Turkish Journal of Fisheries and Aquatic Sciences 11: 87-92 (2011)

www.trjfas.org
ISSN 1303-2712
DOI: 10.4194/trjfas.2011.0112

Appearent Digestibility of Plant Protein Based Diets by European Sea Bass *Dicentrarchus labrax* L., 1758

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Received 17 August 2009
Accepted 03 November 2010

Abstract

Commercial culture of carnivorous fish demands the reduction of environmental impact of feeds; that requires minimal use of dietary animal protein. This study investigated the digestibility of diets formulated exclusively out of plant protein, added rendered ingredients and feed attractants, by the carnivore European sea bass, *Dicentrarchus labrax*. Juvenile European sea bass (14.0±1.0 cm) conditioned to accept artificial dry feed were stocked in polypropylene cages and fed ad libitum in three daily meals, seven experimental diets containing varying levels of vegetable and animal protein sources, added of different feed stimulants. After last daily meal, cages were transferred to cylindrical–conical-bottomed, 200 L aquaria, where faeces were collected by sedimentation into refrigerated containers, preserved and later analysed for chemical composition. Soybean meal can be used as partial substitute of animal protein in diets for European sea bass; the poultry by-product meal shows as a good option as animal protein source in these rations. Control treatment –50PP:50AP– yielded best performances; the requirement for the use of fish meal in the formulation for carnivorous diets is, at least, questionable. Results of the digestibility trials demonstrated the importance of determining the diet digestibility, if precision in the formulation of least-cost feeds for carnivorous fish is the ultimate goal.

Keywords: Carnivorous fish, *Dicentrarchus labrax*, plant protein, rendered ingredients, practical diets.

Bitkisel Protein İçerikli Levrek (*Dicentrarchus labrax* L., 1758) Yemlerinin Görünür Sindirilebilirliği

Özet

Ticari olarak yetiştiriciliği yapılan karnivor balık türlerinin beslenmesinde kullanılan yemlerin çevresel etkisinin azaltılması, yemlerde hayvansal kökenli protein kaynaklarının daha az kullanılmasını gerektirmektedir. Bu çalışmada levreklerin (*Dicentrarchus labrax*) beslenmesinde rendering ürünü hammaddeler ve çeşitli yem katkı maddeleri ile zenginleştirilen bitkisel kökenli hammaddelerin sindirilebilirliği araştırılmıştır. Karma yem alımına adapte edilmiş yavru levrekler (14,0±1,0 cm) polipropilen kafeslere konulmuş, değişik yem katkı maddeleri ve farklı oranlarda bitkisel ve hayvansal protein kaynakları içeren yedi değişik yem ile günde üç kez *ad libitum* beslenmiştir. Kafesler, günlük son beslemenin ardından, silindir konik tabanlı 200 litrelik akvaryuma alınmış, balıklardan gelen dışkılar toplanmış, üstteki sıvı sediment atıldıktan sonra plastik kaplara alınarak, daha sonra kimyasal kompozisyonları analiz edilmek üzere, dondurulmuştur. Soya küspesi ununun levrek yemlerinde tamamen olmasa da hayvansal kökenli protein kaynaklarının yerine kullanılabileceği anlaşılmış, bu yem gruplarında tavuk ununun hayvansal protein kaynağı olarak iyi bir seçenek olduğu görülmüştür. %50 oranında bitkisel protein ve %50 oranında hayvansal protein kullanılan kontrol grubundan en iyi performans verileri elde edilmiş; en azından karnivor balıkların yem formülasyonlarında balık unu gereksinimi bir kez daha sorgulanmıştır. Sindirilebilirlik çalışmaları yem sindirilebilirliğinin önemini bir kez daha gözler önüne sermiş, karnivor balık türlerinin yemlerinde maliyet faktörünün en önemli belirteç olduğunu göstermiştir.

Anahtar Kelimeler: Karnivor balık, *Dicentrarchus labrax*, bitkisel protein, rendering hammadde, ticari yem.

Introduction

Fish meal (FM) is known for its high essential amino acids and fatty acid contents, high digestibility, and low carbohydrates and antinutritional factors contents. There is a general consensus that as aquaculture production will increase to meet expected demand for fisheries products along the next 7–10 years, and if dietary levels of fish meal remain constantly high, annual FM supply will not meet increasing demand. Therefore, the price of FM is going to increase steadily (Cheng *et al*., 2004).

