Apparent Digestibility and Protein Quality Evaluation of Selected Feed Ingredients in Seriola dumerili.

Journal:	*Journal of the World Aquaculture Society*
Manuscript ID	JWAS-17-025.R4
Manuscript Type:	Fundamental Studies
Date Submitted by the Author:	08-Nov-2018
Complete List of Authors:	Tomas Vidal, Ana; Universidad Politecnica de Valencia, Inctituto de Ciencia y Tecnología Animal Monge-Ortiz, Raquel; Universitat Politècnica València, Institute of Animal Science and Technology JOVER, MIGUEL; UNIVERSIDAD POLITECNICA VALENCIA, CIENCIA ANIMAL Martínez-Llorens, Silvia; Polytechnic University of Valencia, Animal Science
Keywords:	yellowtail, protein quality, amino acids, protein and lipid digestibility, Chemical Score

EDITORIAL CERTIFICATE

This document certifies that the article listed below was edited and revised for proper English language, syntax, spelling, punctuation and style by Vanesa Vega Dorado (B.A. English Philology and M.A. Applied Linguistics).

ARTICLE TITLE

Apparent digestibility and protein quality evaluation of selected feed ingredients in *Seriola dumerili*

AUTHORS

Silvia Martínez-Llorens, Ana Tomás-Vidal, Raquel Monge, Miguel Jover-Cerdá

DATE ISSUED

October 10th 2016

Neither the research content nor the author's intention were in any way altered during the editing process. Documents receiving this certification should be English ready for publication. However, the author has the ability to accept or reject the suggestions and changes made to the manuscript.

Apparent Digestibility and Protein Quality Evaluation of Selected Feed Ingredients in Seriola dumerili.

Ana Tomás-Vidal, Raquel Monge-Ortiz, Miguel Jover-Cerdá, Silvia Martínez-Llorens.

Research Group of Aquaculture and Biodiversity. Institute of Animal Science and Technology. Universitat Politècnica de València. Camino de Vera, 14. 46071-Valencia, Spain.

Corresponding author: Raquel Monge-Ortiz, Universitat Politècnica València. Camino de Vera, 14. 46071-Valencia (Spain). Tel.: 34-96-3879752; Fax: 34-96-3877439. E-mail: silmarll@dca.upv.es.

Abstract

The apparent digestibility coefficients (ADC) of dry matter, crude protein, crude lipid, and amino acids in fish, krill, squid, meat, defatted krill, soybean, wheat gluten, wheat, camilina, pea, sunflower and fava bean meals were determined for juvenile Seriola dumerili. The results showed that the ADC of dry matter for yellowtail ranged from 57.7 to 87.2% for animal ingredients, and from 42.2 to 82.2% for plant ingredients. ADC of protein exceeding 90% were observed in fishmeal, while camilina meal and fava bean meal presented the lowest values. Pea meal presented the lowest lipid ADC (83.5%). The availabilities were generally higher in animal ingredients than those in vegetal ones. Except camilina and fava bean meal, the other ingredients appear to be favorable for Seriola dumerili diets, especially the ones from animal sources. The lower case chemical score values (minimum value from AARs) were obtained in some vegetal ingredients (14 to 18%), while the highest ones, appeared in marine ingredients (69 to 88%). According to Oser's Index, the most balanced protein for yellowtail with regard to essential amino acids was in krill, defatted krill, and fishmeal (92 to 96%). So, animal sources are suitable as protein ingredients, but they could be enhanced through some essential amino acid supplementation.

In the last two decades the European aquaculture sector has shown an increasing interest and demand in fast-growing species (Mazzola et al. 2000) such as Mediterranean yellowtail (Seriola dumerili), as they can improve the competitiveness of aquaculture companies.

The most expensive component in feed formulation in aquaculture is protein. This is especially important considering that Seriola species require high protein dietary levels (450550 g kg^{-1}) for maximum growth (Masumoto et al. 1998; Jover et al. 1999; Tomás et al. 2008), and the dietary substitution of proteins by other nutrients—such as lipids or carbohydrates—is not the best solution for the formulation of feeds for S. dumerili (Jover et al. 1999). In this sense, fishmeal is an ideal protein source for carnivorous fish, because it contains a balanced amino acid composition, high content of phosphorous and minerals and high digestibility coefficients. However, its high cost motivated its reduction in aqua feeds, while maintaining the same protein levels from alternative protein sources.

Currently, in order to avoid the dependence on fishmeal, aquaculture diets should contain high amounts of alternative proteins that are more widely available and that have lower and more stable market prices than this marine resource. Alternative proteins include a large range of plant or animal proteins with competitive prices (Martínez-Llorens, Vidal, & Cerdá, 2012). Despite these economic advantages, previous results on Mediterranean yellowtail show that alternative proteins cannot totally replace fishmeal when they are used as the sole ingredient. When soybean is included to replace over 30% of the fishmeal, it resulted in poor growth and showed low nutrient efficiency (Tomás et al. 2005). Total fishmeal substitution presents some limitations in fish diets, such as the anti-nutrients that are particularly contained in vegetable proteins (Krogdahl et al. 2010), causing a protease enzyme activity decrease and, therefore, low protein digestibility (Monge-Ortiz et al. 2016). Another issue related to the use of alternative protein sources is the inadequate profile of amino acids (Cerezo et al. 2012), which results in poor fish growth and protein deficiencies. This forces the use of supplemental diets with synthetic amino acids, which increases the feed price. The best growth and nitrogen retention results when combination of protein sources are used, that include a combination of aminoacids in order to complement one source with limiting ones, that is the case of Sparus aurata (Kissil & Lupatsch, 2004; Sánchez-Lozano et al., 2009), and evidence suggests that for other species too, like Salmo salar (Espe, 2006) or Seriola dumerili (Monge-Ortiz et al, 2017). For this reason, the combination of animal proteins (such as defatted krill and poultry meal) with plant proteins in fish diets could be beneficial to palliate the deficiencies in amino acids of some protein sources, thus reducing the anti-nutrient compounds and avoiding supplementation with synthetic aminoacids (AAs), lack of palatability (Oliva-Teles 2012) and 'green liver' (Takagi et al. 2005). The adequate proportion to simulate the ideal amino acid profile in diets for the Mediterranean yellowtail will require studies on the digestibility and the protein quality of these ingredients.

Therefore, the evaluation of feed ingredients is crucial to nutritional research and feed development for aquaculture species (Glencross et al. 2007). The alternative protein sources and their inclusion levels need to be optimized in fish diets to make aquaculture production more efficient and cost-effective. Digestibility trials are essential to shed light on the potential of these ingredients for fish before their inclusion in diet formulations.

There are numerous factors involved in the digestibility performance of common feed ingredients for fish. Digestibility coefficients are also different for the same ingredient depending on the species under study (Davies et al. 2009). Apparent digestibility coefficient (ADC) values of feed ingredients are normally the result of differences in species, changes in the harvest/catch season of the raw materials and conditions under which they were processed. For that reason, studies on various marine fish species have been conducted (Lupatsch et al. 1997; Tibbets et al.2004; Booth et al. 2005; Tibbets et al. 2006; Davies et al. 2009) in order to assess the quality value of different ingredients.

