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Amino Acid Requirements of Sea Bass (*Dicentrarchus labrax*)

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Abstract

In one trial 120 Sea bass fry received tow different feed mixtures containing tow different levels of chitosan (diet A.1.0 and B 2.0 g kg⁻¹). The average initial live weight was about 0.2± 0.1 g fish⁻¹ in each replicate of all treatments. Twenty fish per aquarium were stocked randomly in 6 glass aquaria (120 l each). Fish were fed with experimental diets at rate 15-6 % of the body weight daily. The amino acids (AA) content was in the diets, whole fish and muscle at the end of the growth period (75 days). The results indicated that, at the end of experiment Sea bass fry had reached an average live weight 4.3 and 4.2 g fish⁻¹ for fish fed diet A and B respectively. Also the increase of chitosan levels in the feed to 2% g fish⁻¹ chitosan caused a significant decrease in the content of indispensable amino acid (IAA) and dispensable amino acid (as %v of whole fish and muscle of wet weight). Tow methods were subsequently used to estimate the quantitative IAA requirements of Sea bass fry based on the hypothesis that (a) the dietary requirement pattern of IAA reflects the tissue pattern and (b) the rate of daily deposition in the fish can be equated with the dietary requirement as percentage of 100 g⁻¹ diet. The quantitative IAA requirement (%) of Sea bass (*Dicentrarchus labrax*) diets as follow: Lysine 2.27, Methionine 1.09, Therionine 0.61, Leucine 2.4, Isoleucine 0.47, Histidine 1.6, Arginine 1.39, Phenylalanine 1.43, Valine 1.22 and Tryptophan 0.42.

1. Introduction

Quantitative data on the requirements for all ten amino acids are available only for a selected species of finfish: chinook salmon, chum salmon, tilapia, channel catfish, common carp, Japanese eel, catla and milkfish. Despite increasing mariculture production of species such as the European sea bass (*Dicentrarchus labrax*), the gilthead sea bream (*Sparus aurata*) and the turbot (*Psetta maxima*), data on the IAA requirements are scarce.

The values, when expressed as a proportion of the diet indicating large variations in the requirements of different species. Dietary amino acids are needed for growth and maintenance and the former is quantitatively much more important in young rapidly growing fish.

The occurrence of an imbalance of amino acids in fish nutrition is nevertheless possible. Indeed if protein - sparing feed is provided to fish and the amino acids supply in the feed must be adjusted so that meet fish needs. Many investigators had varying degree of success in using practical test diets to determine amino acids requirements [22], [6], [32], [2]. 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 sea bass and bream usually ranges between 25-35% making them poor protein converters in comparison to salmonids [29]. The results of various experiments have shown that, the smallest elimination of endogenous N occurs when the used feed has an amino acid makeup most close to the body protein [10].

The present work was undertaken to analyze the whole body amino acid composition of two treatments of Sea bass currently cultivated to draw an estimation of their IAA requirements, based on the relative proportions of whole body IAA.

2. Material and Methods

Food mixture consists of fish meal, soybean meal, yellow corn meal, and rice bran and fish oil according to Table 3. Two different food mixtures were made by combined two different levels of chitosan diets A 1 and B 2 gkg⁻¹ chitosan.

The experiment was conducted in 6-glass aquaria each containing 100 liter of saline water. In each aquarium about one third of saline water volume was daily replaced by aerated fresh saline solution after cleaning and removing the accumulated excreta. All aquaria were supplied with compressed air for oxygen requirements. Fish were exposed to the natural light conditions.

Fish in each aquarium were fed twice daily (six days a week) at a rate of 15% of body weight for the first 30 days then decreased to 12% for 15 days then to 10 and 8% for the rest of experimental period.

Water temperature and dissolved oxygen were measured every other day using an YSI Model 58 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH). Ammonia and nitrite were measured at weekly intervals. Alkalinity was monitored twice weekly using the titration methods of [12] pH was monitored twice weekly using an electronic pH meter (pH pen Fisher Scientific, Cincinnati, OH). During the feeding trial, the water quality parameter averaged ($\pm$ SD): water temperature $27.8 \pm 0.8^\circ\text{C}$ dissolved oxygen $4.8 \pm 0.4 \text{ mg l}^{-1}$; pH 7.4 ± 0.6 ; ammonia $\pm 0.04 \text{ mg l}^{-1}$; nitrite $0.1 \pm 0.05 \text{ mg l}^{-1}$; nitrate $1.5 \pm 0.2 \text{ mg l}^{-1}$; alkalinity $181 \pm 46 \text{ mg l}^{-1}$.