Sustainability of aquaculture will thus depend on the replacement of FM by alternative protein sources in aquafeeds (Francis *et al*., 2001; Zhou *et al*., 2004). Replacing FM by alternative plant protein sources (PPs) in aquafeeds is a rather challenging task. According to Robinson *et al*. (1981), vegetable feedstuffs present high fibre contents. Fibre is basically cellulose, which does not have any value in the nutrition of carnivorous fish, particularly, and should be restricted to less than 7% of fish diets. For instance, McGoogan and Reigh (1996) reported higher digestibility of protein by red drum *Sciaenops ocellatus* for ingredients with less than 2% of fibre; above this dietary fibre level, no relationship could be established between fibre contents and protein digestibility of feeds. Another limitation of PPs is that their amino acid profiles do not match the dietary requirements of fish (Steffens, 1989; Wilson, 1989). Berge *et al*. (1999) reported that concentrated soy protein can replace 45% FM in diets for juvenile Atlantic halibut *Hippoglossus hippoglossus* only when diets are also supplemented with methionine; otherwise, both growth and feed conversion are reduced. In comparison with other vertebrates in the evolutionary scale, carnivorous fish have rather simple, little developed digestive system, and as a consequence, reduced ability to use carbohydrates as an energy source, requiring high dietary protein levels to sustain not only the synthesis of muscular tissue, but also the supply of energy requirements (Millward, 1989). In addition, the nutritional requirements of amino acids by carnivorous fish, quantitative and qualitative, require the use of ingredients of animal origin in the production of diets for these species (Wilson, 1989). The nutritional value of a given feedstuff is based not only in its chemical composition, but also in its nutrients and energy availability. Availability of nutrients for fish should be defined mainly their digestibility, that is, the fraction of nutrients from the ingested feed and feed ingredients that will not be excreted in the faeces (NRC, 1993; Goddard and Mclean, 2001). In the path of results of Portz and Cyrino (2004), this study aims at evaluating the digestibility of animal or plant protein based diets by a model carnivorous fish, the European sea bass *Dicentrarchus labrax*, Linne 1758 and contributing with information which can foster the use of alternative, PPs in diets for intensive production of carnivorous fish.

Materials and Methods

Trials were set up in indoor facilities, open water system with controlled temperature (27.0±1.07°C) and photoperiod (12L:12D). Water quality parameters (pH 7.5; temperature 27±1.07°C; dissolved oxygen 6.8±0.7 mg L^{-1}. Chemical analyses of feedstuffs, diets and fish carcasses were made on a dry matter basis (n=3). Crude protein (CP) was analysed by the Kjeldahl method (N×6.25); lipids were analysed by the Soxhlet method (petroleum ether extract) and crude fibre by the Weende method. The oxidimetric method was used to analyse calcium, and phosphorus was analysed by calorimeter after acid digestion and mineralization of organic matter. Energy was estimated with an adiabatic, calorimetric pump and a benzoic acid standard. Chromic oxide (Cr_2O_3) was analysed following the methodology of Furukawa and Tsukahara (1966). Feed ingredients were ground and homogenized through a 1.0 mm sieve, mixed, moistened with boiled water (2:0.5; w:v) and pelleted (2.0 mm) in a laboratory type of pellet machine. Prepared diets (Table 1) were dried in the dryer oven at 45°C for 36 h, and stored in plastic bags at 4°C in the refrigerator. Six isonitrogenous (40 g 100 g^{-1} CP) and isocaloric (14.64 kJ g^{-1} digestible energy–DE) diets (Sampaio *et al*., 2000), containing different proportions (0–60%) of animal protein sources (APs) and PPs, defined the following treatments: 100PPs : 0APs; 80PPs : 20APs; 60PPs : 40APs; 40PPs : 60APs and 50PPs : 50APs (no fish meal); a Standard carnivorous fish diet served as control and 0.5% of inert marker Cr_2O_3 were added to all diets. Dietary inclusion of fish meal and blood meal were limited to 7%; inclusions of poultry by-product meal and meat meal were limited to 15%. DL-methionine (98%) and L-lysine (80%) were added automatically by the formulation to adjust required dietary amino acids profiles (Portz and Cyrino, 2004). Trials were set up in completely randomized design (n=4); experimental data were submitted to ANOVA and Tukey's test (x = 0.05). Two hundred and eighty juvenile European sea bass (27.0 g), conditioned to accept dry artificial feed, were set to fast for 24 h and then anaesthetized using benzocaine solution (100 mg L^{-1}), weighed on a semi-analytical scale (0.001 g), and randomly assigned to 80-L screen-bottomed cages (mesh size 25 mm; 70 fish per cage) installed in 1,000 L plastic tanks. Fish were fed *ad libitum* three daily meals ($08:^{00}$, $13:^{00}$ and $17:^{00}$ hours) with the experimental diets. After the last meal, fishes were routinely anaesthetized and the cages were transferred to, and held for 10 h in 200-L, aerated, cylindrical–conical fibreglass aquaria, with superficial, partial water exchange (N.N% volume per hour). The faeces were collected by sedimentation into refrigerated containers connected to aquaria bottoms, centrifuged (4°C; 15 min), and stored in freezer to later be subjected to chemical analysis and determination of Cr_2O_3 contents. Determinations of the apparent digestibility coefficients (ADC; %) of dry matter (ADC_{DM}), nutrients (ADC_N) and energy (ADC_{GE}) were performed by the indirect method, using Cr_2O_3 as inert marker (De Silva *et al*., 1997; De Silva and Anderson, 1995), as follows:

