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Apparent digestibility of crude protein and lipids in Brazilian codling, *Urophycis brasiliensis* (Kamp, 1858) (Pisces: Gadiformes), fed with partial replacements of soybean meal and meat meal diets

Digestibilidad aparente de proteína cruda y lípidos en la brótola, *Urophycis brasiliensis* (Kamp, 1858) (Pisces: Gadiformes), alimentada con reemplazos parciales de harina de soja y harina de carne

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Resumen.- El objetivo de este trabajo fue determinar la digestibilidad aparente de dietas (proteína cruda y lípidos) formuladas para brótola. Se investigaron tres dietas (43% proteína cruda): una control con harina de pescado como la principal fuente protéica y otras dos con reemplazo parcial de este ingrediente por harina de soja desgrasada y harina de carne. Los peces se alimentaron diariamente hasta la saciedad y no hubo mortalidades durante los 14 días que duró el experimento. En los días 7 y 14, los peces fueron anestesiados y se extrajeron las heces por masaje abdominal. La digestibilidad de proteína cruda, al séptimo día, fue significativamente diferente al comparar el grupo control con los grupos donde se reemplazó la harina de pescado. Se encontraron diferencias significativas en la digestibilidad de lípidos entre el grupo alimentado con harina de soja y el grupo alimentado con harina de carne durante todo el experimento. Los valores de digestibilidad más altos y los menores porcentajes de humedad en las heces se encontraron en el grupo control, sugiriendo una mejor digestión de esta dieta. En las dietas con reemplazos, la digestibilidad aparente se incrementó de la primera a la segunda semana. Se puede concluir que esta especie puede ser alimentada con dietas con reemplazos de hasta un 30% de harina de pescado utilizando harina de soja desgrasada o de carne; el tiempo necesario para la adaptación enzimática de los peces a esta dieta es de alrededor de dos semanas.

Palabras clave: peces marinos, nutrición, formulación de dietas

Abstract.- The objective of this study was to determine apparent digestibility of crude protein and lipid in Brazilian codling. Three diets (43% crude protein) were tested: a control diet with fish meal as the main protein source, and two others with partial replacement of fish meal by defatted soybean and meat meal. Fishes were fed daily to satiation, and no mortality was observed during the 14 day-experiment. On days 7 and 14, fish were anesthetized and faeces were obtained by stripping. Apparent crude protein digestibility at day 7 was significantly different between the control group and the groups in which fish meal was replaced. Lipid digestibility showed significant differences between soybean group and meat meal group throughout the trial. The highest digestibility values and the lowest moisture percentages in faeces were found in the control group, suggesting an improved digestion of this diet. Apparent digestibility increased from first to second week in diets with replacements. Brazilian codling can be reared on diets in which up to 30% replacement of the fish meal protein can be replaced by defatted soybean or meat meal. Fish need about two weeks for enzymatic adaptation to these diets.

Key words: marine fish, nutrition, diet formulation

Introduction

Brazilian codling, *Urophycis brasiliensis* (Kamp, 1858) is a demersal fish that inhabits shallow coastal waters between Rio de Janeiro State, Brazil (21°30'S) and San Matías Gulf, Argentina (41°10'S). Some studies of nutrition and stress in this species have been conducted at the Universidad de Mar del Plata in Argentina, to evaluate its potential rearing (Bolasina 2002).

Fish meal and oil are essential ingredients in shrimp and fish diets formulation since they bring essential

amino acids like lysine and methionine and fatty acids as eicosapentaenoic and docosahexaenoic acid that are insufficient in other animal and vegetable sources. The use of these ingredients in aquaculture has been increasing steadily over the last years. Intensive fish culturing requires a greater biomass of fish catches than the amount produced. Along the most cultivated species, an average of 1.9 kg of wild fish was necessary for each kilogram of fish produced with artificial diets (Naylor *et al.* 2000). Therefore, one of the solutions for making aquaculture a sustainable activity is to reduce the amounts of fish meal and oil in the fish diets. Research has concentrated on replacing fish meal with cheaper ingredients, like oily seeds or meat industry by-products (Webster *et al.*1999).