Taking into account the relevance of digestibility estimation for diet formulation, the aim of this present study was to compare the apparent nutrient digestibility and protein quality of 12 different commercially available ingredients for Seriola dumerili.

Materials and Methods

Ingredients and Experimental Diets

Yellowtail (S. dumerili) juveniles were caught in the Mediterranean and transferred to the facilities of the Polytechnic University of Valencia, Spain.

Feed ingredients were tested for apparent protein digestibility, availability of digestible amino acids and protein quality in fishmeal, krill meal, squid meal, poultry meal (international feed number 5-03798), defatted krill meal (a product obtained by removing the fat with ethanol), soybean (Glycine max), wheat gluten (Triticum spp), camilina (Camelina sativa) (product given to our facilities by local farmers), pea (Pisum sativum), sunflower (Helianthus annuus) and fava bean (Vicia faba) meals (Table 1 and 3). Test diets were prepared by mixing a basal diet with one of the test ingredients (Table 2) in a ratio of 7 to 3, as described by Cho and Slinger (1979).

The different ingredients of the diets were weighed individually and mixed to form homogeneous dough, and were prepared by cooking-extrusion processing with a a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The conditions under which they were processed are as follows: a screw speed of 100 rpm, a temperature of 110 C, and a pressure of 40-50 atm. The experimental diets were assayed in quintuplicate. The fish were handfed once a day (8.00h), and fecal collection occurred 8 h later (16:00h). The pellet size used was 3mm during the experiment, increasing to 4mm in the final part of the experiment. The pellets were slowly distributed to give all fish time to eat. The uneaten diet was collected when fish had finished feeding.

Digestibility Assay

Fifteen yellowtails (175±2.8 g initial mean live weight) were randomly distributed in five experimental tanks (190 L fiberglass tanks, 88cm length, 62 cm diameter and 188 cm depth) of a marine semi-closed recirculating system designed on the basis of the Guelph system (the fecal material being collected in a settling column). Each tank was loaded with three fish. The velocity of the water flow was adjusted to minimize settling of the feces in the drainpipe and maximize feces recovery in the settling column.

There was a three-month adaptation period (adaptation to dry diets was a very long process, in which yellowtail were first fed anchovy and, later, a semi-moist diet—a mix of anchovy and fishmeal—before being fed the control diet). Once the fish had become accustomed to both the tanks and the dietary regime, collection of feces was initiated.

The fish were fed one meal a day at 10.00 h. The feed was offered only as long as the fish were actively feeding in order to avoid wastage. One hour after the meal, the drainpipe and the settling column were brushed out to remove uneaten feed particles and feces from the system to avoid mixing feed particles and feces in the recollection.

Feces were collected next day from the base of the settling column into a plastic container by gravity at 8.00 h. After feces collection, the fish were fed again at 10.00h, waiting two hours between feces recolection and feeding to avoid stress.

The water temperature was maintained around 20 C (21.5 ± 2.4 C) during the experimental period by a water conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were equipped with aeration and the level of dissolved oxygen was 6.6 ± 1.3 mg L^{-1}. Water salinity was 31.5 ± 4.1 g/L, pH 7.3 ± 0.4, NO_3^- (25–150 mg/L), and NO_2^- (0.05–0.5 mg/L), and the ammonium value was undetectable The photoperiod was maintained at 12-h light and 12-h dark by means of artificial daylight simulation. All these parameters were measured daily from Monday to Saturday. This parameters are optimal for S. dumerili in recirculation system, and are similar that in other Seriola experiments (Jover, 1999; Monge-Ortiz, 2018a, 2018b)

Over a six-month period, the fish groups were fed the experimental diets and feces were sampled from each tank. One tank was fed with the control diet over six months to determine the possible variations in digestibility throughout the experimental period. Experimental periods were 14 days long for each diet and each replicate. The last three days of the two-week period fish fasted in order to avoid mixing feces between diets. Feces were removed immediately to determine their amount and have enough for analysis following which fish continued to be fed the same diet for another week. The switching of dietary treatments was as follows: diets with fava bean, camilina, soybean and pea meal were tested from Week 1 to Week 2, from 7 to 8, from

13 to 14 and from 19 to 20 respectively; diets with sunflower, wheat, wheat gluten and defatted krill meal were assayed from week 3 to week 4, from 9 to 10, from 15 to 16, from 21 to 22 and from 25 to 26, respectively; and diets containing fish, krill, meat and squid meal were tested from Week 5 to 6, from 11 to 12, from 17 to 18, from 23 to 24, and from 27 to 28, respectively of the experimental trial.

The fecal material collected was dried to a constant weight in an oven at 60 C for 48 h prior to analysis and stored in airtight plastic containers until nutrient component and inert marker (acid insoluble ash, AIA) analysis. The AIA content of feeds and feces was determined by the method of Atkinson et al. (1984), to calculate the apparent digestibility coefficient, (ADC).

Apparent digestibility coefficients (ADC_{diet}, %) of each specific nutritional variable (protein (ADCp, %), lipid (ADCl, %), and AA) of the diets were calculated using the following formulae:

$$ADC_{diet} = 1 - \left(\frac{Marker_{diet} \times Nutrient_{faeces}}{Marker_{faeces} \times Nutrient_{diet}} \right)$$

In this equation, the terms $Marker_{diet}$ (g kg^{-1}) and $Marker_{faeces}$ (g kg^{-1}) represent the marker content of the diet and the feces, respectively, and $Nutrient_{diet}$ (g kg^{-1}) and $Nutrient_{faeces}$ (g kg^{-1}) are the nutritional parameters of concern (e.g. protein or energy) in the diet and the feces, respectively.

The apparent digestibility coefficients of dry matter, protein, lipid and amino acids for the test ingredients (30% replacement level) were calculated as follows:

$$ADCN_{ing} = \frac{[(a+b) \times ADCN_{test} - a \times ADCN_{ref}]}{b}$$

In the equation above, "*a*" is nutrient contribution of reference diet to nutrient content of test diet, "*b*" is nutrient contribution of test ingredients to nutrient content of combined diet, *"a + b"* is the level of nutrient in combined diet (%); $ADCN_{test}$ (%) = apparent digestibility coefficient of a nutrient in combined diet and $ADCN_{ref}$ (%) = apparent digestibility coefficient of a nutrient in reference diet (Forster 1999).

Chemical Analysis

Chemical analysis of the dietary ingredients was performed prior to diet formulation (Table 1). Diets and their ingredients were analyzed according to AOAC (1995) procedures: dry matter (105 C to constant weight), ash (incinerated at 550 C to constant weight), crude protein (N x 6.25), assessed by the Kjeldahl method after acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden), and crude lipid, extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator).

Following the aforementioned method by Bosch et al. (2006), the amino acid content of the fish carcasses and the diets were established using a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) with two pumps (Model 515, Waters), an auto-sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. Before hydrolysation, alpha aminobutyric acid was added as an internal standard. The derivatization of amino acids was made by using AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteic acid after oxidation with performic acid. Amino acids were separated with a C-17 reverse-phase column Waters Acc. Tag (150 mm x 3.9 mm) and subsequently transformed to Met and Cys.

