Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*

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Abstract

Rainbow trout juveniles (*Oncorhynchus mykiss*) were fed diets containing high levels (50, 70 or 100%) of vegetable proteins for 8 weeks. Vegetable protein sources employed were soybean meal, lupin seed meal and corn gluten meal, as well as potato protein concentrate. Nutritional evaluation showed that diets containing 50 or 70% plant protein supported growth rates, fish acceptance and nutritive utilization similar to those of both a whole fish meal diet and a commercial trout feed. A taste panel proved that organoleptic properties of fish were not affected by feeding on diets containing high levels of vegetable feedstuffs.

Keywords : Nutrition, trout *Oncorhynchus mykiss*, vegetable proteins.

Valeur nutritive des aliments à forts pourcentages de protéines végétales pour la truite, Oncorhynchus mykiss.

Résumé

Des juvéniles de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été nourris au moyen d'aliments contenant de forts pourcentages (50, 70 ou 100 %) de protéines végétales pendant 8 semaines. Les sources protéiniques utilisées sont les farines de soja, de lupin, et de gluten et maïs ainsi qu'un concentré de protéines de pomme de terre. L'évaluation nutritionnelle montre que les aliments contenant 50 ou 70 % de protéine végétale induisent des taux de croissance, des degrés d'acceptabilité par les poissons et une utilisation nutritive similaires à la fois à un aliment à base de protéine de poisson et à un aliment commercial destiné à la truite. Les propriétés organoleptiques du poisson ne sont pas affectées par une alimentation à hauts niveaux de protéines végétales.

Mots-clés : Nutrition, truite, *Oncorhynchus mykiss*, protéines végétales.

INTRODUCTION

Formulation of fish feeds containing high levels of plant proteins has become an important objective in fish nutrition research. The possibility of designing practical fish diets using vegetable feedstuff has been tested utilizing a great variety of seeds: soya, sunflower, carob, cotton, etc. (*see* reviews by Tiews *et al.*, 1979, Tacon and Jackson, 1985; Tacon, 1987; Alexis, 1990). Assays aimed to reach substitution levels of more than 50% of fish meal protein, by mixing two or more of such protein sources, have been scarce, although some of the results obtained look promising (Jackson *et al.*, 1982; Ross and McKinney, 1987; Smith *et al.*, 1988). Even in those cases, soybean meal has been the major ingredient utilized, being a very common protein source whose utilization for terrestrial animals is practised widely. Nowadays, availability of proteinaceous seeds containing low levels of antinutritional factors has combined with the development of new processing technologies, allowing increased levels at which such ingredients can be utilized in fish diets. Moreover, it is demonstrated that nutritive quality of protein obtained when mixing cereals and legumes may be similar to that of casein, which is recognized as a good quality protein (Yadav and Liener, 1978). In this sense, the aim of the present work was to carry out a nutritional evaluation of fish diets including different mixtures of vegetable feedstuffs, substituting from 50 to 100% of dietary fish meal protein, and not using soybean meal as major ingredient. The possible influence of diet composition on fish's acceptance by the consumer, was also tested by a taste panel.