Determination of nutrient digestibility is the first step in evaluating the potential of an ingredient for use in the diet of reared species (Allan *et al*. 2000). Information on digestibility coefficients of feed ingredients is very useful not only to enable formulation of diets that maximize fish growth by providing appropriate amounts of available nutrients but also to reduce fish waste products (Lee 2002).

Most of the digestibility determinations have been made using chromic oxide, Cr2O3 (Austreng 1978). Nose (1960) and Inaba *et al.* (1962) used this method to determine crude protein digestibility in rainbow trout. They observed that the digestibility estimations obtained with fecal collection from the tanks were 10% greater compared with that obtained by stripping, indicating that some nitrogen compounds were lost in the water. Similar results were found by Singh & Nose (1967). The method used to determine digestibility can affect the value of the coefficients obtained (Cho *et al*. 1982). Lee (2002) compared apparent nutrient digestibility of a diet by using a chromic oxide indicator according to the various fecal collection methods (dissection, stripping or using fecal collection column attached to fish rearing tank), and suggested that stripping or fecal collection column could be a reliable procedure for measuring nutrient digestibility in rockfish.

The objective of this study was to determine crude protein and lipid digestibility in Brazilian codling. A control diet with fish meal as the primary protein source was compared with others two, using soybean and meat meal as replacements.

Materials and methods

Fishes were caught from the Mar del Plata coast (38° 00' S) with a trawling net, transported to J.J. Nágera Research Station (Playa Chapadmalal, Mar del Plata, Argentina) and placed in a 2000 L fiberglass tank for 20 days. During this acclimatization period, juvenile fish of similar size (total length 24.1 ± 2.67 cm, n=54) were fed daily. Initial fish weight was 123.27 g ± 9.99 (mean ± SD). After this period fishes were distributed in three groups and placed in 300 L fiberglass tanks in triplicate. Seawater temperature was 13.8 ± 1.82ºC and salinity was 32.3 ± 0.60 psu. Three experimental diets (43% crude protein) were formulated (Table 1): a control diet (C) with fish meal as the main ingredient, and two diets partially replacing fish meal by defatted soybean meal (DM) and meat meal (M), respectively. Fishes were fed daily to satiation. The experiment lasted 14 days. On days 7 and 14, fishes were anesthetized in a benzocaine bath (ethyl aminobenzoate, 20 mg/L); faeces were obtained by stripping, following this procedure (Hemre *et al*. 2003):

Table 1

Composition and energy content of the diets used during the experiment

Composición y contenido energético de las dietas utilizadas durante el experimento

	CD (%)	DSD (%)	MD (%)
Fish meal	55	38.5	38.5
Soybean meal	7	4.9	4.9
Deffated soybean meal	-	30	-
Meat meal	-	-	30
Wheat gluten	3	2.1	2.1
Wheat starch	20	14	14
Wheat bran	8	5.6	5.6
Fish oil	2	1.4	1.4
Fish soluble	2	1.4	1.4
Vitamin complex†	2	1.4	1.4
Chromic oxide	1	1	1
Energy (Kcal/ 100g diet)	439.77	412.89	454.59
Total energy/protein (Kcal/ g protein)	10.08	9.42	10.44

CD: control diet, DSD: deffated soybean meal diet, MD: meat meal diet

†Composition (g/kg): vitamin D_3, 1.8 g; thiamine, 8.2g; riboflavin, 7.8 g; pyridoxine, 10.7g; calcium pantothenate, 12.5g; biotin, 12.5 g; niacin, 25 g; folic acid, 1.3 g; cyanocobalamin, 1 g; ascorbic acid, 39.1 g; menadione, 1.7 g

First, the bladder was emptied by slight pressure and the area around the anus dried with a towel. Then, faeces were collected by exerting gentle pressure on the area from the anal fin to the anus. No fish mortalities or health problems were encountered during the experiment.