Protein Quality

The values of the following indices were calculated:

- Amino acid ratio (AAR, %) = $(AA_{sample})/(AA_{reference})*100$, where AA_{sample} and $AA_{reference}$ are the digestible amino acid contents in the test sample and whole S. dumerili (Fig. 1), the one taken as reference (average values of 20 samples of fish weighing 100 to 500g).
- Chemical score (CS, %): minimum value from AARs calculated for digestible essentials amino acids (EAA: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val).
- Limiting amino acid: the digestible amino acid corresponding to CS in the test sample.
- Oser's Index (OI, %): used as index of nutritional quality and achieved as the geometric mean ratio of digestible amino acids in samples of the ones detected in Seriola dumerili, which were taken as a reference according to the following formula:

$$OI\ (\%) = (10^{\,(1/n*(\log(AAR_1)+\log(AAR_2)\ldots+\log(AAR_n)))}),$$

in which AAR_1, AAR_2,…AAR_n are the ratios of digestible essential amino acids, and "n" is the number of detected digestible essential amino acids. When the ratio lay above 100, this was taken as reference (Oser 1951).

Statistical Analysis

Digestibility coefficients were evaluated using one-way analysis of variance (ANOVA). ACD data were $\log_{10}$ transformed to meet the assumptions of statistical tests (normality, linearity and homosdasticity). The Newman-Keuls test was used to assess specific differences among diets at a level of $P<0.05$ (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

Results

Proximate Composition and Amino Acid Content

The proximate composition of the 12 test feed ingredients are reported in Table 1. The greatest protein values were observed in animal meals; fishmeal (713.2 g kg^{-1}) wheat gluten (810 g kg-1), squid meal (718.8 g kg-1) and extracted krill meal (723.1 g kg-1), while the lowest were found in vegetable meas like pea (215.9 g kg-1), fava bean (236.6 g kg-1) and sunflower meal (291.3 g kg-1).

Vegetal meals presented the poorest amino acid balance, with serious deficiencies in EAA, such as in arginine, threonine, lysine and methionine (Table 3). Arginine, lysine and leucine were the predominant essential amino acids in animal ingredients, of which fish and defatted krill meal had a relatively similar content. The principal non-essential amino acids present in animal and vegetal meals were aspartate and glutamine.

Digestibility

The ADCs of dry matter for yellowtail ranged from 71.88 to 89.53% for animal ingredients, and from 65.89 to 79.98% for plant products. The higer protein ADCs appeared in fishmeal (91.3%), wheat gluten (87%), defatted krill meal (86.7%), squid meal (85.2%) and poultry meal (85.0%); mid-range in krill meal (75.8%), soybean meal (73.2%) and sunflower and pea meal (63%); and low in camilina (48.5%) and fava bean meal (54.9%).

Apparent digestibility coefficients of lipids in all the treatments were above 88%, except in the case of pea meal (83.5%).

For the animal ingredientss, the availabilities of amino acids (Table 5) in fish, squid, meat and defatted krill meal were generally higher than those in krill meal. Among all the plant ingredients, the availabilities of amino acids in wheat gluten were the highest, followed by soybean-, pea- and sunflower meal. Camilina and bean meal presented the lowest AAs ADC.

<u>Protein Quality Evaluation</u>

Defatted krill meal was the most balanced digestible protein ingredient (Figs. 1, 2). It was only deficient in histidine, threonine and valine (AAR: 70 (CS), 86 and 87%). Fishmeal was slightly deficient in isoleucine, leucine, lysine, phenylalanine and valine (AAR: 90, 94, 95, 97 and 88 %, respectively). Krill meal was deficient in histidine, lysine and valine (AAR inferior to 90%). Squid and poultry meals presented 60 to 85% ARRs in all the digestible amino acids, except for arginine, which was the most balanced of them all, while isoleucine was the least balanced (AAR<50%). With respect to the vegetal ingredients, wheat gluten showed the lowest CS (13.98% for lysine), followed by sunflower, which was found to be deficient in Met (CS: 17.97%), and camilina meal (poor in methionine, CS 30.8%). Pea meal proved to be a balanced amino acid ingredient (its CS, Met, was above 50%).

Oser's Index (OI, Fig. 2) calculated for protein meals was positively related with CS, presenting the lowest values of OI those presented the lowest CS, such in the case of wheat meal and sunflower meal (50.41% OI, 13.89% CS and 67.96% OI, 17.97% CS respectively). On the contrary, defatted krill and fishmeal (92 to 96 %) showed the most balanced protein with regard to essential amino acids, whereas all the other ingredients presented lower levels (50-78%).

Discussion

The results obtained in the present study confirmed the very high digestibility of certain animal proteins for carnivorous marine fish (Lupatsch et al., 1997; Tibbets et al. 2006). ADCs of animal proteins were close to those obtained for fishmeal concerning crude protein digestibility, and that fact is probably the result of their very high protein concentrations. Only krill meal ADCp was lower than the other animal protein sources, probably because of the unusually high amount of fat of the krill meal sample used in this study (and consequent low protein level). This was the main difference between the krill meal and the defatted krill meal, which has a lower fat and a higher protein level, making it a higher quality product for yellowtail diets. Tibbetts (2006) obtained similar results with shrimp, with low ADC, due to the high ash content of the shrimp meal.

Protein ADCs of fishmeal assessed in yellowtail are also similar to the ones encountered in turbot (Psetta maxima), seabass (Dicentrarchus labrax), sea bream (Sparus aurata), Atlantic cod (Gadus morhua), red drum (Sciaenops ocellatus), Australian snapper (Pagrus auratus) and Atlantic halibut (Hippoglossus hippoglossus), at 91 to 96% (Gaylord and Gatlin 1996; Lupatsch et al. 1997; Gomes da Silva and Oliva-Teles 1998; Burel et al. 2000; Peach 2005; Tibbetts et al. 2006; Davies et al. 2009; Booth et al. 2010).

Protein ADCs were high for poultry meal (85%). Animal by-product meals can vary greatly in their proximate composition, depending on several factors (raw material source and freshness, production processes and storage) and, due to its very nature, the values reported for protein ADCs are also highly variable in studies on fish. In many fish species, poultry meal has received positive results in diets (ADCp 85.5% in sea bass, 79 to 80% in sea bream, 78.4% in turbot, and 80% in Atlantic cod), and is regarded as the most effective animal protein (Lupatsch et al. 1997; Tibbets et al. 2006; Davies et al. 2009).

The ADCp of krill hydrolysate found in Atlantic halibut (Peach 2005) and Atlantic cod (Tibbetts et al. 2006) was nearly 100%, very different indeed to the krill ADCp in yellowtail (76%), but that krill meal was obtained through finely grinding whole krill that had previously been freeze-dried (Euphausia superba) and, therefore, the proximate composition was fairly different from the one found in commercially produced krill meals like the one used in the present work. Those discrepancies are explained by the differences in product quality, as in the case of the defatted krill meal (full-fat krill meal) with a higher protein level.