A set of 120 Sea bass (*Dicentrarchus labrax*) fry obtained from Fish Hatchery, National Institute of Oceanography & Fisheries Alexandria Branch, Egypt were used in the present study. Fish were placed randomly in 6 glass aquaria; four replicates per treatment were used in this study. Each aquarium stocked with twenty fry of Sea bass with an average initial body weight of $0.21 \pm 0.01 \text{ g}$ fish. To minimize stress of handling, fish from each aquarium were weighed every 2 weeks and at the end of the feeding trial (75 days). There after, six fish per replicate for each treatment under study were killed and kept in the freezer. For preparation of samples the frozen sea bass were slightly cut into parts minced and then homogenized. The dry matter (DM) and

crude protein (N x 6.25) were determined according to [1].

According to [18] three hydrolyses were carried out for each sample, the amino acid composition in each hydrolysate was determined with the help of the automatic amino acid analyzer AAA 400 amino acid analyser (Ingos Ltd., Czech Republic) equipped with a Watrex Polymer 8 ion-exchange column (20 x 3.7 cm) for amino acids and an Ostion LG ANB ion-exchange column (6 x 3.7 cm) for biogenic amines. Free amino acids and biogenic amines were separated by stepwise gradient elution using Li⁺ buffer systems for amino acids and using Na⁺/K⁺ buffer system for biogenic amines. Colorimetric detection was accomplished at 570 and 440 nm after postcolumn derivatisation with ninhydrin reagent [5]. The analyses were done in duplicate. The average values $\pm$ the relative standard deviations. The amino acid determination of the feed ration was done in an analogue way to the method for the animal carcasses. Tryptophan was determined according to the method of [3].

The IAA value were evaluated with the help of the SAS program package as one way classification analysis of variance with application of the student Newman keuls test.

The IAA pattern of whole fish and muscle was then determined by expressing each of the ten EAA as a percentage of the sum of IAA. This pattern is required in the diet of Sea bass was calculated by assuming the lysine requirement to be 2.0% of diet [28] and adjusting the levels of the other IAA accordingly.

[21] Proposed a method for determination of the quantitative IAA requirements of fish based on determination the rate of deposition of each of ten IAA as g/100g fish/day. Samples of fish, fed 45% protein feed, were analyzed for IAA as described before and the daily rate of deposition calculated. According to feeding rate of 10 % of the body weight/day of 45 % protein feed with a protein digestibility of 90% were assumed.

3. Results and Discussion

The mean contents of indispensable and dispensable amino acid acids, given as % of diets and % wet weight of carcass for each of the two treatments, are shown in Tables 1 & 2. The one way statistical evaluation showed that, differing in chitosan supplies have a significant effect on the crude protein content and thus on all amino acid values in Sea bass. The relative amino acid content at the chitosan level 1.0 g kg⁻¹ diet was higher than it was lower chitosan level 2.0 g kg⁻¹ diet.

The results (Tables 1&2) demonstrate, however, that changes in the amino acid content depend on the protein content in Seabass. The degree to which the amino acid pattern of Seabass protein was affected can be observed by the value of amino acids content per 100 g fish weight. Thus the content of indispensable and dispensable amino acid acids clearly decreased by increasing the chitosan levels in the feed from 1.0- 2.0 g kg⁻¹ diet. A variable dietary protein and energy supply significantly affects the protein content

and protein retention of Carp [24], [35]; [36].

