Note

Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets

Jorge Dias [(1, 2)], Emídio F. Gomes [(2)] and Sadasivam J. Kaushik [(1*)]

[(1)] *Fish Nutrition Laboratory, Unité Mixte INRA-IFREMER, IFREMER, Centre de Brest, 29280 Plouzané, France.*

[(2)] *Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, 4000 Porto, Portugal.*

[(*)] *Corresponding author: E-mail: kaushik@st-pee.inra.fr*

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Abstract

The incorporation of an attractant mixture by substitution of an equivalent amount of basal dietary mixture was studied in terms of feed intake and growth performance in European seabass juveniles (mean initial weight : 17 g) fed plant-protein rich diets during 21 days. Three diets had as main protein sources; fish meal, or one of two soy protein concentrates (S and E). To the basal mixture of these last two diets, as well as to a corn-gluten based diet, an attractant amino acid mixture was added at a 2.5% level. Daily feed intake was measured throughout the experimental period. Feed intake and weight gain were highest in seabass fed the fish meal based diet. In those fed the soy protein concentrate diets, the addition of the attractant mixture improved growth significantly; voluntary feed intake and feed efficiency were also ameliorated in these groups. In terms of growth, feed intake and protein utilisation, lowest values were found in fish fed with the corn-gluten rich diet, supplemented with the amino acid mixture.

Keywords: *Dicentrarchus labrax*, plant proteins, feed intake, amino acids, feed attractants.

INTRODUCTION

Studies on the total or partial replacement of fish meal by alternative protein sources in diets of teleosts are numerous (Tacon, 1994). Despite the existence of some variability between and within fish species in the utilisation of plant products, most studies confirm that for successful replacement of fish meal by plant protein-rich ingredients, special attention should be paid to the use of appropriate technological processes for the deactivation/removal of endogenous anti-nutritional factors (ANF) and for increasing nutrient availability, as well as to the dietary amino acid/mineral supplementation to overcome possible nutritional imbalances (Kaushik, 1990; Rumsey *et al.*, 1993; Tacon, 1994). Besides, several studies have reported that high levels of substitution of fish meal with plant protein sources lead to reduced growth, due mainly to a lower voluntary feed intake (VFI), that may be related to feed palatability (Reigh and Ellis, 1992; Davis *et al.*, 1995; Gomes *et al.*, 1995). Therefore, the beneficial effects of incorporation of an attractant amino acid mixture (AA Mix) by substitution of an equivalent amount of basal dietary mixture on feed intake, growth performance and protein utilisation were studied in a marine teleost, the European seabass (*Dicentrarchus labrax*), fed with plant-protein rich diets.

MATERIAL AND METHODS

Experimental diets

Six experimental diets were formulated to be isoproteic (42 % crude protein (CP)) and isoenergetic (20 kJ/g dry matter) (Table 1). Three diets had as main protein sources; fish meal (F), or one of two soy protein concentrates (S and E), supplemented with L-methionine. To the basal mixture of these last two diets, as well as to a corn-gluten based diet supplemented with some essential amino acids, an attractant mixture (Table 2; Mackie and Mitchell, 1982) was added at a level of 2.5 % (diets SA, EA and GA).

Growth trial

Duplicate groups of 42 juvenile European seabass (mean body weight: 17.0 g) were fed one of six experimental diets during 21 days. Fish were grown in cylindro-conical tanks (volume: 60 l; water-flow rate: 4 $l.min^{-1}$). Water temperature was maintained at 20 °C and salinity at 35 ppm. After one-week of adaptation, a fixed ration size, largely in excess, was distributed by hand, 3 times a day (08:00, 14:00 and 18:00 h). During and after feeding, uneaten feed was collected over 30 min by the continuous filtration system (Choubert et al., 1982), originally used for faeces collection in digestibility trials. Daily voluntary feed intake (VFI) was quantified on a dry matter basis throughout the experimental period. Fish were group weighed at the beginning and at the end of the experimental period.