MATERIAL AND METHODS

Duplicate lots of approximately 45 rainbow trout (*Oncorhynchus mykiss*), 35 g mean initial weight, were daily fed at 2% initial body weight for 8 weeks. Diets included several mixtures of vegetable feedstuffs; lupin seed meal (LSM), soybean meal (SBM), corn gluten meal (CGM) and potato protein concentrate (PPC). Proximate composition of the utilized feedstuffs is presented in *table* 1. Formulation of diets was aimed to reach a range of substitution (50 to 100%) of fish meal protein, using several combinations of these vegetable sources. A diet including fish meal as sole protein source was prepared at the laboratory in the same way as the rest of the experimental feeds and utilized as control diet (C). A commercial diet was also used as reference (diet COM), therefore diets were formulated to be proximately isocaloric in gross energy and similar to the levels of crude protein (50.2%) and ether extract (13.8%) present in such feed (*table* 2). Levels of essential amino acids existing in the utilized feedstuffs were considered in the formulation of diets as the main factor determining the proportion of each protein ingredient in the mixture. Addition of crystalline l-methionine to fish-meal-free diets was necessary in order to cover reported requirements for salmonids (Ogino, 1980). Amino-acid profile of the utilized protein sources was determined by HPLC, after previous hydrolysis with HCl at 122°C for 22 h in nitrogen atmosphere (*table* 3). Determinations were carried out with a CHROMAKON 500 analyzer. Using those data, levels of EAA in the experimental diets were calcultated (*table* 4). A 0.5% of a commercially available chemoattractant (TROFIC. S.A. "crustaceo" TM) was added to all diets, in order to avoid a possible refusal of food in the case of fish-meal-free diets. Fish were initially stocked at a density of 8 kg/m^3 in polyethylene tanks, provided with a continuous flow (2 l/min) of dechlorinated tap water at 15°C ± 1°C. Food intake was registered daily and weight increase of each lot was measured every two weeks. At the end of the experimental period, after 24 h starving, 10 animals of each group were killed and, together with an initial sample of 10 fish, utilized for body composition analysis. Protein content of feedstuffs and fish was determined by Kjeldahl (% N × 6.25), ether extract by Soxhlet method and fish whole-body energy balance by calculation, using the values detailed in Brafield (1985) for complete combustion of carbohydrate, protein and fat; 17.2, 23.6 and 36.2 kJ/g, respectively. Data were subjected to one-way analysis of variance; when the differences were significant at $P < 0.05$, the least significant difference, LSD test was used to compare mean values of individual treatments. Palatability test was carried out using a taste panel and a nine-point hedonic scale (Peryam and Pilgrim, 1957; Amerine *et al.*, 1965). The test was conducted only for those fish belonging to the groups showing an adequate final growth. All samples were prepared by dissecting 4 × 2 cm portions of fish dorsal muscle and cooked in a microwave oven for exactly 2 min. After removal of the skin, samples were randomly numbered and presented to the panelists, who assigned a score to each one within

Table 1. – Proximate composition of the feedstuffs utilized in the elaboration of the experimental diets (g/100 g).

	Fish meal	Soybean meal	Lupin meal	Gluten meal	Potato concentrate
Dry matter	93.1	86.9	86.9	91.1	92.2
Crude protein	70.4	48.2	40.9	74.2	83.9
Ether extract	10.8	3.9	12.7	2.6	1.1
Ash	18.8	7.9	3.6	0.9	2.6
Free nitrogen Extract	–	40.0	42.7	22.2	12.4

Table 2. – General composition of the experimental diets in g/100 g dry matter.

	50-1	50-2	70	100-1	100-2	100-3	C
Fish meal	31.4	31.4	19.1	–	–	–	62.7
Soybean meal	–	–	–	18.8		28.2	–
Lupin seed meal		32.6	26.6	27.2	32.6		
Corn gluten meal	23.7	11.9	20.8	23.7	32.7	32.7	–
Potato concent.	5.5	–	5.5	8.2	8.2	8.2	–
L-Methionine			–	0.7	0.6	0.4	
Fish oil	3.6	3.6	4.5	6.0	6.0	6.0	–
Maize oil	5.7	1.5	2.1	1.5	1.2	4.8	6.0
Vit. premix (1)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix (1)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Starch	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Cellulose	16.4	5.3	8.5	–	5.1	5.9	16.3
Flavouring (2)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Binder (3)	2.45	2.45	2.45	2.45	2.45	2.45	2.45
Crude protein	46.9	46.8	46.7	47.3	47.1	46.9	48.0
Ether extract	11.9	11.9	12.1	11.7	11.9	12.2	11.8
Gross energy (kJ) (4)	2090	2080	2110	2120	2160	2120	2110

(1) As detailed in De la Higuera *et al.*, 1988.
(2) Described in Material and Methods.
(3) Carboxy-methyl-cellulose.
(4) Using values detailed in Brafield (1985).

Table 3. – Levels of essential amino acids in the protein sources utilized for the elaboration of the experimental diets (g/16 g N). LSM: Lupin seed meal; SBM: Sobbean meal; CGM: Corn gluten meal; PPC: Potato concentrate; FM: Fish meal.

