

Table 2. Amino acid (AA) composition of experimental microcapsules (sum of free and protein-bound AA) and percentage present in the free form [calculated for each AA as: free AA × (free AA + protein-bound AA)$^{-1}$ × 100].

	IAAsup diet		**DAAsup diet**	
AA	**Total AA** (mg AA g^{-1} DW)	AA in free form	**Total AA** (mg AA g^{-1} DW)	AA in free form
His	**11 ± 3**	**3%**	9 ± 3	ND
Thr	**27 ± 6**	**34%**	16 ± 4	ND
Arg	**24 ± 8**	**4%**	22 ± 6	ND
Val	30 ± 8	ND	30 ± 7	ND
Met	**13 ± 4**	**13%**	12 ± 2	ND
Ile	23 ± 6	ND	22 ± 4	ND
Leu	39 ± 10	ND	38 ± 8	ND
Phe	18 ± 5	ND	18 ± 3	ND
Lys	31 ± 8	ND	29 ± 8	ND
Tyr	18 ± 5	2%	18 ± 4	2.6%
Cys	**4 ± 1**	**15%**	0.2 ± 0.2	100%
Asx	26 ± 5	0.6%	24 ± 8	ND
Glx	70 ± 18	ND	**72 ± 19**	**2%**
Ser	32 ± 8	ND	**35 ± 8**	**5%**
Gly	26 ± 7	ND	26 ± 7	ND
Ala	24 ± 6	ND	**23 ± 5**	**6%**
Pro	49 ± 12	ND	**52 ± 12**	**3%**

Results are expressed as means ± SD (n = 3). DW = dry weight; ND = not detectable or vestigial. AA that were supplemented to the microcapsules are given in bold.

Table 3. Leaching of each free amino acid (FAA) from the experimental microcapsules after 5 and 60 min of immersion in water.

	IAAsup diet		**DAAsup diet**	
AA	5 min	60 min	5 min	60 min
His	**61**	**62**	-	-
Thr	**6**	**10**	-	-
Arg	**10**	**33**	-	-
Tyr	100	100	100	100
Met	**43**	**95**	-	-
Cys	**40**	**40**	100	100
Asx	50	59	100	100
Glx	100	100	**100**	**100**
Ser	-	-	**40**	**93**
Gly	100	100	52	52
Ala	-	-	**50**	**89**
Pro	-	-	**100**	**100**

Note: the loss of each specific FAA was calculated as a percentage of that FAA in the microencapsulated diet: (micrograms of each FAA found in water) × (micrograms of each FAA in microdiet before immersion)$^{-1}$ × 100. AA that were supplemented to the microcapsules are given in bold.

into the water within 5 min of immersion. However, when expressed as the total loss of free amino acids from the microcapsules [(micrograms of total FAA in water) × (micrograms of total FAA in microdiet before immersion)$^{-1}$ × 100], the leaching losses represent only 20 and 31% of the total initial free amino acid content in the IAAsup diet, after 5 and 60 min of immersion in water, respectively. On the contrary, the total loss of free amino acids in the DAAsup diet was very high: after 5 min of immersion in water the leaching losses accounted for 75% of the total initial free amino acid content, and this value increased to 94% after 1 h of immersion. It should be noted that the larval seabream readily accepted both microcapsules and the majority of the larvae were picking up the feed from the water surface within 5 min after deliver.

Table 4. Survival and growth of *Sparus aurata* larvae fed microcapsules supplemented with amino acids (IAAsup and DAAsup) or *Artemia* metanauplii. RGR: relative growth rate.

Treatments	**Survival (%)**	**Total length (mm)**	**Dry weight (mg)**	**RGR (% day^{-1})**
IAAsup	75 ± 6 ^{a}	12.0 ± 1.2 ^{b}	2.3 ± 0.7 ^{b}	9.0 ± 1.6 ^{ab}
DAAsup	52 ± 9 ^{b}	11.8 ± 1.1 ^{b}	2.0 ± 0.7 ^{b}	7.5 ± 0.5 ^{b}
Artemia	87 ± 5 ^{a}	13.0 ± 1.6 ^{a}	3.2 ± 1.3 ^{a}	12.9 ± 2.8 ^{a}

Values are means ± SD (n = 3) and different superscripts within the same column indicate significant differences between treatments (p < 0.02). Initial total length and dry weight were, respectively, 9.9 ± 1.2 mm and 1.1 ± 0.1 mg for all treatments.