$$ADC_{DM} = 100[1-(Cr_2O_3 \text{ diet} / Cr_2O_3 \text{ faeces})],$$

$$ADC_{N \text{ and } GE}=100[1-(Cr_2O_3 \text{ diet}/Cr_2O_3 \text{ faeces})(\text{nutrient or energy faeces / nutrient or energy diet})]$$

Table 1. Composition of ingredients and experimental diets (dry matter basis)

Ingredients[1]	100PP:0AP	80PP:20AP	60PP:40AP	40PP:60AP	50PP:50AP	Control
Fish meal	0.00	70.0	70.0	70.0	0.00	50.0
PbyPM	0.00	54.9	119.6	150.0	150.0	150.0
Meat meal	0.00	0	0.00	148.8	150.0	0.00
Blood meal	0.00	0.00	50.0	50.0	50.0	70.0
Corn meal	86.8	111.6	241.1	148.9	135.5	0.00
Corn gluten meal	298.2	179.9	29.5	25.1	88.3	25.3
Sorghum meal	0.00	0.00	0.00	0.00	0.00	184.7
Wheat meal	150.0	150.0	5.7	150.0	150.0	95.0
Soybean oil	56.9	45.5	38.7	13.1	19.6	41.5
Fish oil	0.00	0.00	0.00	0.00	0.00	20.8
Soybean meal	369,4	364.3	429.0	225.8	234.4	349.7
Di-base calcium phosphate	14.9	5.3	0.00	0.00	0.00	0.00
DL-Methionine	2.2	2.2	3.4	3.6	4.0	0.00
L-Lysine	8.6	3.4	0.00	1.7	5.2	0.00
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0
Premix[2]	8.0	8.0	8.0	8.0	8.0	8.0
Nutrients Composition						
Protein, %	432.7	429.7	432.4	414.4	420.9	410.6
Lipid, %	78.1	84.1	84.8	69.8	70.5	107.1
Crude fibre, %	21.9	20.3	25.1	23.4	19.8	22.6
Calcium, %	8.6	11.4	39.6	41.6	37.0	13.5
Phosphorus, %	8.3	9.5	21.9	22.2	21.6	9.4
Mineral, %	55.4	65.0	136.8	137.0	128.4	67.8
Gross energy, kcal kg^{-1}	193.4	194.2	210.4	191.0	182.2	206.0
Chromic oxide	4.9	4.6	4.7	4.7	5.0	5.1