Feed samples were finely ground in a hammer mill using a 1 mm screen. Faecal samples were oven dried at 60ºC before storage, ground with mortar and pestle and kept at 4 ºC. Formulated diets and faecal samples were analyzed for proximate composition using standard methods (AOAC 1995). All analyses were done in triplicate. Crude protein was determined by estimating Kjeldahl nitrogen (N x 6.25) and lipid by chloroform: methanol extraction (Fenucci 1981). Ash content only from food samples was determined by burning in a muffle furnace at 550ºC for 24 h because of the insuficient faeces amount. Food and faeces containing Cr_2O_3 were digested in a mixture of perchloric acid, concentrated sulphuric acid and Na-molybdate in Kjeldahl digestion flasks at 250ºC. The resulting dichromate was determined at 360 nm against Cr_2O_7 standard solutions.

For the determination of apparent nutrient digestibility of diets (AD_d), the following equation was used (Austreng 1978):

$$AD_d\,(\%) = 100 - 100\,[(Cr_2O_3 \text{ in diets}) / (Cr_2O_3 \text{ in faeces})] \times [(\%\text{ nutrient in faeces})/ (\%\text{ nutrient in feed})]$$

The equation for determining apparent nutrient digestibility of ingredients (AD_i) was:

$$AD_i\,(\%) = (AD_{td} - 0.7\,AD_{cd}) / 0.3$$

where:

AD_{td}: apparent digestibility of test ingredient

AD_{cd}: apparent digestibility of control diet

Data were subjected to one-way analysis of variance (P<0.05). Differences in digestibility coefficients were assessed by independent samples t-test (P<0.05) (Sokal & Rohlf 1995).

Results and discussion

Studies comparing different methods of faeces collection to establish apparent digestibility coefficient values in rainbow trout have shown that stripping or abdominal massage results in lower values when compared to collection of faeces in the water column (St. Pee system and Guelph system), explained by rapid leakage of some nutrients into the water (Vandenberg & De La Nouee 2001). Hemre *et al.* (2003) analyzed the digestibility in cod (*Gadus morhua*) using stripping and dissection of faecal matter. They concluded that the sampling method did not influence the digestibility coefficients obtained, giving the opportunity to freely choose stripping or dissection of intestinal contents for digestibility measurements. This conclusion is in agreement with earlier results on Atlantic salmon (Storebakken *et al.* 1998).

The proximal composition of diets and faeces is shown on Table 2, and apparent digestibility of diets and ingredients, in Table 3. The control group showed the lower moisture content (65%) at day 14. This fact was already checked at the time of sampling because faeces were more solid in the control groups compared with the rest. Apparent crude protein digestibility of diets at day 7 was significantly different (P<0.05) comparing control group (C) with defatted soybean (SB) and meat meal (M) groups respectively. DS at day 7 showed significant differences (P<0.05) with C and M at day 14. Apparent crude lipid digestibility between the soybean diet and the meat meal diet showed significant differences (P<0.05) all over the trial. Apparent crude protein digestibility of ingredients registered significant differences between DS at day 7 and M at day 14 and, in the case of lipids, in all comparisons between DS and M.

The highest digestibility values were obtained in the control diet. The lowest moisture level in faeces was found in this group, suggesting an improved digestion. As a general trend, apparent digestibility increases from the first to the second week in the test diets (DS and M). Using defatted soybean meal diet, crude protein digestibility was lower than in meat meal diet during the first week, but in the next one this condition was reverted. Watanabe *et al.* (1996), studying apparent crude protein digestibility and using the same ingredients on rainbow trout (*Onchorhynchus mykiss)*, carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and ayu

Table 2

Proximal composition in diets and faeces (mean ± SD of three replicates)

Composición proximal en las dietas y en las heces (media ± desviación estándar de tres réplicas)