Figure 1 shows that the IAAsup diet seems to have A/E ratios more similar to the ones of larval seabream than the DAAsup diet. The IAAsup diet still seems to be deficient in arginine and lysine, while these same amino acids plus threonine and sulphur amino acids seem to be deficient in the DAAsup diet. The comparison of the A/E ratios of larval seabream with the A/E ratios of *Artemia* metanauplii (results not shown), suggest

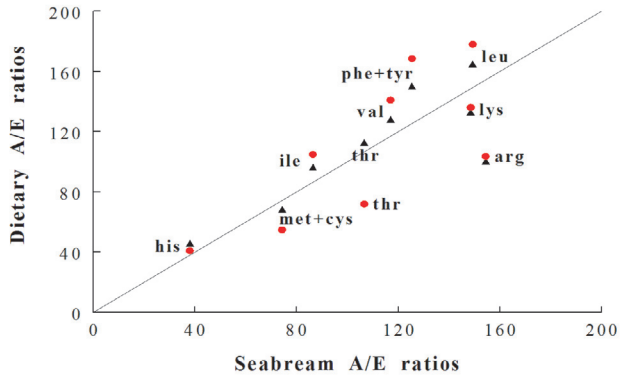

Fig. 1. Comparison of the A/E ratios of *Sparus aurata* larvae (corrected for the different amino acid bioavailabilities) with the dietary A/E ratios (corrected for the leaching losses of free amino acids after 5 min of immersion in water). ▲: Microcapsules IAAsup (supplemented with Indispensable amino acids); •: microcapsules DAAsup (supplemented with Dispensable amino acids). The comparison with the A/E ratios of *Artemia* metanauplii is not shown, since most of the points overlap with the DAAsup diet.

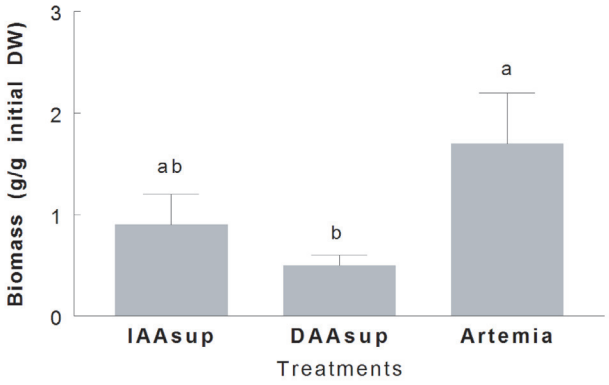

Fig. 2. Total biomass production (g per g initial dry weigh) of *Sparus aurata* larvae fed microcapsules supplemented with amino acids (IAAsup and DAAsup) or *Artemia* metanauplii. Means ± SD (n = 3). Different letters indicate significant differences among treatments (p = 0.01).

that *Artemia* is deficient in threonine, sulphur amino acids, and arginine for the seabream larvae. These dietary deficiencies are in the same order of magnitude as the ones found for the DAAsup diet, with the exception of arginine that seems to be less deficient in *Artemia* than in both microcapsules.

After four days of experiment, larval groups eating both microcapsules started to display moderate mortality. The pattern of mortality was similar in both treatments, but larvae fed the DAAsup diet had a higher mortality during the experimental period. This resulted in a significantly higher mortality at the end of the experiment in larvae fed the DAAsup than the IAAsup diet or *Artemia* (Table 4). In 10 days of experiment, larvae from all treatments more than doubled their average dry weight (Table 4). At the end of the experiment, length and weight between groups fed with microcapsules was similar, but significantly lower than in larvae fed with *Artemia*. When growth is expressed as RGR (Table 4) or total biomass production (Fig. 2), it is seen that both parameters tended to be higher, although not statistically significant, in larvae fed the IAAsup than the DAAsup diet. Only in this last treatment growth was significantly lower in comparison with larvae fed with *Artemia*.