[1]Chemical composition of ingredients (g kg^{-1} dry matter): Corn Meal crude protein:104, lipid: 42.1; Soybean meal crude protein: 514.0, lipid: 67.1; Corn gluten meal crude protein: 712.4, lipid: 36.0; Wheat meal crude protein: 171.2; Sorghum meal crude protein: 94.7, lipid: 26.5; Fish meal crude protein: 715.2, lipid: 132.2; Meat meal crude protein: 411.9, lipid: 160.1; Poultry by product meal crude protein: 617.9, lipid: 103.2; Blood meal crude protein: 802.8, lipid: 74.4.
[2]Premix (Supplementation kg^{-1} of the diet): Vitamin A 8000 IU; Vitamin D_3 5000 IU; Vitamin E 15000 mg; Vitamin K 27 mg; Vitamin C 550 mg; Thiamin 10 mg; Riboflavin 20 mg; Pyridoxine 15 mg; Vitamin B_{12} 3 mg; Pantothenic acid 150 mg; Niacin 250 mg; Choline chlorid 2500 mg; Biotin 50 mg.

Results and Discussion

The average values of faeces composition and the diets digestibility coefficients for juvenile European sea bass are presented in Table 2. Independent of treatments, the collected faeces presented solid form; survival rate was 100% and no cases of refusal of the different diet types were registered. The treatment 100PP presented the highest values (61.91%) of ADC_{DM}, and differed ($P<0.05$) from treatments 60PP:40AP (55.34%), 40PP:60AP (50.47%), control (50.48%) and 50PP:50AP (49.63%). The best values of apparent digestibility of protein coefficient (ADC_{CP}) were also registered for treatment 100PP (90.15%) which, however, did not differ ($P>0.05$) from treatments 80PP:20AP (89.25%) and 60PP:40AP (89.92%). ADCCP of treatments 40PP:60AP (80.49%), control (84.70%) and 50PP:50A (80.36%) also did not differ among themselves ($P>0.05$), but differed from the first group of treatments ($P<0.05$). Apparent digestibility coefficients of energy (ADC_{GE}) and lipid (ADC_{L}) also presented the same tendency of ADC_{DM} and ADC_{CP}, that is, best results were registered for treatment 100PP, respectively 67.94% and 84.00%; only treatments control (58.57%) and 50PP : 50AP (54.95%) differed from all others ($P<0.05$) regarding ADC_{GE}. Fish use around 80% of dietary dry matter; ADC_{DM} describes especially how efficiently the feeds or feed ingredients are digested, and how much of their nutrient contents can be made available to fish for maintenance and growth. Also, ADC_{DM} generally provides a better estimate of the quantity of indigestible materials in the feeds or feed ingredients, rather than that of the individual nutrient (Eusebio *et al*., 2004).

Nutritional values of proteins and protein sources vary as a function of amino acids profile and digestibility. Even though some feed ingredients have high CP (i.e. nitrogen) contents, a large proportion of this CP can be made out of non-protein nitrogen. These ingredients will thus not contribute with enough amino acids to meet the nutritional requirements of fish; actually, these ingredients will increase the production of ammonia and the rate of nitrogen excretion by the fish, lowering productivity and water quality of production systems (Cho, 1990; Altan, 2002; Koprucu and Ozdemir, 2005). Sugiura *et*

Table 2. Chemical composition of faeces and appearent digestibility coefficients of dietary nutrients by juvenile European sea bass fed different animal and plant protein based diets

