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Note

Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles

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Abstract – A number of feedstuffs was tested to obtain the apparent digestibility coefficients (ADC) of dry matter, protein, energy and phosphorus for seabass juveniles (individual mean weight of 40 g). The effect of the inclusion level (15 and 30 %) of a feedstuff in a reference diet on the ADC was also evaluated. The feedstuffs tested in this trial were two fish meals, a fish protein hydrolysate, blood meal, meat meal, soybean meal and yellow dextrin. The ADC of dry matter were consistently lower than those of energy, but there was a significant positive correlation between them. The ADC of protein were high (range 89–97 %) in all feedstuffs. The ADC of energy of animal feedstuffs (range 86–96 %) and dextrin (93 %) were also high, while that of soybean was lower (70 %). The ADC of phosphorus of animal feedstuffs averaged 81 % whereas that of soybean was only 38 %. There were no significant differences in the ADC of protein and energy of each feedstuff at the two inclusion levels tested. © Ifremer/Elsevier, Paris

Digestibility / seabass / feedstuffs

1. INTRODUCTION

As nutrients are not available to an animal before they are absorbed in the digestive tract, the nutritional value of a feedstuff depends not only on its chemical composition but also on the digestibility of its nutrients and energy. Therefore, knowledge of the apparent digestibility coefficients (ADC) of nutrients of feedstuffs is essential for correct diet formulation. However, the available data on the ADC of feedstuffs used in fish diets is scarce, particularly for marine fish species [20].

Regarding energy, both digestible energy (DE) and metabolizable energy (ME) have been used to express feedstuff values for fish [19]. Theoretically, it is recognized that ME is a more appropriate measure of the physiologically available energy for the animal, however, as ME is more influenced by nutritional and physiological factors than DE, it is not recommended for the evaluation of individual feedstuffs for fish diets [6].

The overall digestibility of a diet can be estimated by adding the digestible values that have been determined for the individual feedstuffs that comprise the diet, since it is assumed that there are no significant interactions among ingredients in their digestibility [5]. However, this assumption may not always be true, especially when feedstuffs are rich in carbohydrates or when antinutritional factors are present [15].

The determination of the ADC of feedstuffs is complicated, as very few of them are accepted as the sole component of a fish's diet [19]. Therefore, the ADC of feedstuffs is usually determined by comparing the digestibility of a reference diet with that of a test diet, obtained by blending a proportion of the reference diet (usually 70 %), containing an indigestible indicator such as chromic oxide, with a proportion (usually 30 %) of the test ingredient. Thereafter, the ADC for nutrients and energy of the reference and the test diets are calculated by an indirect method [19] and on the individual ingredients themselves [8]. This approach assumes that there are no interactions among dietary

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ingredients during digestion and that the ADC of feedstuffs is additive [8].

The present study was undertaken to estimate the ADC of dry matter, protein, energy and phosphorus of several feedstuffs that are commonly used in diets for seabass and to assess whether the inclusion level of the feedstuffs (15 or 30 %) influenced these coefficients.

2. MATERIAL AND METHODS

This experiment was carried out using a thermoregulated recirculation water system in which there were eight 50 L fiberglass tanks designed according to the Guelph System [8]. A faeces settling column was connected to the outlet of each tank. Twelve seabass (*Dicentrarchus labrax*) juveniles with an average individual weight of 40 g were randomly distributed to each tank and acclimatized to the experimental conditions for more than one week. The average batch weight of the fish in each tank at the start of the experiment was 480 ± 1 g and at the end of the experiment was 1 037 ± 41 g.

Proximate constituents, phosphorus and energy of the test ingredients used in this experiment is provided in *table I*. The reference diet was formulated to contain 50 % protein and 12 % lipid and its composition is presented in *table II*. The test diets contained 70 % of the reference diet and 30 % of the test ingredient (trial 1) or 85 % of the reference diet and 15 % of the test ingredient (trial 2). Proximate composition of the test diets is presented in *table III*. The diets, after mixing the ingredients homogeneously, were cold pelleted in a laboratory pellet mill (CPM) equipped with a 2 mm die.

Each trial comprised three 10-day faecal collection periods. In each period, the reference and each of the seven test diets were allocated to one group of fish. Then, the diets were randomly changed between tanks and the procedure repeated twice, giving a total of three pooled faecal samples for each diet. The fish were fed by hand to satiation twice a day, except in the morning of the first day of each period, when they were batch-weighed after application of a mild anaesthetic (ethylene glycol monophenil ether, 0.3 $mL \cdot L^{-1}$). In each 10-day period, the fish were allowed to acclimatize to the feed for the first three days and faeces were collected over the next seven days. All faeces collected from each tank in each period were pooled for analysis.