Sampling and analytical procedures

At the beginning of the trial, 10 fish from the initial stock were sampled for whole-body composition analysis. At the end of the trial, ten fish from each group were sampled for the same purpose. Chemical composition analysis of the diets and whole fish was made using the following procedures: dry matter after drying at 105 °C for 24 h; ash by combustion at 550 °C for 12 h; fat by dichloromethane extraction (Soxhlet), and gross energy in an adiabatic bomb calorimeter (IKA). Protein (N × 6.25) was determined by an automatic flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (Nitrogen Analyser 2000, Fisons Instruments). Amino acid analysis of the diets was assessed by HPLC after an acidic hydrolysis (6 N HCl for 24 h at 110 °C). Amino acids were derivatised with phenylisothiocyanate and were analysed by the PICO-TAG method according to Bidlingmeyer et al. (1984).

Table 1. – Ingredient and composition of the experimental diets (%).

	F	S	E	SA	EA	GA
Soluble fish protein concentrate	9.5	9.5	9.5	9.0	9.0	9.0
Fish meal	38.0					
Soy protein concentrate S[2]		42.8		40.5		
Soy protein concentrate E[3]			42.8		40.5	
Corn gluten						40.5
Extruded peas	31.4	22.8	22.8	21.5	21.5	23.0
Fish oil	11.4	15.1	15.1	14.2	14.2	12.5
L - methionine		0.1	0.1	0.1	0.1	0.1
L - arginine						0.2
L - tryptophan						0.05
Vitamin Mix[4]	1.9	1.9	1.9	1.8	1.8	1.8
Mineral Mix[5]	1.9	1.9	1.9	1.8	1.8	1.8
Binder	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose				2.5	2.5	2.5
Attractant mixture[6]				2.5	2.5	2.5
Crude Protein (% DM)	42.1	41.7	40.8	42.0	41.4	42.2
Crude Energy (kJ/g DM)	20.3	20.2	20.3	20.2	20.2	20.3
EAA Index [7]	111.0	103.9	100.5	98.4	98.0	85.6
Chemical score[8]	82.8	68.7	68.1	69.1	66.8	36.9

[1] CPSP G from Sopropêche, France : 71 % CP, 19.4 % Lip.
[2] From Sogip, Belgium : 68 % CP, 1.2 % Lip.
[3] From Sopropêche, France : 63 % CP, 1.6 % Lip.
[4] According to NRC (1993).
[5] Mineral mixture (g or mg/kg diet): calcium carbonate (40 % Ca), 2.15 g; magnesium oxide (60 % Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75 % I), 0.4 mg; zinc sulphate (36 % Zn), 0.4 g; copper sulphate (25 % Cu), 0.3 g; manganese sulphate (33 % Mn), 0.3 g; dibasic calcium phosphate (20 % Ca, 18 % P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30 % Se), 3 mg; KCl, 09 g; NaCl, 0.4 g.
[6] See Table 2 for definition of attractant mixture.
[7] Essential amino acid index (Oser, 1959), calculated by comparison to whole body EAA profile according to Mambrini and Kaushik (1995).
[8] Chemical score (Mitchell and Block, 1946), calculated by comparison to whole body EAA profile according to Mambrini and Kaushik (1995).

Table 2. – Attractant amino acid mixture in g/100 g diet [AA Mix, according to Mackie and Mitchell (1982)].

Taurine	0.185	L - Isoleucine	0.016
L - Aspartic acid	0.010	L - Leucine	0.030
L - Glutamic acid	0.029	L - Arginine HCl	0.125
L - Proline	0.805	L - Lysine HCl	0.016
Glycine	0.490	L - Histidine HCl	0.008
DL - Alanine	0.150	L - Tyrosine	0.012
L - Threonine	0.024	L - Phenylalanine	0.016
Serine	0.020	Betaine base	0.500
L - Valine	0.020	Inosine	0.014
DL - Methionine	0.020	Hypoxanthine	0.026

Statistical analysis

A one-way analysis of variance model was used to test differences between dietary treatments. When appropriate, means were compared by the Duncan's multiple range test. Statistical significance was tested at a 0.05 probability level. All statistical tests were performed using the SAS (1987) statistical package.