Table 1. Composition and proximate analysis of the experimental diets

Ingredients (%)	Diets	
	A	B
Fish meal (C.P.70%)	50.0	50.0
Soybean meal (C.P.44%)	19.0	19.0
Yellow corn meal	7.0	7.0
Rice bran	11.7	11.7
Fish oil	3.0	3.0
Sunflower oil	3.0	3.0
Vit. & Min premix ¹	2.0	2.0
Calcium diphosphate	1.0	1.0
Molasses ²	2.0	2.0
Colin	0.2	0.2
Vitamin C	0.3	0.3
Lysine	0.3	0.3
Methionine	0.3	0.3
Antitoxins	0.1	0.1
Enzymes	0.1	0.1
Chitosan (g/kg)	1.0	2.0
Proximate analyses (%) ³		
Dry matter	95.4	92.3
Crude protein	45.1	45.1
Crude fat	10.9	10.7
Ash	10.1	10.4
Crude fiber	3.5	3.8
NFE ⁴	30.4	30.0
Gross energy Kcal	496.8	494.8

¹Vitamin and mineral premixed according to (17).

²Molasses was used as a binder and attractant according to (9).

³Values represent the mean of three sample replicates.

⁴NFE = 100 - (% protein + % fat + %fiber + % ash).

Table 2. Content of essential and none essential amino acids in the experimental diets with varying levels of chitosan supply g kg⁻¹

Amino acids	Diets supplemented with chitosan	
	A (1.0 g kg ⁻¹)	B (2.0 g kg ⁻¹)
Indispensable amino acids (%)		
Lysine	2.66	2.66
Methionine	1.47	1.47
Therionine	0.7	0.7
Leucine	3.06	3.07
Isoleucine	0.46	0.46
Histidine	1.5	1.51
Arginine	1.58	1.59
Phenylalanine	1.5	1.51
Valine	1.63	1.63
Tryptophan	0.3	0.31
Dispensable amino acids (%)		
Alanine	4.69	4.7
Aspartic acid	5.7	5.73
Cystine	0.08	0.09
Glycine	5.88	5.89
Glutamic acid	8.86	8.89
Proline	0.06	0.06
Serine	1.62	1.63

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 [7] 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. In contrast, the various protein and energy level had no influence on the amino acid pattern of Sea bass protein. This result agrees well with our results of work in which the amino acids composition of the body protein could not be changed by varying the levels of chitosan. Deviations in the amino acids compositions of the body protein are only conceivable under extreme conditions for example, when a shift in tissue ratio is accompanied by a varying amino acids pattern. Yet, in this work has been shown that Sea bass with different live weight (4.3 g to 200 g) have the same amino acids pattern. Similarly, it was demonstrated that, Carp with different live weights have the same amino acids composition in their entire bodies [25].

The sparse available data which are concerned with the amino acid composition of protein in the carcasses or edible portions of different salmonids, tilapia and marine fish [33]; [11]; [23] do exhibit general agreement with the work presented here. In addition, a more recent study done with 12 different salt water fish species, also confirms the quite uniform amino acid pattern [19]. The studies of [33] showed higher indispensable amino acids content in the edible portions of carp and trout as compared to the indispensable amino acids in whole fish.

As mentioned earlier, [10]; [12]; [8] provided clues to optimum amino acid requirements and composition of feed protein. But it should be discussed to what degree the amino acids composition of carcass protein can be used in metabolism and serve as building blocks for further body substances as methionine, and phenylalanine. In addition, maintenance metabolism and the rate of re-utilization of individual amino acid must be taken into account [26]. The comparison of the amino acid pattern in the whole fish and muscle with that of the feed protein should at least make manifest any gross amino acids metabolism. In Table 2 Leucine, was presented in feed protein in relatively high amount in comparison to lysine.

The amino acid pattern which corresponds to the recommendations based upon requirement and adjustments of these amino acids can be also regarded as oriented in the amino acid compositions of Sea bass protein. Leucine is clearly deficient and is regarded as necessary in significantly lower amounts. In addition, methionine and phenylalanine appear in somewhat lower relative amounts (Table 3). While, [16] reported that 50% of tyrosine can substitute for phenylalanine and 60% of cystine can substitute for methionine.