On faecal collection days, the faeces from each tank were collected directly into 50 mL centrifuge tubes before the first daily feeding. Faeces were centrifuged for 30 min at 2 100 × *g*, the supernatant was discarded and the faeces were frozen at –20 °C until analysed. After the second daily meal, the tanks and the settling columns were thoroughly cleaned to eliminate all feed waste and faecal residues. In this procedure, about 50 % of the water from each tank was replaced. The average water temperature and salinity during the experiment was 21.6 °C and 33.4, respectively.

Digestibility determinations were done by an indirect method, using chromic oxide as a marker included in the reference diet at 1 % of dry weight. The ADC of nutrients and energy in the reference and test diets were calculated by the following formula:

$$ADC\ (\%) = [1 - (ID \times NF)/(IF \times ND)] \times 100$$

where: ID is % indicator in diet, IF is % indicator in faeces, ND is % nutrient or energy in diet and NF is % nutrient or energy in faeces.

The ADC of a nutrient or energy in the feedstuffs was calculated according to the following expression:

$$ADC = (ADC_{test\ diet} - [(1-Y) \times ADC_{reference\ diet}])/Y$$

where Y is % test ingredient in the test diet.

Analyses of feedstuffs, diets and faeces were conducted as follows: water content, by drying in an oven at 104 °C to constant weight; ash, by combustion of samples in a muffle furnace at 450 °C for 16 h; protein N(6.25) according to the Kjeldhal method, using Kjeltec digestor and distillation units; lipid by petro-

Table I. Proximate analysis, energy and phosphorus of the feedstuffs used in the digestibility trial.

Feedstuff	Dry Matter (%)	Protein (%DM)	Lipids (%DM)	Ash (%DM)	Phosphorus (%DM)	Energy ($kJ \cdot g^{-1}$DM)
Fish meal A (1)	92.5	74.9	13.7	11.4	1.8	22.7
Fish meal B (2)	89.5	70.1	11.5	18.3	3.1	21.2
Soybean meal (3)	87.7	51.6	2.5	7.5	0.6	20.5
CPSP G (4)	96.7	71.7	21.3	4.5	0.8	27.5
Blood meal (5)	90.4	97.1	1.0	1.9	–	24.4
Yellow dextrin (6)	88.3	–	–	0.3	–	17.3
Meat meal (7)	96.5	75.1	14.7	10.2	3.5	22.4

(1) Standard fish meal from Norway (70 % protein), supplied by Sopropêche, France.
(2) Portuguese brown fish meal, obtained from sardine, supplied by Olfaixe, Portugal.
(3) Toasted, dehulled, solvent extracted soybean meal, supplied by Sorgal, Portugal.
(4) Fish protein concentrate, supplied by Sopropêche, France.
(5) Supplied by Aprocat, Spain.
(6) Commercial yellow maize dextrin.
(7) Meat meal, steam cooked, defatted by pressing, sieved, supplied by Rogério Leal e filhos, Portugal.

Table II. Composition and chemical analysis of the basal diet (dry matter basis).

Ingredients		($g.kg^{-1}$)
Fish meal A		555
CPSP G		50
Blood meal		50
Yellow dextrin		263
Cod liver oil		32
Vitamin premix (1)		5
Mineral premix (2)		10
Choline chloride (50 %)		5
Binder (lignin)		20
Chromic oxide		10
Chemical analysis:		
Water content (%)	9.7	
Protein (N × 6.25) (%)	50.2	
Lipid (%)	12.1	
Ash (%)	9.8	
Phosphorus (%)	1.0	
Gross energy ($kJ{\cdot}g^{-1}$)	21.8	

(1) Vitamins: ($mg{\cdot}kg^{-1}$ diet): alpha-tocopherol 100; menadione 20; thiamin 5; riboflavin 5; Ca pantothenate 40; nicotinic acid 100; pyridoxine 10; folic acid 5; cyanocobalamin 0.05; biotin 0.5; ascorbic acid 200; inositol 300; ($IU{\cdot}kg^{-1}$ diet): retinol 18 000; cholecalciferol 2 500.
(2) Minerals ($mg{\cdot}kg^{-1}$ diet): cobalt sulphate 1.9; copper sulphate 19.6; iron sulphate 200; sodium fluoride 2.2; potassium iodine 0.8; magnesium oxide 830; manganese oxide 26; sodium selenite 0.7; zinc oxide 37.5; bicalcium phosphate 8 020; potassium chloride 1 150; Sodium chloride 440.

leum ether extraction in a Soxhlet extraction system; total phosphorus, by a spectrophotometric method, using the vanado-molybdate reagent; chromic oxide as in Groop and Tacon [9]; gross energy by burning the samples in an adiabatic bomb calorimeter (model PARR 1261).