Digestibility (ADC);g kg^{-1}	100PP:0AP	80PP:20AP	60PP:40AP	40PP:60AP	50PP:50AP	Control
Dry matter	619.1^{a}	571.9^{a}	553.4^{ab}	504.7^{b}	496.3^{b}	504.8^{b}
Protein	901.5^{a}	892.5^{ab}	899.2^{a}	804.9^{b}	803.6^{b}	847.0^{b}
Digestible protein	390.1^{a}	287.6^{b}	291.6^{b}	333.5^{bc}	338.2^{bc}	260.8^{bc}
Energy	679.4^{a}	615.6^{a}	648.5^{a}	659.3^{a}	549.5^{ab}	585.7^{ab}
Digestible energy, kcal g^{-1}	131.4^{a}	89.7^{ab}	102.3^{a}	122.4^{ab}	100.1^{b}	90.5^{ab}
DE:DP	80.5^{a}	74.2^{a}	83.8^{a}	87.6^{a}	70.5^{b}	82.9^{a}
DE:CP	72.6^{a}	66.5^{a}	75.4^{a}	70.5^{a}	56.8^{ab}	70.2^{a}
Lipid	840.0^{a}	849.9^{a}	858.2^{a}	888.8^{a}	819.7^{a}	837.4^{a}
Nutrient Composition (Digested Nutrient, g kg^{-1})						
Protein	112.9	111.1	97.0	163.8	164.9	132.2
Lipid	33.2	31.0	27.7	15.8	24.6	38.0
Crude fibre	93.4	80.0	86.1	64.8	70.0	92.7
Calcium	27.1	21.6	15.9	66.8	71.6	20.7
Phosphorus	20.7	12.4	7.2	33.3	31.1	6.5
Mineral	123.7	90.6	80.7	241.9	216.0	75.1
Gross Energy, kcal g^{-1}	162.8	174.1	167.2	138.7	161.9	176.0
Chromic oxide	13.1	11.4	10.6	9.6	10.3	10.3

ADC, Appearent digestibility coefficient; DE, Digestible energy; DP, Digestible protein; CP, Crude protein.

al. (1998) did not observe significant differences among ADC_{CP} of herring meal (94.6%), menhaden meal (89.8%) and poultry by-product meal (95.9%) for rainbow trout; similar results were found by Portz and Cyrino (2004) – ADC 98.21% for fish meal and 98.19% for poultry byproduct meal – working with largemouth bass; Ellis and Smith (1984) report that ADC for lipid of herring meal was 89.8%. Jointly, these research reports and results of this study allow inferring that the use of fish meal in carnivorous fish diets is not a strict requirement.

Poultry by-product meal, for instance, is a choice of protein source. Portz and Cyrino (2004) infer that both soybean meal and corn gluten meal can partially replace APs in diets for European sea bass; poultry by-product meal also can be considered a good alternative animal protein source in feeds for European sea bass because of its good digestibility, adequate amino acid profile and phosphorus availability. Works developed by Britz and Hecht (1997), Catacutan and Coloso (1995), Dias *et al*. (1998), El Sayed and Teshima (1992), Garling and Wilson (1976), Lee and Putnam (1973), Rojas and Verdegen (1994), Samantary and Mohanty (1997) and Tibaldi *et al*. (1996), using carnivorous fish as biological material, determined that the best energy: to protein ratio for feeding carnivorous species lies between 0.03 and 0.04 kJ of DE g^{-1} of protein. Sampaio *et al*. (2000) observed that juvenile of peacock bass, Cichla sp. (10 g), feeding on a dry diet with 41% of CP and 14.64 kJ g^{-1} feed (DE:CP ratio = 0.04 kJ g^{-1}) gained 20 g in weight in 60 days, with feed conversion ratio (FCR) of 1.2:1. Considering that protein requirements decrease with increasing fish size, the author inferred that larger fish consuming diets with 36–37% CP and 1.44 kJ g^{-1} will not present reduced weight gain nor alteration on carcass composition, with consequent reduction in feed costs and, by extension, production costs. Working with juvenile European sea bass (14 g), Portz *et al*. (2001) and Cyrino *et al*. (2000) demonstrated that the species nutritional requirements are 43.59% of CP and 16.19 kJ DE g^{-1} and limits of DE:CP ratio between 0.032 and 0.037 kJ g^{-1} feed. In these conditions, the European sea bass presents increased hepatic glycogen and hepato-somatic index, that is, high capacity of energy storage, both as readily available – glycogen and organic reserve–Lipid. In spite of being strictly carnivorous, the European sea bass can make efficient use of diets containing levels of carbohydrates as high as 24–30%. In that case, dietary lipid shall remain between 10% and 12%, so that energy to protein ratio will not exceed requirements and induce accumulation of visceral fat and reduced carcass quality (fatness).