	LSM	SBM	CGM	PPC	FM
Arg	10.59	7.58	3.29	5.00	6.13
His	2.42	2.90	2.50	2.10	2.91
Ile	4.53	5.12	3.70	5.40	4.47
Leu	9.53	9.48	18.23	10.00	9.21
Lys	4.87	6.80	1.99	8.00	8.55
Met	0.20	0.73	2.30	2.10	3.06
Phe	4.72	5.73	6.94	6.20	4.54
Thr	3.75	4.18	3.83	5.50	4.46
Trp	1.20	1.00	1.00	1.20	1.12
Val	4.18	5.17	4.84	6.70	5.35
Cys (*)	2.43	2.04	2.55	1.50	1.42
Tyr (*)	4.52	3.72	5.31	5.20	3.68

(*) Semi-indispensable amino acid.

a 0-9 scale. A total number of 36 panelists were utilized. Statistical analysis of such data was carried out using the non-parametric Spearman range test (Steel and Torrie, 1980).

RESULTS

Acceptance of the experimental diets was good in all cases. Daily food intake ranged between 1.3 and 1.5% of body weight, when expressed in relation to a mean value calculated between consecutive biweekly weighings (*table* 5). It was noticeable that fish fed on diets including such different quantities of vegetable protein as in the 50-1 and 100-2 diets presented a similar food intake level. Final weights of fish fed on 100% plant protein diets were significantly lower than the rest (*table* 5). A plot of growth, expressed as % increase over initial weight of fish versus time, supported these results, showing clear differences existing between growth obtained in lots 100-1 or 100-3 and the rest of the experimental groups (*fig.* 1). Values of specific growth rate closely followed the same above-mentioned tendencies (*table* 5). Feed and protein efficiencies were lowest when using fish-meal-free diets with the exception of diet 100-2. Nevertheless, results obtained in fish fed on diets containing levels of 50 or 70% plant protein were similar to or better than when using a commercial diet or a whole fish meal diet, respectively. Efficiency in dietary protein retention, expressed by the protein productive value (PPV), was abnormally low in those fish fed on the commercial diet (14.5%). This value was 40% less than, for example, that determined in fish fed on diet 70 (39.3%). Proximate analysis of fish partially explained such results (*table* 5). Only protein content in fish fed on commercial or control diets was significantly different from the rest, being the lowest and highest values obtained, respectively. This fact conditioned protein productive values calculated for such fish. Significant differences in fat contents were only established when comparing fish fed on diet 100-1 with those fed on 100-2 or 100-3. A similar analysis in relation to body moisture revealed that fish fed on the commercial diet contained significantly more

Table 4. — Calculated levels of essential amino acids present in the protein of experimental deits. C: control diet, COM: commercial diet (g/100 g diet d. m.).

	50-1	50-2	70	100-1	100-2	100-3	C	(1)	COM
Arg	2.44	3.00	2.69	3.40	2.41	2.82	2.76	2.04	2.80
His	1.33	1.41	1.34	1.26	1.26	1.21	1.31	0.86	1.30
Ile	2.12	2.26	2.22	2.24	2.19	2.10	2.01	1.33	2.10
Leu	6.45	5.51	6.25	5.80	7.18	7.18	4.14	2.42	5.50
Lys	2.93	3.35	2.90	1.93	2.16	3.75	3.85	2.81	3.00
Met	2.66	2.21	2.13	1.46	1.78	1.64	1.38	0.91	1.50
Phe	2.83	2.69	2.92	3.04	3.23	3.08	2.04	1.38	2.20
Thr	2.15	2.12	2.13	1.70	2.09	2.03	2.01	1.53	2.00
Trp	0.15	0.50	0.50	0.50	0.50	0.50	0.50	0.28	
Val	2.64	2.59	2.63	2.53	2.61	2.46	2.41	1.58	2.50
Cys (*)	0.94	0.91	0.98	1.12	1.12	1.18	0.64	0.33	
Tyr (*)	2.24	2.01	2.20	2.37	2.41	2.53	1.66	1.09	-

(1) Requirements of EAA for fish as calculated in Tacon (1987).
(*) Semi-indispensable amino acids.

Table 5. — Growth, food and protein utilization indexes, body composition and cost of fish obtained for each experimental diet, control diet (C) and for commercial diet (COM). Values not sharing a common superscript differ significantly with $p<0.05$ (1).