Statistical analyses of the data were done by one- or two-way ANOVA, with a probability level of 0.05 for rejection of the null hypothesis [31]. Significant differences among means were determined by Tukey's multiple range test.

3. RESULTS AND DISCUSSION

The apparent digestibility coefficient (ADC) of the feedstuffs determined at 30 % and 15 % inclusion levels are presented in *table IV.* Due to insufficient sample size, faecal samples from the three periods of each series were pooled for phosphorus analysis. In the present study, differences in the ADC values of the reference diet determined in the two trials were not statistically significant ($P > 0.05$), and ave-raged 85.0 % for dry matter, 92.8 % for protein and 93.3 % for energy.

The ADC of a feedstuff reflect the digestive capability of the species and is influenced by biotic and abiotic factors such as fish size, physiological condition, ration level and water temperature [20]. Moreover, the processing technology applied to the feedstuff or to the diet manufacture considerably affects the ADC of a feedstuff. The apparent digestibility of a feedstuff may also be strongly affected by the method employed to obtain faecal samples, which can lead to an over- or under estimation of the ADCs. However, the method used in this study (Guelph system) along with that of rotating screen (INRA system) were recently recommended as the most adequate methods for faecal collection [12].

Although Hajen et al. [13] consider that when faeces are collected in salt water, a considerable amount of salt in the dried faeces will dilute the concentrations of all faecal constituents and will lead to underestimation of dry matter digestibility. In this trial, as collected faeces were centrifuged and the supernatant discarded, it was assumed that contamination of faeces with salt would be insignificant and could be disregarded.

Table III. Proximate composition, phosphorus (% dry matter) and energy content of the test diets used in trial 1 (30 % replacement level of the reference diet) and in trial 2 (15 % replacement level of the reference diet).

Test diets:	Water content (%)	Ash	Protein	Lipids (% DM)	Phosphorus	Gross Energy ($kJ{\cdot}g^{-1}$ DM)
Trial 1 :						
Blood meal	10.3	7.4	64.2	8.7	–	22.3
Fish meal A	9.8	10.1	57.7	12.6	1.26	21.9
Soybean meal	10.9	8.8	50.5	9.2	0.91	21.3
CPSP G	10.7	8.3	56.5	15.6	0.97	23.2
Fish meal B	9.6	12.4	56.0	11.9	1.66	21.5
Yellow dextrin	7.5	7.0	35.1	8.4	–	20.0
Meat meal	9.5	9.8	57.5	12.8	1.78	21.8
Trial 2:						
Blood meal	10.3	8.7	57.2	10.4	–	22.0
Fish meal A	11.9	9.9	53.8	9.9	1.15	21.7
Soybean meal	10.4	9.5	50.2	10.7	0.98	21.4
CPSP G	10.7	9.2	53.3	13.8	1.01	22.7
Fish meal B	11.6	11.1	53.1	12.0	1.35	21.5
Yellow dextrin	7.8	8.4	42.7	8.4	–	20.8
Meat meal	10.8	10.0	53.8	12.4	1.41	21.7

Table IV. Apparent digestibility coefficients (%) of the feedstuffs for seabass, tested at the 30 % and 15 % replacement levels. (Mean ± standard deviation; at each replacement level, in the same column numbers followed by different letters are significantly different, $P < 0.05$).

Feedstuff:	Dry matter	Protein	Energy	Phosphorus
30 % replacement level:				
Fish meal A	86.0 ± 1.6^{b}	96.0 ± 0.9^{a}	94.5 ± 1.3^{ab}	79.9
Fish meal B	71.4 ± 1.0^{d}	89.5 ± 0.6^{b}	86.2 ± 0.2^{c}	75.4
Soybean meal	65.5 ± 2.5^{e}	89.8 ± 1.5b	69.3 ± 2.3^{d}	36.1
CPSP G	85.6 ± 1.3^{b}	96.5 ± 0.5^{a}	94.6 ± 0.4^{a}	83.5
Blood meal	90.4 ± 2.3^{ab}	90.6 ± 1.6^{b}	92.1 ± 1.7^{b}	–
Yellow dextrin	92.1 ± 0.7^{a}	–	93.6 ± 0.9^{ab}	–
Meat meal	79.6 ± 2.2^{c}	92.0 ± 1.2^{b}	86.4 ± 1.6^{c}	79.6
15 % replacement level:				
Fish meal A	86.7 ± 0.9^{b}	95.3 ± 1.7^{ab}	96.5 ± 0.4^{a}	81.7
Fish meal B	72.2 ± 3.1^{d}	90.5 ± 1.9^{cd}	89.1 ± 1.4^{d}	78.4
Soybean meal	61.4 ± 2.0^{e}	88.6 ± 1.6^{d}	69.8 ± 0.9^{e}	40.9
CPSP G	88.5 ± 0.3^{ab}	97.8 ± 0.7^{a}	95.7 ± 0.6^{ab}	86.5
Blood rmeal	92.9 ± 1.7^{a}	93.1 ± 2.6^{bc}	93.5 ± 1.7^{bc}	–
Yellow dextrin	90.0 ± 2.8^{ab}	–	92.6 ± 1.5^{c}	–
Meat meal	79.9 ± 1.7^{c}	91.0 ± 1.8^{cd}	84.6 ± 2.8^{d}	82.6

Except for yellow dextrin, the feedstuffs tested in this study were all protein concentrates, and as seabass is a carnivorous species, the ADC of protein of the tested feedstuffs were high, as expected, ranging from 89 % to 97 %.