There are numerous studies on digestibility of soybean meal on various fish species. The protein ADC, however, varied a great deal (76 to 98%). Yellowtail is within this range, although on the lower side, i.e. among the species that present a lower soybean protein digestibility. Other authors observed a decrease in protein digestibility (Robaina et al. 1995; Refstie et al. 1999), mainly attributed to the presence of phytate.

Protein ADC was high for wheat gluten meal (87%), but inferior to that reported for other marine fish (100 %), like seabass (Robaina et al. 1999) and Atlantic cod (Tibbetts et al. 2006). The high palatability and digestibility, together with the absence of anti-nutritional factors, make wheat gluten meal, supplemented with some amino acids (lysine, methionine and arginine), a suitable fishmeal replacement candidate in yellowtail diets.

Pea meal had a middle ADCp. This ingredient appears to have some potential for its use in marine fish diets (Sánchez-Lozano et al. 2009, 2011); nonetheless it should be pre-extruded in order to boost the digestibility of non-protein ingredients.

Lipid ADC results indicate that lipids from both animal and plant sources were well digested by Seriola dumerili. There were only slight differences in this parameter between soybean meal and most of the raw materials. Several studies have concluded that dietary soybean meal decreases the lipid digestibility in salmonids (Romarheim et al. 2008). This fact must be attributed to the bile acid level, which is reduced by dietary soybean meal (Romarheim et al. 2006; Yamamoto et al. 2007).

Regarding the availability of AAs, it should be noted that the digestibility of each AA within a feed ingredient is variable. This variability, nonetheless, increases in those ingredients with a lower protein ADC (AAs ADC of camilina or fava bean meals were between 38 and 66%). In general, apparent digestibility coefficients of AAs reflect the apparent CP digestibility coefficients of some test ingredients. In some of them, however, there are differences, like in wheat gluten, in which apparent digestibility coefficients of AAs were lower than ADCp. On the contrary, krill meal, pea meal or poultry meal presented higher AA ADCs. Thus, amino acid availabilities cannot always be estimated from the ingredient ADCp. Fishmeal and defatted krill meal are the most balanced digestible amino acid ingredients and have one advantage: they benefit from having a higher protein content and CS than krill. However, the defatted krill meal also has one shortcoming: the need for histidine supplementation. Regarding other animal sources, squid meal was the most balanced amino acid in arginine and poultry meal protein quality was similar to camilina and soybean meal.

Despite the high protein digestibility observed in present work for some protein meals, the biological value of the protein should also be taken into account before diet formulation to include them in fish diets. For example, wheat gluten digestibility resulted very high, however it shows a low OI and the lowest CS (in this case Lysine) and therefore it cannot replace the fishmeal in high dietary levels for Mediterranean yellowtail feeding.

It has been observed in studies performed with sea bream (Lupatsch et al. 1997) that, when using ACDp values obtained for individual ingredients to calculate the ACDp for compounds diets, both values were very similar, and consequently, protein digestibility can be considered to be additive. Therefore, ACDp results obtained in this present experiment, along with the protein quality assessments, should be taken into account for formulating diets for Mediterranean yellowtail. This is a useful tool to simulate the ideal protein in diets because, by combining different ingredients, the inclusion of synthetic amino acids could be avoided or diminished, which would reduce the cost of fish diets and improve protein digestion.

In conclusion, animal sources are evidently suitable as protein ingredients in yellowtail diets, although the protein quality indices present in them could be enhanced through some essential amino acid supplementation. None of the vegetal meals assayed could offer a good nutritional balance on their own and, therefore, they would either need to be supplemented or need to be used together with other raw materials.

Acknowledgments

Project financed by the “Ministerio de Ciencia e Innovación” (reference AGL2011-30547-C03). We would also thank IEO (Instituto Español de Oceanografía), Centro Oceanográfico de Murcia (España) and Dr. Fernando de la Gándara for providing us the fish used in the present study.

Literature Cited

AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists 16th Edition (16th ed.)AOAC Inc., Arlington, Virginia, USA

Atkinson J.L., Hilton J.W. and S.J. Slinger. 1984. Evaluation of acid insoluble ash as an indicator of feed digestibility in rainbow trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences, 41:1384–1386

Booth M.A., Allan G.L. and A.J. Anderson. 2005. Investigation of the nutritional requirements of Australian snapper Pagrus auratus (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research, 36:378–390

Booth M.A., Allan G.L. and I. Pirozzi. 2010. Estimation of digestible protein and energy requirements of yellowtail kingfish Seriola lalandi using a factorial approach. Aquaculture, 307:247-259.

Bosch L., Alegria A. and R. Farré. 2006. Application of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), reagent to the RP-HPLC determination of amino acids in infant foods. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 831:176–178.

Bureau, D.P., Harris, A.M. and Cho, C.Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). Aquaculture, 180: 345-358.

Burel C., Boujard T., Tulli F. and S.J. Kaushik. 2000. Digestibility of extruded peas, extruded lupin and rapeseed meal in rainbow trout (Oncorhynchus mykiss) and turbot (Psetta maxima). Aquaculture, 188:285–298

Cerezo Valverde J., Martínez-Llorens S., Tomás Vidal A., Jover M., Rodríguez C., Estefanell J., Gairín J.I., Domingues P.M., Rodríguez C.J. and B. García. 2012. Amino acids composition and protein quality evaluation of marine species and meals for feed formulations in cephalopods. Aquaculture International, 21:413-433

Cho C. Y. and S.J. Slinger. 1979. Apparent digestibility measurement in feedstuffs for rainbow trout, Pages 239-247 in J. Halver, K. Tiews (Eds.), Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, vol. 2 Heenemann, Berlin.

Davies J., Gouveia A., Laporte J., Woodgate S. and S. Nates. 2009. Nutrient digestibility profile of premium (category III grade) animal protein by-products for temperate marine fish species (European sea bass, gilthead sea bream and turbot). Aquaculture Research, 40:1759-1769.

Forster I. 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquaculture Nutrition, 5:143–145.

Gaylord T.G. and D.M. Gatlin. 1996. Determination of digestibility coefficients of various feedstuffs for red drum (Sciaenops ocellatus). Aquaculture, 139:303–314.

Glencross B.D., Booth M. and G.L. Allan. 2007. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition, 13:17–34.

Gomes da Silva J. and A. Oliva-Teles. 1998. Apparent digestibility coefficients of feedstuffs in seabass (Dicentrarchus labrax) juveniles. Aquatic Living Resources, 11:187–191.

Jover M., García-Gómez A., Tomás A., De la Gándara F. and L. Pérez. 1999. Growth of the Mediterranean yellowtail (Seriola dumerili) fed extruded diets containing different levels of protein and lipid. Aquaculture, 179:25-33.

Krogdahl Å., Penn M., Thorsen J., Refstie S. and A.M. Bakke. 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquaculture Research, 41:333–344.

Lupatsch I., Kissil G., Sklan, D. and E. Pfeffer. 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, Sparus aurata L. Aquaculture Nutrition, 3:81–89.