	50-1	50-2	70	100-1	100-2	100-3	C	COM
Mean initial weight (g)	32.8	30.9	31.8	30.3	32.9	30.1	30.5	31.5
Mean final weight (g)	50.5^{a}	52.7^{a}	51.4^{a}	42.6^{b}	42.5^{b}	42.8^{b}	49.3^{ab}	52.6^{a}
PFDW (%)	22.6	37.1	26.8	9.7	2.2	5.4	20.5	21.5
SGR	0.77	0.95	0.86	0.61	0.46	0.63	0.86	0.91
Mean food intake (g/100 g fish. day)	1.38	1.51	1.37	1.32	1.35	1.29	1.48	1.44
FE (g/g fish)	0.68	0.78	0.81	0.58	0.73	0.61	0.70	0.80
PER (g fish/g prot.)	1.45	1.67	1.74	1.22	1.55	1.29	1.42	1.59
PPV (%)	35.7	30.7	39.3	28.2	35.7	31.0	35.8	14.5
Composition (g/100 g l. wt.)								
Protein	19.48^{a}	17.29^{ab}	19.09^{a}	19.68^{a}	18.99^{a}	18.86^{a}	19.97^{a}	15.72^{b}
Fat	7.32^{ab}	8.11^{a}	8.47^{a}	5.67^{b}	9.45^{a}	9.00^{a}	8.95^{a}	6.43^{b}
Moisture	70.32^{ab}	72.32^{ab}	69.53^{a}	72.32^{ab}	67.80^{a}	69.50^{a}	68.73^{a}	76.16^{b}
ER (%)	33.9	24.4	30.4	18.1	30.9	29.6	30.1	27.0
Economics (2)								
Cost/kg fish	111.9	95.7	95.7	143.1	116.7	135.4	120.8	100.0
Cost kg fish prot.	45.4	45.2	35.3	73.6	47.4	63.3	48.9	100.0

PFDW: Percentage of fish that duplicate weight; SGR: Specific growth rate; FE: Feed efficience; PER: Protein efficiency ratio; PPV: Protein productive value; ER: Energetic performance ratio.
(1) Statistical treatment of paired data, with only one degree of freedom, was not considered relevant enough. The values expressed were the arithmetic means.
(2) Calculated considering cost of commercial diet as 100.

water than the rest of experimental groups, except 50-2 and 100-1. Taking into account the data mentioned, as well as coefficients described in the Material and Methods section, an energetic performance ratio relating gross energy intake and energy deposition was calculated (*table* 5). Energetic balances, ranging from 30 to 34%, were especially favourable in fish fed on diets 50-1, 70, 100-2 and control.

Statistical analysis of data using the Spearman range test showed no significant differences among panelists' appreciations of the different samples. Nevertheless, samples of fish could be ordered considering the score obtained within the established scale; those belonging to groups 50-2 and 70 received best qualifications, whereas samples of the commercial group showed the opposite tendency (*fig.* 2).

An economic analysis of our results was carried out, taking into account that contribution of feeding to final price of fish is determined both by feed cost and its efficiency and considering a mean annual market price of the utilized feedstuffs in Spain. The cost of producing a kg of fish using high levels of

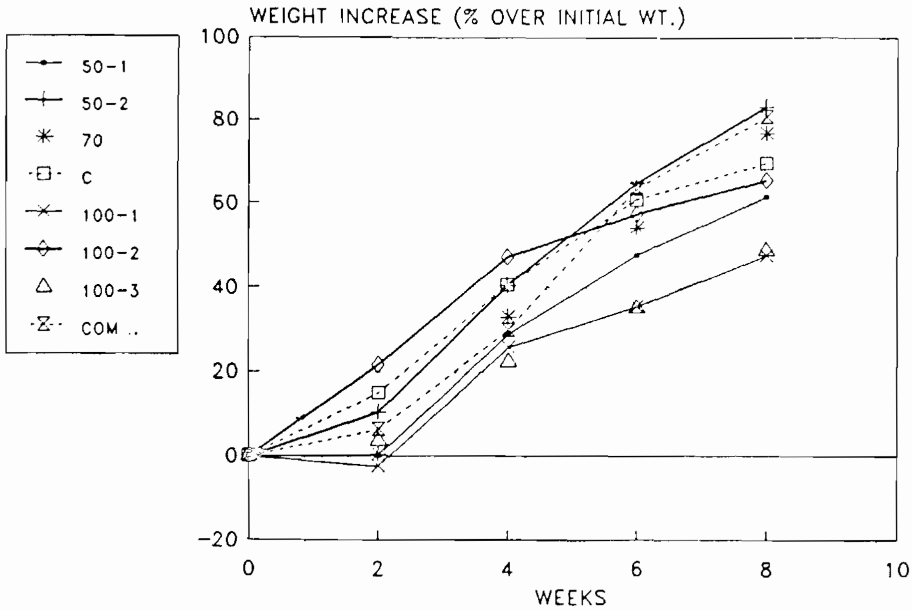

Figure 1. — Growth of fish during the experimental period.