Regarding the use and digestibility of feeds, feed ingredients, and nutrients, and nutrient use by fish, available and related scientific literature allow to present a consolidated, core discussion. Most proteic ingredients are highly digestible by fish. The digestibility coefficients of proteic ingredients vary between 75% and 95%, smaller values being usually associated to larger carbohydrates concentrations (Rychly and Spannhof, 1979) and overheating during ingredient or feed processing, especially for fish meal; overheating degrades lysine and consequently lowers of quality of protein. On the other hand, insufficient heating of soybean meal reduces the protein digestibility and amino acids availability (NRC, 1993). Smith (1976) showed that increasing processing temperature from 127°C to 204°C can increase digestibility of soybean protein from 45% to 75%. The soybean meal can substitute up to 90% the fish meal in extruded diets for rainbow trout without causing reduction in the growth rate or net protein utilization (Watanabe and Pongmaneerat, 1993).

However, these authors also noticed that trouts fed diets containing high levels of soybean meal presented poorer FCR, a fact attributed to the low starch digestibility, determined to be approximately 54%, independently of the inclusion level in the diet. Apparent digestibility coefficient of raw starch for rainbow trout is only 49% (NRC 1993); the amylase activity in rainbow trout can be affected, i.e. reduced, by dietary carbohydrate type and contents. Actually, Aksnes and Opstvedt (1998) determined that the digestible energy of soybean meal for rainbow trout is lower than that determined of fish meal. According to the authors, carbohydrate contents of soybean meal are higher, this nutrient presents low digestibility for salmonids. On the other hand, Wilson and Poe (1985) reported that the channel catfish, an omnivorous species with clear preference for APs, can digest in excess of 70% of raw starch gross energy contents. Dietary plant ingredients can affect gastrointestinal transit time of feed as a result of the presence of fibres and sugars, and alter the digestibility of nutrients ingested by the fish (Storebakken *et al*., 1999; Eusebio *et al*., 2004; Zhou *et al*., 2004). According to Eusebio *et al*. (2004), as dietary fibre is part of the carbohydrate component of plant ingredients, most fish cannot utilize it. However, low dietary concentrations of dietary fibre (3–5%) may have a beneficial effect on fish growth. High dietary fibre (>8%), by its turn, may decrease dry matter digestibility of the diet and reduce the availability of other nutrients.

Allan *et al*. (2000) evaluated the digestibility of alternative ingredients to fish meal in diets for silver perch and found ADC of 99% for wheat meal, 12–15% for protein, and 80% of carbohydrate; the specie was considered relatively efficient in the digestion and use of carbohydrates, what can explain there was no relationship between dietary carbohydrate contents and protein digestibility of nitrogen fixation, especially in ingredients of animal source origin. Hepher (1985) suggested that the protein digestibility would be negatively related with the content of carbohydrate in the diet. As a matter of fact, Falge *et al*. (1978) had already reported that high dietary carbohydrate contents reduce the activity of proteolitic enzyme in fish. McGoogan and Reigh (1996) reported that the digestibility of protein for red drum was larger for ingredients containing less than 2% of crude fat, but above this level, no relationship between the crude fat content and protein digestibility was detected. In fact, indigestible carbohydrates pass quickly through the gastrointestinal tract carrying along intact proteins, thus affecting the protein digestibility. However, Storebakken *et al*. (1998) shed some controversy in the subject reporting that increased dietary carbohydrate contents (10–20%) reduces digestibility of dry matter, energy and fat, but has little effect in the protein digestibility for rainbow trout.

This study registers that ADC_{GE} and ADC_L presented the same tendency of ADC_{DM} and ADC_{CP}, that is, best results were registered for fish-fed diets containing 100% plant protein plus an attractant, supplemented with methionine and lysine, and only the treatment containing 50% animal protein plus 50% plant protein (control) differed from all others, presenting smaller ADC_{GE} ($P<0.05$). Therefore, based on these arguments, results of the trials on digestibility of different diets based on animal and plant protein by European sea bass, allow to infer that soybean meal can be used as partial substitute of APs in formulated diets for the species, and the poultry by-product meal can be considered a good option as animal protein source for this and other carnivorous fish diets, once it has good digestibility, good amino acids profile and high phosphorus availability. The need for using fish meal in carnivorous fish diets is, at least, questionable, but the determination of digestibility of diets and dietary ingredients targeting improved precision in formulation of minimum cost diets for carnivorous fish, should be constituted in routine practice in the animal feed industry.

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