Martínez-Llorens, S., Vidal, A. T. and M.J. Cerdá. 2012. A new tool for determining the optimum fish meal and vegetable meals in diets for maximizing the economic profitability of gilthead sea bream (Sparus aurata, L.) feeding. Aquaculture Research, 43:1697–1709.

Masumoto T., Itoh Y., Ruchimat T., Hosokawa H. and S. Shimeno. 1998. Dietary amino acids budget for juvenile yellowtail (Seriola quinqueradiata). Bulletin of Marine Science and Fisheries, 18:33-37.

Mazzola, A., Favaloro, E. and G.L. Sarà. 2000. Cultivation of the Mediterranean amberjack, Seriola dumerili (Risso, 1810), in submerged cages in the Western Mediterranean Sea. Aquaculture, 181:257-268.

Monge-Ortiz R., Martínez-Llorens S., Márquez L., Moyano F.J., Jover-Cerdá M. and A. Tomás-Vidal. 2016. Potential use of high levels of vegetal proteins in diets for market-sized gilthead sea bream (Sparus aurata). Archives of Animal Nutrition, 70 http://dx.doi.org/10.1080/1745039X.2016.1141743

Monge-Ortiz R., Tomás-Vidal A., Rodriguez-Barreto D., Martínez-Llorens S., Pérez J.A., Jover-Cerdá M., Lorenzo A. 2018. Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (Seriola dumerili) juveniles: Effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. Aquculture Nutrition. 24:605-615.

Monge-Ortiz R., Tomás-Vidal A., Gallardo-Álvarez F.J., Estruch G., Godoy-Olmos S., Jover-Cerdá M., Martínez-Llorens S. 2018. Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for Seriola dumerili: Effects on performance and nutrient efficiency. Aquaculture Nutrition, 24:1163-1174.

Oliva-Teles A. 2012. Nutrition and health of aquaculture fish. Journal of Fish Diseases, 35:83–108.

Oser B.L. 1951. Methods for integrating essential amino acid content in the nutritional evaluation of protein. Journal of the American Dietetic Association, 27:396–402.

Peach R.W. 2005. Protein and energy utilization by juvenile Atlantic halibut (Hippoglossus hippoglossus). M.Sc. Thesis. Dalhousie University, Halifax/Nova Scotia Agricultural College, Truro, Nova Scotia.

Refstie S., Svihus B., Shearer K.D. and Storebakken, T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. Animal Feed Science and Technology, 79:331–345.

Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D. and H. Fernández-Palacios. 1995. Soybean and lupin meals as protein sources in diets for gilthead sea bream (Sparus aurata): nutritional and histological implications. Aquaculture, 130:219–233.

Robaina L., Corraze G., Aguirre P., Blanc D., Melcion J.P. and S. Kaushik. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (Dicentrarchus labrax) fed pelleted or extruded diets with or without wheat gluten. Aquaculture, 179:45-56.

Romarheim O.H., Skrede A., Gao Y., Krogdahl Å., Denstadli V., Lilleeng E. and T. Storebakken. 2006. Comparison of white flakes and toasted soybean meal partly replacing fishmeal as protein source in extruded feed for rainbow trout (Oncorhynchus mykiss). Aquaculture, 256:354–364.

Romarheim O.H., Skrede A., Penn M., Mydland L.T., Krogdahl A. and T. Storebakken. 2008. Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (Oncorhynchus mykiss) fed diets containing defatted soybean meal. Aquaculture, 274:329–338

Sánchez-Lozano N.B., Martínez-Llorens S., Tomás-Vidal A. and M. Jover Cerdá. 2009. Effect of high-level fishmeal replacement by pea and rice concentrate protein on growth, nutrient utilization and fillet quality in gilthead sea bream (Sparus aurata, L.). Aquaculture, 298:83–89.

Sánchez-Lozano N., Martínez-Llorens S., Tomás-Vidal A. and M. Jover-Cerdá. 2011. Amino acid retention of gilthead sea bream (Sparus aurata, L.) fed with pea protein concentrate. Aquaculture Nutrition, 17:e604–e614.

493 **Tibbetts S.M., Lall S.P. and J.E. Milley.** 2004. Apparent digestibility of common feed ingredients by juvenile haddock, Melanogrammus aeglefinus. L. Aquaculture Research, 35:643-651.

496 **Tibbetts S.M., Milley J.E. and S.P. Lall.** 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, Gadus morhua (Linnaeus, 1758). Aquaculture, 261:1314–1327.

499 **Tomás A., De la Gándara F., García-Gómez A., Pérez, L. and M. Jover.** 2005. Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, Seriola dumerili. Aquaculture Nutrition, 11:333–340.

502 **Tomás A., De la Gándara F., García-Gómez A. and M.J. Cerdá.** 2008. Effect of the protein/energy ratio on the growth of Mediterranean yellowtail (Seriola dumerili). Aquaculture Research, 39:1141–1148.

505 **Takagi S., Murata H., Goto T., Ichiki T., Munasinghe D.M., Endo M., Matsumoto T., Sakurai A., Hatate H., Yoshida T., Sakai T., Yamashita H., Ukawa M. and T. Kuramoto.** 2005. The green liver syndrome is caused by taurine deficiency in Yellowtail, Seriola quinqueradiata fed diets without fishmeal. Aquaculture Science, 53:279-290.

510 **Yamamoto T., Suzuki N., Furuita H., Sugita T., Tanaka N. and T. Goto.** 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout Oncorhynchus mykiss. Fisheries Science, 73:123–131

TABLE 1. Proximate composition of ingredients used in the digestibility trial (expressed as *g kg* $^{-1}$ of dry matter basis, dm).

Ingredients[a] *(g kg^{-1})*	Dry matter	Crude Protein	Crude lipid	Ash	N-free extract
Plant protein ingredients					
Fava bean meal	890	237	12	33	718
Camilina meal	918	391	45	61	503
Soybean meal	882	499	22	71	408
Pea meal	866	216	10	39	736
Sunflower meal	896	291	15	67	627
Wheat meal	890	116	15	18	851
Wheat gluten	933	810	9	9	173
Animal protein ingredients					
Defatted krill meal	878	723	24	102	151
Fish meal	903	713	93	168	26
Krill meal	888	561	225	104	111
Poultry meal	970	531	153	269	47

Squid meal	928	719	30	110	142

[a] Wheat (Triticum spp), Sunflower (Helianthus annuus L.), pea (Pisum sativum L.), fava bean (Vicia faba L.) and soybean meal (Glycine max L. Merr.) were provided by DESCO, Museros, Valencia (Spain); Defatted krill meal was provided by LUDAN RENEWABLE ENERGY, Valencia (Spain); Wheat gluten and Camilina (Camelina sativa, L. Crantz) were provided by DADELOS AGRÍCOLA, Valencia (Spain); Poultry meal was provided by VALGRA S.A., Beniparrell, Valencia (Spain); Krill meal was provided by AKER SEAFOODS ANTATARCTIC, S. A. Lysaker (Norway); Squid meal was provided by MAX NOLLERT, Utrecht (Netherlands).