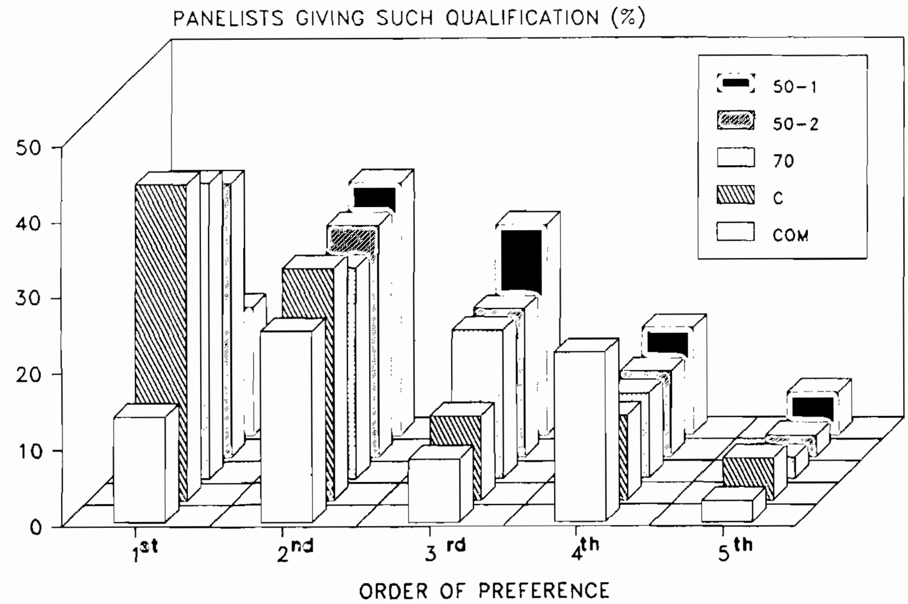

Figure 2. — Order of fish samples by panelists' preference, e.g. 15% of panelists gave commercial diet fish (COM) the best qualification, 25% the second qualification.

plant protein in diets (50 or 70% of total protein) was 25 or 5% lower than when using whole fish meal or commercial diets, respectively.