Table 3. Indispensable amino acid and dispensable amino acid in whole and muscle of Sea bass (*Dicentrarchus labrax*)

Parameters	Diets supplemented with chitosan			
	A		B	
Final fish weight (g fish ⁻¹)	4.3±0.2		4.2±0.1	
Chitosan level (g kg ⁻¹ diet)	1.0		2.0	
Treatments (%)	Whole fish	Muscle	Whole fish	Muscle
Protein content (%)	17.82	18.51	16.92	17.81
Moisture content (%)	72.8	73.4	72.2	73.5
Indispensable amino acids (%)				
Lysine	0.85	1.26	0.82	1.21
Methionine	0.38	0.5	0.36	0.49
Therionine	0.24	0.29	0.23	0.28
Leucine	0.85	1.18	0.82	1.15
Isoleucine	0.22	0.22	0.21	0.21
Histidine	0.68	1.01	0.65	1.0
Arginine	0.58	0.68	0.56	0.66
Phenylalanine	0.62	0.7	0.5	0.6
Valine	0.58	0.6	0.56	0.58
Tryptophan	0.2	0.2	0.24	0.26
Dispensable amino acids (%)				
Alanine	0.77	1.91	1.7	1.84
Aspartic acid	2.25	2.52	2.17	2.42
Cystine	0.76	0.82	0.61	0.64
Glycine	3.16	2.15	3.06	2.07
Glutamic acid	2.78	3.51	2.69	3.38
Proline	0.03	0.02	0.03	0.02
Serine	0.57	0.61	0.55	0.59

Table 4. The quantitative EAA requirement (%) of Sea bass (*Dicentrarchus labrax*) diets

Amino acid	Basic of whole fish pattern ¹	Calculation muscle pattern ¹	Daily deposition ²	Determined IAA for Sea bass as % diet	Determined IAA for as g 16 gN	Required ³ g16 N
Lysine	2.0	2.0	2.82	2.27	5.05	4.7
Methionine	0.91	0.8	1.56	1.09	2.42	2.2
Therionine	0.58	0.5	0.74	0.61	1.35	2.4
Leucine	2.06	1.9	3.24	2.4	5.32	4.1
Isoleucine	0.52	0.5	0.49	0.47	1.12	2.5
Histidine	1.6	1.6	1.59	1.6	3.54	1.3
Arginine	1.39	1.1	1.68	1.39	3.08	3.7
Phenylalanine	1.58	1.12	1.59	1.43	3.17	4.1
Valine	1.39	0.96	1.73	1.22	2.7	2.8
Tryptophan	0.47	0.48	0.32	0.42	0.94	0.5

¹Whole fish or muscle = 2.0/determined lysine x amino acid % (28).

²IAA deposition = protein efficiency ratio x amino acid % (21).

³According to (28).

Table 4 shows the results of experiments, where the method of [21] equating the deposition rate of each of IAA with requirement was used. The results are in close agreement with those obtained in Table 3. Neither the method of [4] nor [21] take into account the metabolic rate of IAA other than for protein synthesis is particularly no allowance is made for the maintenance requirement for the IAA. However, it is unlikely that maintenance will change the requirement pattern [4] and should only have slight influence on the absolute dietary requirements.

In conclusion, given the quantitative importance of dietary proteins, of the ideal dietary AA supply in nitrogen utilization affecting fish growth, its implications on nitrogenous losses and given the strong necessity for use of alternatives to fish meal in aqua feeds. The quantitative IAA requirement (%) of Sea bass (*Dicentrarchus labrax*) diets as follow: Lysine 2.27

Similar conclusions can be drawn from the work of [12], to what degree the amino acid composition of Sea bass and fish feed protein is actually useful in determining a need-oriented amino acid supply in food.

Data in table 4 showed that values of indispensable amino acids in whole fish and muscle and daily deposition of Sea bass relation to lysine (lys.=2.0) is corresponding to the recommendations for minimum amino acids requirements [21]; [31]; [28]; [37]. The feed protein in question must be considered to be high grade based upon its composition and high digestibility of 87-94% [34]. It is obvious that the amino acid composition of the feed protein corresponds well with the amino acid composition of Sea bass protein [29]; [15]. [4] showed that for the chick a direct correlation existed between the tissue IAA pattern and dietary requirements pattern. [6] showed that, this was also true for carp. Generally, the requirements based on the two tissues were close agreement with main exception low methionine requirement is predicted from muscle tissue analysis [27].

Methionine 1.09 Therionine 0.61 Leucine 2.4 Isoleucine 0.47 Histidine 1.6 Arginine 1.39 Phenylalanine 1.43 Valine 1.22 Tryptophane 0.42 (g100g⁻¹ diet).

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