RESULTS

Weight gain, feed efficiency and feed intake were highest in seabass fed with a fish meal based diet (F) (Table 3). In those fed the soy protein concentrate diets (S and E), the incorporation of the attractant AA Mix improved the daily growth coefficient significantly ($p<0.05$); VFI (daily voluntary feed intake) and FE (feed efficiency) were also ameliorated in these groups (SA and EA). In terms of growth, voluntary feed intake and protein utilisation, lowest values were found in fish fed with the corn-gluten rich diet, supplemented with the amino acid mixture.

In terms of overall protein utilisation, best results were achieved in fish fed with a fish meal based diet. In those fed the soy protein concentrate diets, nitrogen deposition was significantly improved by AA supplementation. The dietary use of the AA Mix improved protein retention as well as protein efficiency values, leading to a reduction of nitrogen loss.

DISCUSSION

The results of this study indicate that the high replacement level of fishmeal by plant-proteins in diets for European seabass juveniles reduces growth performance and protein utilisation. Previous studies with this species on fish meal replacement by plant-proteins had also reported reduced growth and lower protein utilisation when soy protein concentrate or soybean meal dietary incorporation exceeded 20 - 30 % level (Alliot *et al.*, 1979; Langar, 1992).

The use of plant protein-rich ingredients may necessitate amino acids supplements to restore the dietary amino acid profile to a level that matches the essential amino acid (EAA) requirements of a given species. In the present study, the poor overall growth performance of fish fed the corn-gluten based diet is probably related to an EAA imbalance (chemical score of about 37), with lysine being the first limiting EAA. However, the essential amino acid index of soy protein concentrate based diets (supplemented or not) were high and similar (ranging from 103 to 98) suggesting a good dietary amino acid balance. The addition of the attractant amino acid mixture to these diets enhanced voluntary feed intake, weight gain and protein utilisation. Mackie and Mitchell (1982), tested this attractant amino acid mixture (which was based on the composi-

Table 3. – Weight gain, voluntary feed intake, feed efficiency and protein utilisation in juvenile seabass (Initial body weight, IBW = 17.0 ± 0.5 g. Values are means ± sd. Figures in the same row with different superscript letters are significantly different ($p<0.05$).

	F	S	E	SA	EA	GA
Final body weight, FBW (g)	25.0 ± 0.5 ^{a}	22.9 ± 0.3 ^{b}	23.7 ± 0.1 ^{ab}	24.5 ± 1.2 ^{ab}	23.2 ± 0.2 ^{b}	22.9 ± 1.1 ^{b}
Daily weight gain (% IBW)	2.35 ± 0.22 ^{a}	1.74 ± 0.15 ^{ab}	1.64 ± 0.18 ^{ab}	2.10 ± 0.16 ^{ab}	1.93 ± 0.22 ^{ab}	1.36 ± 0.26 ^{b}
Daily growth coefficient, DGC[1]	1.86 ± 0.03 ^{a}	1.39 ± 0.02 ^{c}	1.35 ± 0.05 ^{cd}	1.65 ± 0.05 ^{b}	1.57 ± 0.01 ^{b}	1.25 ± 0.08 ^{d}
VFI (g IBW/day)	2.19 ± 0.06 ^{a}	1.82 ± 0.05 ^{bc}	1.79 ± 0.13 ^{bc}	2.03 ± 0.05 ^{abc}	1.98 ± 0.10 ^{abc}	1.64 ± 0.17 ^{c}
Feed efficiency, FE[2]	1.16 ± 0.03 ^{a}	1.00 ± 0.01 ^{ab}	0.98 ± 0.01 ^{b}	1.05 ± 0.03 ^{ab}	1.01 ± 0.02 ^{ab}	0.98 ± 0.02 ^{b}
Protein intake (mg/kg ABW[3]/day)	7.02 ± 0.06 ^{a}	6.11 ± 0.07 ^{ab}	5.73 ± 0.32 ^{ab}	6.74 ± 0.09 ^{a}	6.47 ± 0.20 ^{ab}	5.31 ± 0.43 ^{b}
Protein efficiency ratio, PER[4]	2.58 ± 0.18 ^{a}	2.31 ± 0.14 ^{ab}	2.34 ± 0.09 ^{ab}	2.45 ± 0.12 ^{ab}	2.37 ± 0.15 ^{ab}	2.13 ± 0.19 ^{b}
Protein retention (% intake)	44.7 ± 2.8 ^{a}	40.5 ± 2.2 ^{ab}	42.6 ± 1.2 ^{ab}	44.9 ± 1.9 ^{a}	43.8 ± 2.3 ^{a}	37.6 ± 2.8 ^{b}
N Gain[5] (g/kg weight gain)	27.8 ± 0.2 ^{a}	28.1 ± 0.2 ^{cd}	29.1 ± 0.3 ^{cd}	29.4 ± 0.2 ^{b}	29.5 ± 0.4 ^{b}	28.3 ± 0.5 ^{d}
N Loss[6] (g /kg weight gain)	34.5 ± 4.1 ^{a}	41.4 ± 4.1 ^{ab}	39.3 ± 2.3 ^{ab}	36.1 ± 3.1 ^{a}	38.0 ± 4.0 ^{ab}	47.2 ± 6.4 ^{b}