DISCUSSION

Low acceptance of diets containing a high proportion of vegetable feedstuffs has been reported by several authors (Jackson *et al.*, 1982; Roselund, 1986). In our experiment, addition of a chemoattractant to diets contributed to ensure similar acceptance of all diets and avoided problems that might arise when using partially unpalatable feedstuffs. Differences in growth response could be appreciated from the beginning of the experiments, resulting in a higher weight increase of fish fed on 50-2, 70, commercial and control diets. Results obtained in fish fed 100% plant protein diets agreed with those previously reported in tilapia, where the use of fish-meal-free diets determined a lower performance than obtained when feeds included fish meal protein (Jackson *et al.*, 1982). Considering that our experimental diets theoretically covered amino-acid requirements of fish, nutritive utilization of such diets could be affected by factors other than an amino-acid imbalance. Plant protein-based diets usually contain antinutritive factors that may affect food digestion (Krogdahl, 1989). Such factors are not only specific substances with a particular ability to inactivate digestive enzymes as is the case for soybean trypsin inhibitor or tannins found in carob seeds (Alexis, 1990), but also oligosaccharides and starches that may be mistaken as available carbohydrates, as occurs in soybean makes up 20% of dry matter, but the soluble oligosaccharides stachyose and raffinose must be considered physiologically as fibre (Saini, 1989), as are the α-galactosides (8.8% of dry matter) present in lupin (Champ *et al.*, 1991). Alcohol-soluble carbohydrates have proved to affect digestibility of soybean in fish (Arnesen *et al.*, 1989). Values of protein digestibility for soybean meal (around 77%; Akiyama, 1988) and lupin seed meal (85%; De la Higuera *et al.*, 1988) point to the fact that digestible energy in whole plant protein diets containing any of those seeds may be less than expected, resulting in a lower dietary utilization for growth. Also, a poorer nutritive utilization of dietary protein when crystalline amino acids are added to the formula (as was the case for methionine in fish-meal-free diets) has been reported by several authors (Andrews and Page, 1974; Murai *et al.*, 1981). Nevertheless, it seems that a diet including around 70% of plant protein may be adequate for feeding salmonids, as has been reported by Smith *et al.* (1988), when they assayed a combination of full-fat soybean meal, cottonseed meal and corn gluten meal. In our experiment, best results were obtained using a diet (70) similar to that of Smith *et al.* but including corn gluten meal as the main vegetable ingredient, potato protein concentrate and lupin seed meal instead of soybean meal. Results have confirmed the great interest of using lupin seed meal in combinations, considering good results obtained when tested as a sole partial substitute of fish meal (Moyano *et al.*, 1985; De la Higuera *et al.*, 1988; Gomes and Kaushik, 1989). Furthermore, it is confirmed that high levels of corn gluten meal can be incorporated into rainbow trout diets (20.8% in diet 70). This result is in agreement with other reports on the possibilities of using this vegetable source in feeds for salmonids (Fauconneau, 1988; Moyano *et al.*, 1991). On the other hand, the very low value of protein retention determined in fish fed on a commercial diet was noticeable: from 30 to 50% lower than the rest. This result could be based on an increased catabolism of a large proportion of dietary protein, due to its poor nutritive quality. Nevertheless, as shown in *table* 5, fish fed on this diet showed normal values of weight increase (mean final weight; 52.6 g) and feed efficiency (0.80). There was no significant influence of the type of dietary protein source on the proximate composition of fish, with the only exception of fish fed on the commercial diet. Those fish presented a lower fat deposition, a higher water content and 10-20% less protein, expressed in live weight, than fish fed on the rest of experimental diets. A possible explanation is that fish fed on the commercial diet utilized for this experiment increased weight by means of a high water retention, in a manner quite similar to that observed in cattle when feeds include hormonal components. This result, considering the number of samples utilized in body analysis (25% of total population), would mean that fish culturists may be paying for a feed that increases fish weight, but not muscle weight in the same proportion. A similar result can be deduced from data, reported by Alexis *et al.* (1986) from comparison of a series of experimental diets with a commercial one, indicating a 4% higher water content in fish fed on this latter diet. Thus, the hypothesis of an abnormal water retention in fish fed on some commercial feeds, perhaps involving hormonal components, should be considered. In this sense, possible influence of steroidal components present in fish meal should be discarded because the above mentioned effect was not observed in fish fed on a whole fish meal diet.

This kind of modification in fish composition might affect their organoleptic properties. In fact, flesh quality of fish fed on the commercial diet was less appreciated by members of the taste panel than the rest of samples tested, althought not in a statistically significant way. Low fat and high water contents indubitably influenced score. On the other hand, feeding fish on high levels of plant proteins did not negatively affect their taste quality, being well accepted by all the panelists, in agreement with the results reported by Smith *et al.* (1988). Therefore, the type of protein seems to have no negative influence on flavour and other organoleptic of fish, fats being a much more important diet component in this sense (De la Higuera *et al.*, 1977).

An economic evaluation of the results, given an adequate selection of protein feedstuffs (from animal or plant origin), will be determined by market prices and must take into account the high proportion that feed represents on the total cost of fish production (FAO, 1983). In this sense, the use of low-price vegetable proteins is an interesting way of reducing such costs, also considering that in the future, fish meal shortages might increase its price (Anonymous, 1988). In our experiment, low cost of diets 50-2 and 70 in relation to control or commercial diet seemed to make them suitable for the producer. Saving was within the range that can be calculated from data presented in Smith *et al.* (1988) (from 8.5 to 13.5%) or Alexis *et al.* (1986) (from 16 to 30%). Furthermore, a less favourable utilization of protein was observed in fish fed on the commercial diet in relation to the rest of the experimental groups. Based on the calculation of the cost of fish protein produced, it is shown that the utilization of the mentioned diets may be profitable, not only in terms of cost but also in relation to their efficiency, resulting in less than half the price calculated for commercial diet (*table* 5). From the customer's perspective, the utilization of high vegetable protein diets could mean a lower price of fish and no alterations in their organoleptic properties.

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