TABLE 2. Formulation and apparent digestibility coefficients of the experimental diets.

		Plant protein ingredients						Animal protein ingredients				
Test ingredients (g/kg)	Control	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal
Fava bean meal		300										
Camilina meal			300									
Soybean meal				300								
Pea meal					300							
Sunflower meal						300						
Wheat meal	240	168	168	168	168	168	168	168	168	168	168	168
Wheat gluten							300					
Defatted krill meal								300				
Fish meal	665	465.5	465.5	465.5	465.5	465.5	465.5	465.5	765.5	465.5	465.5	465.5
Krill meal										300		

Poultry meal											300	
Squid meal												300
Fish oil	75	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Multivitamin and mineral mix	20	14	14	14	14	14	14	14	14	14	14	14
Crude Protein (% w.w.)	46.2	37.4	41.3	40.3	35.8	37.4	55.2	56.0	53.0	45.4	51.3	49.5
Crude Lipids (% w.w.)	12.2	9.0	10.1	8.8	8.7	9.0	9.0	13.0	13.9	13.8	14.8	10.9
AIA (*g kg* $^{-1}$ dry matter)	2.2	2.2	3.1	3.1	2.8	1.6	1.8	2.0	3.5	3.4	3.6	3.0
Dry matter ADC (%)	84.8	72.1	76.4	75.8	76.5	78.9	84.1	78.6	83.9	76.6	80.2	81.0
Protein ADC (%)	96.9	85.8	83.6	90.4	88.5	90.2	94.5	93.5	97.5	90.0	93.8	96.1
Lipid ADC (%)	98.5	96.2	97.5	94.9	96.1	97.2	98.2	96.6	98.2	96.8	98.1	96.7

522 TABLE 3. Amino acid composition of ingredients (expressed as *g kg* $^{-1}$ of dry matter basis, dm).

	Plant protein ingredients							Animal protein ingredients				
	Fava bean meal	Camilina meal	soybean meal	Pea meal	Sunflower meal	Wheat meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal
EAA[a] *(g kg* $^{-1}$*)*												
Arginine	17.7	36.7	32.3	15.2	27.7	4.6	25.7	53.9	60.5	42.2	37.2	63.3
Histidine	6.6	10.3	12.5	5.0	8.4	2.4	14.5	13.0	29.1	12.8	10.8	12.0
Isoleucine	9.2	15.1	20.5	8.5	14.7	3.6	30.1	34.2	32.2	32.5	10.1	15.2
Leucine	18.1	28.6	37.2	15.4	24.7	6.7	57.9	57.5	55.5	47.4	26.2	32.6
Lysine	17.0	17.9	30.5	16.6	13.7	3.2	12.1	58.0	56.9	38.4	25.6	33.8
Methionine	2.8	3.2	8.1	3.1	1.8	6.3	8.8	17.5	20.2	16.8	7.5	8.4
Phenylalanine	9.8	19.1	23.0	9.6	16.2	4.5	43.1	33.2	30.8	30.3	15.8	15.5
Threonine	8.3	16.9	17.5	7.5	13.0	3.2	19.5	31.1	34.2	27.8	16.8	18.0
Valine	10.1	22.4	20.3	9.2	18.0	4.7	32.6	35.6	37.6	31.7	17.6	23.6

NEAA[b] (g kg $^{-1}$)												
Alanine	9.8	18.8	19.0	8.3	14.8	3.5	20.0	41.4	45.8	33.1	36.8	52.6
Aspartate	25.9	39.4	57.7	23.6	43.6	5.4	22.3	81.0	65.1	60.3	50.8	46.0
Cystine	2.1	4.5	4.1	2.0	2.9	7.1	11.2	3.5	6.6	3.8	7.3	35.8
Glutamine	41.3	76.8	94.1	36.7	74.9	28.9	319.8	110.5	92.2	75.2	69.9	102.6
Glycine	10.2	23.3	18.6	8.4	20.2	4.7	24.5	30.6	48.3	27.8	75.4	106.9
Proline	8.9	22.7	21.7	7.4	16.0	9.4	108.2	23.8	29.7	23.0	42.7	53.6
Serine	12.1	17.3	24.2	9.6	15.4	4.9	36.7	29.1	29.9	24.8	22.8	22.9
Tyrosine	4.2	9.7	12.4	3.5	6.7	1.3	22.9	29.3	23.0	27.3	11.2	11.8
Ratio EAA/NEAA	0.87	0.80	0.80	0.91	0.71	0.60	0.43	0.96	1.05	1.02	0.53	0.51

[a]EAA: Essential amino acids.

[b]NEAA: Non-essential amino acids.

TABLE 4. Apparent digestibility coefficients (ADCs) for dry matter, crude protein and crude lipid in test ingredient consumed by yellowtail.

	Plant protein ingredients						*Animal protein ingredients*					
ADC (%)	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal	SEM[a]
Dry matter	42.2^{d}	58.8^{c}	54.9^{c}	57.2^{c}	65.7^{c}	82.2^{ab}	68.0^{c}	87.2^{a}	57.7^{c}	70.2^{bc}	70.1^{bc}	3.4
Protein	54.9^{d}	48.5^{e}	73.2^{c}	63.0^{d}	62.9^{d}	87^{b}	86.7^{b}	91.3^{a}	75.8^{c}	85.0^{b}	85.2^{b}	1.3
Lipid	90.2^{ab}	91.3^{ab}	91.1^{ab}	83.5^{c}	94.0^{a}	90.8^{ab}	88.4^{b}	94.2^{a}	92.0^{ab}	93.9^{a}	88.3^{b}	1.2

[a] Standard error of the mean. Means of quintuplicate groups. Data Log Different letters in the same line denote significant differences (P < 0. 05). Newman–Keuls test.

TABLE 5. Apparent digestibility coefficients (ADCs) of amino acids in test ingredient consumed by yellowtail.

	Plant protein ingredients						*Animal protein ingredients*					
ADC (%)	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal	SEM^{a}
EAA^{b}												
Arginine	41.0^{g}	37.7^{h}	68.8^{f}	79.2^{d}	72.3^{e}	84.1^{c}	89.1^{a}	90.0^{a}	86.6^{b}	82.7^{c}	90.3^{a}	0.8
Histidine	47.4^{e}	42.9^{ef}	85.4^{b}	77.0^{cd}	81.0^{bc}	77.6^{cd}	90.2^{a}	90.3^{a}	81.8^{bc}	91.5^{a}	92.1^{a}	1.5
Isoleucine	53.6^{e}	39.7^{f}	68.8^{d}	64.4^{d}	69.3^{d}	78.0^{c}	89.0^{a}	91.0^{a}	80.7^{bc}	85.8^{ab}	85.5^{ab}	1.5
Leucine	51.5^{f}	46.7^{g}	72.6^{d}	63.4^{e}	70.6^{d}	79.4^{c}	85.4b	87.6^{ab}	77.0^{c}	91.4^{a}	90.9^{a}	1.2
Lysine	64.2^{d}	41.2^{e}	79.0^{bc}	69.9^{cd}	78.7^{bc}	70.1^{cd}	91.6^{ab}	93.7^{a}	78.1^{bc}	83.8^{ab}	90.7^{ab}	2.5
Methionine	61.4^{f}	65.5^{e}	47.4^{g}	66.5^{e}	63.3^{ef}	93.5^{ab}	86.6^{c}	92.0^{b}	72.3^{d}	95.4^{a}	88.2c	0.9
Phenylalanine	57.1^{f}	57.1^{f}	65.1^{e}	78.1^{d}	81.0^{d}	85.2^{c}	87.0^{bc}	93.6^{a}	80.7^{d}	89.3^{b}	80.4^{d}	1.0
Threonine	45.8^{d}	50.2^{d}	62.6^{c}	64.1^{c}	67.8^{c}	79.7^{b}	69.2^{c}	90.0^{a}	84.1^{ab}	90.8^{a}	93.1^{a}	2.3
Valine	53.1^{c}	55.2^{c}	69.7^{bc}	63.2^{bc}	70.7^{bc}	76.3^{ab}	86.5^{a}	92.0^{a}	88.0^{a}	90.4^{a}	90.8^{a}	4.3