		Crude protein (%)	Crude lipid (%)	Moisture (%)	Cr_2O_3 (%)	Ash (%)
C		43.61 ± 0.15	7.30 ± 0.47	5.25 ± 0.54	0.68 ± 0.32	14.44 ± 0.58
DS		43.81 ± 0.24	5.95 ± 0.87	4.77 ± 1.03	0.68 ± 0.41	10.68 ± 0.11
M		43.54 ± 0.11	7.83 ± 0.38	4.36 ± 1.71	0.69 ± 0.38	14.00 ± 0.65
FC	day 7	25.24 ± 0.37	9.56 ± 0.18	93.22 ± 0.53	3.85 ± 0.21	-
	day14	34.18 ± 0.26	8.54 ± 0.26	64.80 ± 0.34	3.76 ± 0.11	-
FDS	day 7	33.11 ± 0.19	2.15 ± 0.21	90.06 ± 0.85	1.25 ± 0.49	-
	day 14	34.31 ± 0.20	1.62 ± 0.18	91.80 ± 1.08	1.20 ± 0.70	-
FM	day 7	31.04 ± 0.16	9.06 ± 0.30	91.46 ± 0.45	1.38 ± 0.33	-
	day 14	34.14 ± 0.21	13.70 ± 0.43	90.94 ± 0.76	2.98 ± 0.56	-

C: control diet, DS: defatted soybean meal diet, M: meat meal diet, FC: faeces of control diet group, FDS: faeces of defatted soybean diet group, FM: faeces of meat meal diet group

Table 3

Apparent crude protein and lipid digestibility of diets and ingredients[†]

Digestibilidad aparente de proteína cruda y lípidos de las dietas y los ingredientes

		AD_{pd} (%)	AD_{ld} (%)	AD_{pi} (%)	AD_{li} (%)
C	day 7	89.25 ± 1.09^{a}	76.50 ± 0.97^{ab}	-	-
	day 14	85.65 ± 1.52^{ac}	78.28 ± 1.11^{ab}	-	-
DS	day 7	59.06 ± 1.00^{bd}	80.28 ± 1.35^{b}	-11.37 ± 1.19^{b}	89.10 ± 2.43^{a}
	day14	84.16 ± 1.25^{ade}	84.59 ± 1.40^{b}	80.70 ± 7.74^{ab}	99.31 ± 7.10^{a}
M	day 7	65.13 ± 0.85^{bce}	42.71 ± 2.64^{ac}	8.84 ± 5.15^{ab}	-36.12 ± 11.06^{b}
	day 14	81.63 ± 1.30^{ae}	59.82 ± 1.04^{ac}	72.25 ± 1.30^{ac}	16.76 ± 2.35^{b}

[†] Values (mean ± SD of three replicate groups) in each column with the same superscripts are not significantly different ($P<0.05$). AD_{pd} and AD_{pi}: apparent crude protein digestibility of diet and ingredient respectively, AD_{ld} and AD_{li}: apparent crude lipid digestibility of diet and ingredient respectively, C: control, DS: defatted soybean meal, M: meat meal

(*Plecoglossus altivelis*), found similar results to ours obtained at day 14. Zhou *et al.* (2004) determined the apparent digestibility of different feed ingredients in cobia (*Rachycentron canadum*). The apparent protein and lipid digestibility ranged from 87.21 to 96.27% and from 91.59 to 96.86%, respectively, for animal products, and from 88.97 to 94.42% and from 92.38 to 96.93%, respectively, for plant products. In Brazilian codling, the lipid digestibility of meat meal is much lower than in cobia. This was probably due to the difficulty of digestion of the highly saturated fatty acids present in meat meal (Takeuchi *et al.* 1979).

Crude protein digestibility of defatted soybean and meat meal diets increased during the second week, showing no significant differences with the control diet. The use of soy protein, considered as one of the most important protein sources of plant origin, is very frequent at present but replacements have to be under 50% due of the its lack of some essential amino acids like methionine. The presence of trypsin inhibitor factors (Webster *et al.* 1995) makes necessary to cook it previously. In conclusion, this study indicates that Brazilian codling can be reared on diets in which up to 30% of the fish meal protein is replaced by defatted soybean or meat meal, reducing feed costs. The time needed for the fish enzymatic adaptation to these diets is about two weeks.

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