4 Discussion

The casein-based microencapsulated diet used in the current study has been used successfully in previous studies with larval gilthead seabream (Yúfera et al. 1999, 2000, 2003). However, this is the first time that it is used in latter stages of development. In previous studies, growth in seabream feeding microcapsules from 6 to 22 DAH was similar to that observed using live food, but only at 22 °C; at 18 °C the growth was significantly lower (Yúfera et al. 2000). Since the current experiment was conducted at 19 °C, this may be one of the reasons for the lower growth in larvae eating both microcapsules than in larvae eating *Artemia*. Moreover, it has been proposed that several metabolites released by *Artemia* stimulate ingestion rates in fish larvae (Kolkovski et al. 1997b). Therefore, it is likely that after the change from *Artemia* to microcapsules the larvae had a lower food intake, which probably affected the larval growth rate during the first days of experiment.

When working with living larvae of marine fish, the assessment of an experimental microdiet with modified formulation in terms of potential growth enhancement has many constrains. Growth response depends of several variables not only related to microparticle characteristic (i.e. formulation or leaching rate of soluble compounds) but also with the larvae, such as feeding behaviour, digestive and absorptive capacities (Yúfera et al. 2003). All these aspects are especially relevant in the case of dietary proteins, peptides, and amino acids (Kvåle et al. 2007). In addition, the final response can be restricted by the deficiency in other nutrient. Taken into account these restrictions and experimental difficulties, two assumptions were considered in the present study to have a realistic scenario for the diet comparison: a) the amino acid leaching in seawater is similar or lower than that determined in freshwater, b) the microdiet composition after 5 min of immersion in water is representative of the ingested composition.

In this study the effect of amino acid supplementation on individual larval growth was not as notable as expected. Nevertheless, the average dry weight doubled during the experimental period and therefore dietary deficiencies are probably reflected. This is further supported by the significant differences in mortality between treatments. A balanced dietary amino acid profile has been shown to increase the amino acid retention in Senegalese sole postlarvae (Aragão et al. 2004c) and since growth is essentially protein deposition (Carter and Houlihan 2001), an improvement in growth performance was expected in seabream larvae fed the IAAsup diet. Dietary

amino acid imbalances were significantly reduced in the IAAsup compared with the DAAsup diet, although arginine may still be limiting growth. Moreover, amino acid imbalances affect more seriously the fish when the dietary protein content is low, since in this case protein synthesis is seriously compromised.

The amino acid supplementation resulted in different IAA:DAA ratios and studies with rainbow trout revealed that feed intake and feed efficiency ratio increase with increasing dietary IAA:DAA ratios (up to 1.33; Green et al. 2002). As a result, higher nitrogen retention and growth rates and lower nitrogen excretion were observed in that study. However, in the present study, besides the reduction in dietary amino acid deficiencies and the increased IAA:DAA ratio in IAAsup diet, only a tendency for a higher growth rate and total biomass production in larvae fed this than the DAAsup diet was observed. This may be linked with the possible decrease in growth rates during the first days of experiment, as already mentioned. Further studies using long-term experiments or introducing the microcapsules earlier in order to avoid a long period of feeding with *Artemia* seem necessary.

The indispensable amino acid supplementation was not sufficient efficient to increase larval growth rates in short term, however it resulted in an increased survival of seabream larvae eating these microcapsules. Dietary deficiencies in amino acids may have major implications for larval development, other than effects on growth. For instance, a dietary tryptophan deficiency has been reported to induce scoliosis in salmonids (Cahu et al. 2003), while dietary amino acid profile has been shown to affect swim bladder inflation in striped bass larvae (Hughes 2003). On the other hand, scale deformities and vertebral abnormalities were found in rainbow trout fed with a high leucine diet (Choo et al. 1991). Regarding survival, some studies in fish juveniles show an increase in survival when the dietary arginine or sulphur amino acid requirements are met (Alam et al. 2002; Goff and Gatlin III 2004).

Therefore, dietary supplementation with indispensable amino acids in order to obtain a more balanced amino acid profile significantly increased survival. Further studies introducing microcapsules earlier in the development seem necessary in order to optimise growth performances.

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