NEAA^{c}												
Alanine	61.6^{e}	49.2^{f}	69.4^{d}	59.9^{e}	73.3^{cd}	79.4^{bc}	85.2^{ab}	91.7^{a}	79.8^{bc}	91.2^{a}	91.3^{a}	2.2
Aspartate	40.4^{d}	63.5^{c}	73.6^{bc}	72.7^{c}	70^{c}	80.0^{ab}	84.7^{a}	91.7^{a}	82.7^{a}	88.8^{a}	86.7^{a}	2.6
Cystine	57.7^{c}	60.6^{c}	35.6^{d}	75.2^{abc}	62.9^{bc}	95.3^{a}	82.5^{abc}	91.0^{ab}	67.6^{bc}	75.7^{abc}	94.8^{a}	6.4
Glutamine	62.6^{c}	45.9^{d}	84.6^{a}	64.3^{c}	74.2^{b}	85.1^{a}	88.0^{a}	93.3^{a}	65.9^{c}	84.9^{a}	87.9^{a}	2.7
Glycine	48.6^{c}	42.2^{d}	68.7^{b}	69.0^{b}	69.3^{b}	88.9^{a}	88.4^{a}	92.3^{a}	71.8^{b}	88.8^{a}	95.1^{a}	2.0
Proline	55.5^{d}	46.6^{e}	66.2^{c}	73.8^{bc}	68.3^{c}	88.4^{a}	82.6^{ab}	90.3^{a}	83.4^{ab}	85.3^{ab}	87.9^{a}	3.0
Serine	55.1^{e}	49.9^{f}	69.2^{c}	71.1^{c}	64.5^{d}	81.0^{b}	90.1^{a}	86.3^{ab}	78.9^{b}	83.6^{ab}	91.0^{a}	2.2
Tyrosine	60.8^{f}	53.9^{g}	66.4^{e}	70.5^{d}	75.3^{c}	85.1^{b}	88.4^{a}	89.3^{a}	84.7^{bc}	84.4^{a}	84.7^{a}	0.8

[a] Standard error of the mean. Means of quintuplicate groups. Different letters in the same line denote significant differences ($P < 0.05$). Newman–Keuls test

[b] EAA: Essential amino acids.

[c] NEAA: Non-essential amino acids.

FIGURE 1. Amino acid ratios (%) for essential digestible amino acids in vegetable ingredients (A) and in animal ingredients (B). EAA of whole body of Seriola dumerili were used to estimate this index as $AA_{reference}$ (expressed in g/100 g of protein): Arg (7.14), His (2.65), Iso (4.76), Leu (7.50), Lys (8.14), Met (2.37), Phe (4.32), Thr (3.85), Val (5.60).