The ADC of protein of fish meal A was identical to the values reported for a similar feedstuff in other studies with this species [1, 27], but it was higher than reported by Spyridakis et al. [26]. The digestibility of fish meal B protein, and of the fish protein hydrolysate were similar to the values reported for rainbow trout [4, 11]. The ADC of protein of the fish by-products were inversely correlated to the ash content, which agrees with the results of other studies on fish [2, 18]. The digestibility of blood meal protein was higher than values reported in the literature for other carnivorous species [4, 14, 20, 22, 28], but lower than the 100 % observed in red drum [17]. However, differences in the ADC of blood meal protein are not unexpected, as the processing conditions applied to this feedstuff may strongly affect its digestibility [7, 20]. The ADC of meat meal protein was also similar to that obtained for meat and bone meal [1] in sea bass, and to values reported in the literature for meat meal in other species [11, 29]. It was, however, higher than values reported for meat and bone meal in other species [10, 17, 20, 28]. The ADC of soybean meal protein was also high, and similar to that reported by Alexis [1] for this species.

In this study, while the ADC of energy of the animal feedstuffs were high (range: 86–96 %) that of soybean meal was considerably lower (70 %), which may be attributed to the higher crude fiber and carbohydrate content of this feedstuff [20]. The ADCs of energy observed in this trial were within the range of values reported for other carnivorous species [4, 10, 14, 20, 28], except that of blood meal which had a high energy digestibility, as it was already the case for protein digestibility.

In seabass, the ADC of energy of dextrin was not very different from that obtained in rainbow trout (88.2 %) [11], although it was high if compared with values reported previously for that species [24, 25]. Moreover, in this trial the ADC of energy of dextrin was not affected by the dietary inclusion level, while in rainbow trout, carbohydrate digestibility is negatively correlated to the dietary inclusion level [3, 22, 24].

According to Cho and Kaushik [6], the ADC of energy of feed ingredients closely correlates to that of dry matter. In this trial, the ADCs of dry matter were in all cases lower than those of energy, although there was also a significant positive correlation between them ($R^2 = 0.773$; $n = 14$; $P < 0.01$).

The average ADC for phosphorus in the animal by-products was high, averaging 81 % (range: 77–85 %). In the literature, there is considerable variability in the reported values of phosphorus digestibility for animal feedstuffs, especially those of fish meal. The values obtained in this study are within the range of values reported for salmonids [16, 21] but are higher than those reported for common carp [21] or red drum [10]. Although the phosphorus digestibility of fish meal for rainbow trout was considerably lower [23] than values obtained in this as well as in other studies, this may be attributed to methodological differences in faeces collection. Soybean phosphorus availability determined in the present study was also comparable to values reported for this feedstuff in other fish species [10, 16, 30].

Two-way analysis of variance of the parameters tested in this trial showed that there was a significant effect ($P < 0.05$) of the replacement level on the ADC of dry matter. On the contrary, the ADCs of protein and energy of each feedstuff were not significantly different at the two inclusion levels, although a significant ($P < 0.05$) interaction between feedstuffs and inclusion level was noticed.

In chinook salmon [14], the ADCs of protein and energy of extruded wheat and soybean protein isolate were not significantly different when tested at 15 % or 30 % incorporation levels, although data variability was higher when the test ingredients were incorporated at the lower level. Contrary to these findings, Spyridakis et al. [26] stated that the ADC of protein for seabass varies with the dietary inclusion level and, in rainbow trout, Pfeffer et al. [22] concluded that the effect of replacement level on the ADCs of protein and energy depended upon the feedstuff considered.

The calculation of the ADC of the reference diet, by applying the ADCs of the feedstuffs that compose it, showed that the determined and estimated values were very similar (ratio determined:estimated ADCs were 1.00 for dry matter, 1.02 for protein and 0.98 for energy). The same has been previously checked in different species [8, 29]. This suggest that the ADC of each feedstuff was not significantly affected due to interactions among feedstuffs in the diet, and that for practical purposes the ADC of a diet may be calculated from the ADC of the ingredients that compose it.

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