[1] DGC, Daily growth coefficient : $((FBW)^{1/3} - (IBW)^{1/3}) / 21$ days × 100.
[2] FE, Feed efficiency : (wet weight gain / dry feed intake).
[3] ABW, Average body weight : (IBW + FBW) / 2.
[4] PER, Protein efficiency ratio : (wet weight gain / crude protein intake).
[5] N Gain : (final carcass nitrogen content - initial carcass nitrogen content).
[6] N Loss : Total N intake - N retained.

tion of squid mantle tissue extract) with juvenile seabass fed a vitamin-free casein diet and found that the feeding stimulant activity resided essentially in the neutral L-amino acid fraction. The selective testing of the different amino acids groups or non-amino acid components indicated synergistic effects.

Besides amino acids, fish are known to have gustatory and olfactory sensitivity to other substances such as bile salts, steroid hormones, prostaglandins, carboxylic acids, nucleotides, and even to water pH and CO_2 level. The receptor sites and response mechanisms to all these substances seem to be independent and when more than one stimulatory substance is conjugated, additive effects have been reported (Hara, 1982; 1994). Other specific commercial products containing betaine and some amino acids have been tested with salmonids as feed attractants (Clarke *et al.*, 1994). But the results in terms of an increase in daily voluntary feed intake or in terms of growth improvement are far from conclusive.

CONCLUSION

Besides the limiting essential sulphur-amino acid level of most plant protein-rich ingredients, these products are considered less suitable than fish meal as a major protein source due to their high content in several anti-nutritional factors (ANFs: tannins, lectins, protease inhibitors, oligosaccharides, phytic acid, antigenic proteins, alkaloids, etc.) which influence nutrient availability. In teleosts, the specific effects of endogeneous ANFs on feed intake are still poorly understood, although a number of other effects have been reported (Krogdhal, 1989; Rumsey *et al.*, 1993, 1994; Kaushik *et al.*, 1995). In higher vertebrates, some of the legume seed components, especially tannins (highly polymerised phenolic compounds) and alkaloids (the majority of studies were conducted on lupin alkaloids) are found to be feeding deterrents (Hill and Pastuzewska, 1993; Troszynska *et al.*,1993). Besides, little is known on the endocrine regulation of appetite in fish. Recent studies however, show a link between satiation and growth hormone secretion (Fairbridge and Leatherland, 1992; Pérez-Sánchez, *et al.*, 1995). More investigations are needed to understand the effects of nutrient balance on voluntary feed intake, as the complex mechanisms which control feed intake and satiety are little understood in fish.

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