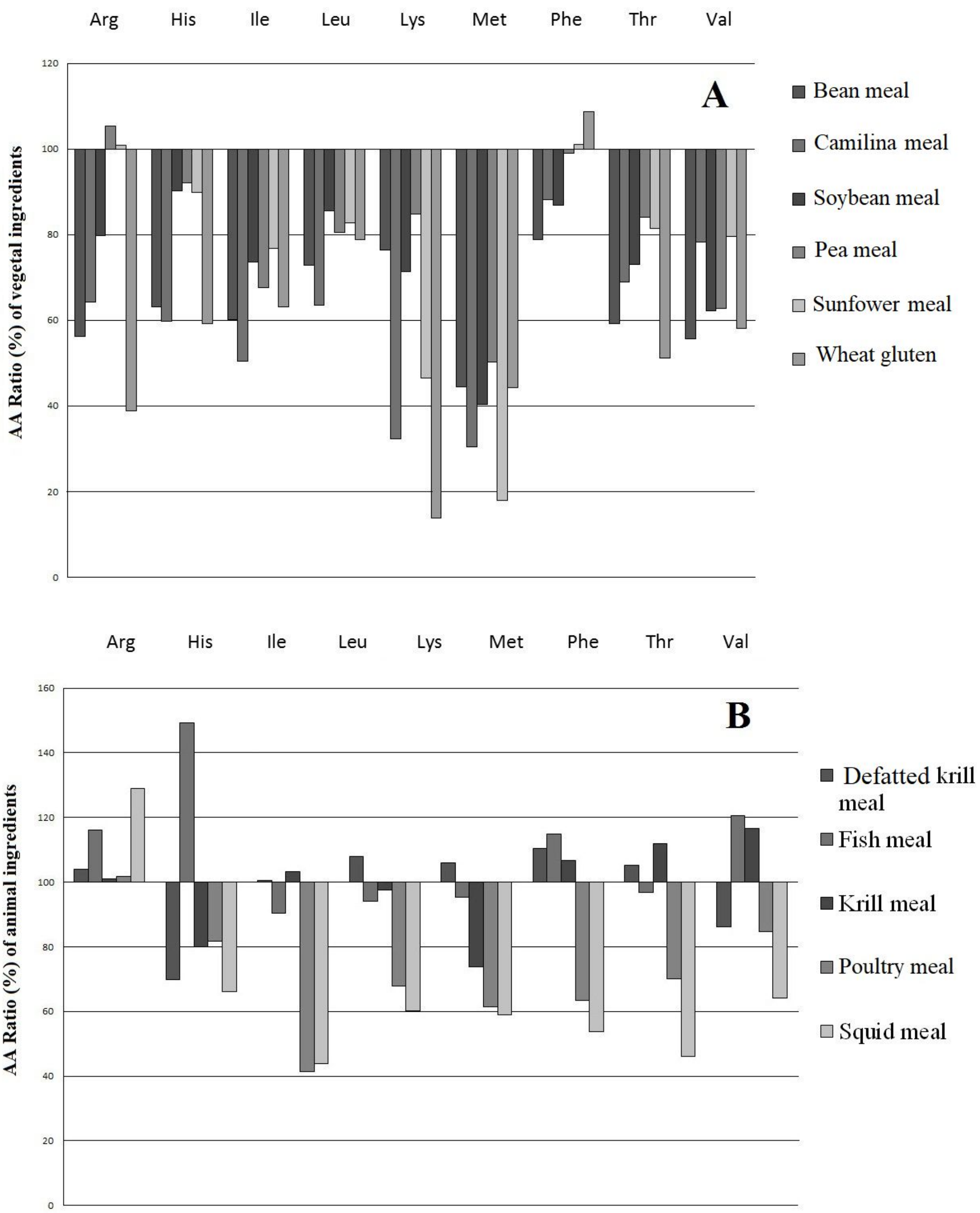
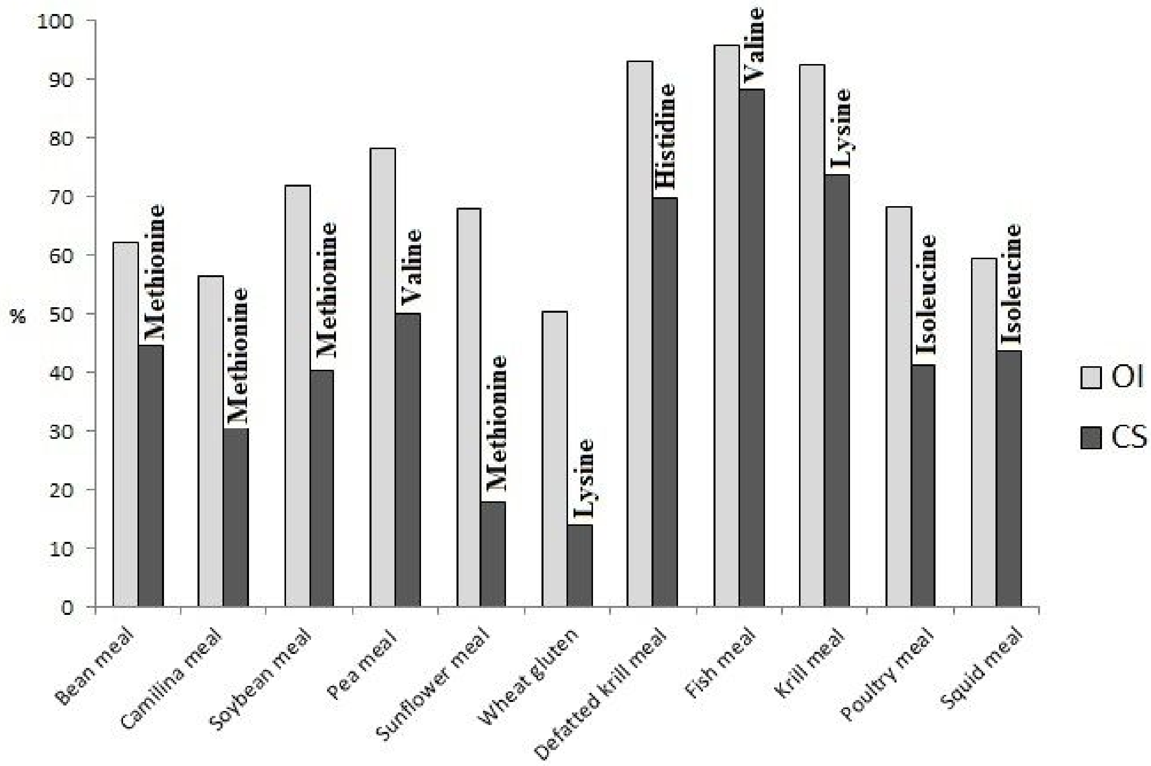

Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Val
A
AA Ratio (%) of vegetal ingredients
0
20
40
60
80
100
120
Bean meal
Camilina meal
Soybean meal
Pea meal
Sunfower meal
Wheat gluten
Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Val
B
AA Ratio (%) of animal ingredients
0
20
40
60
80
100
120
140
160
Defatted krill meal
Fish meal
Krill meal
Poultry meal
Squid meal

FIGURE 2. Oser´s index (OI), Chemical Score (CS) and limiting amino acid in test ingredients for digestible amino acids.


%
100
90
80
70
60
50
40
30
20
10
0
Methionine
Methionine
Methionine
Valine
Methionine
Lysine
Histidine
Valine
Lysine
Isoleucine
Isoleucine
Bean meal
Camilina meal
Soybean meal
Pea meal
Sunflower meal
Wheat gluten
Defatted krill meal
Fish meal
Krill meal
Poultry meal
Squid meal
OI
CS

Apparent Digestibility and Protein Quality Evaluation of Selected Feed Ingredients in <u>Seriola</u> <u>dumerili.</u>

Ana Tomás-Vidal, Raquel Monge-Ortiz, Miguel Jover-Cerdá, Silvia Martínez-Llorens.

Research Group of Aquaculture and Biodiversity. Institute of Animal Science and Technology. Universitat Politècnica de València. Camino de Vera, 14. 46071-Valencia, Spain.

Corresponding author: Raquel Monge-Ortiz, Universitat Politècnica València. Camino de Vera, 14. 46071-Valencia (Spain). Tel.: 34-96-3879752; Fax: 34-96-3877439. E-mail: silmarll@dca.upv.es.

Abstract

The apparent digestibility coefficients (ADC) of dry matter, crude protein, crude lipid, and amino acids in fish, krill, squid, meat, defatted krill, soybean, wheat gluten, wheat, camilina, pea, sunflower and fava bean meals were determined for juvenile Seriola dumerili. The results showed that the ADC of dry matter for yellowtail ranged from 57.7 to 87.2% for animal ingredients, and from 42.2 to 82.2% for plant ingredients. ADC of protein exceeding 90% were observed in fishmeal, while camilina meal and fava bean meal presented the lowest values. Pea meal presented the lowest lipid ADC (83.5%). The availabilities were generally higher in animal ingredients than those in vegetal ones. Except camilina and fava bean meal, the other ingredients appear to be favorable for Seriola dumerili diets, especially the ones from animal sources. The lower case chemical score values (minimum value from AARs) were obtained in some vegetal ingredients (14 to 18%), while the highest ones, appeared in marine ingredients (69 to 88%). According to Oser's Index, the most balanced protein for yellowtail with regard to essential amino acids was in krill, defatted krill, and fishmeal (92 to 96%). So, animal sources are suitable as protein ingredients, but they could be enhanced through some essential amino acid supplementation.

In the last two decades the European aquaculture sector has shown an increasing interest and demand in fast-growing species (Mazzola et al. 2000) such as Mediterranean yellowtail (Seriola dumerili), as they can improve the competitiveness of aquaculture companies.

The most expensive component in feed formulation in aquaculture is protein. This is especially important considering that Seriola species require high protein dietary levels (450550 g kg^{-1}) for maximum growth (Masumoto et al. 1998; Jover et al. 1999; Tomás et al. 2008), and the dietary substitution of proteins by other nutrients—such as lipids or carbohydrates—is not the best solution for the formulation of feeds for S. dumerili (Jover et al. 1999). In this sense, fishmeal is an ideal protein source for carnivorous fish, because it contains a balanced amino acid composition, high content of phosphorous and minerals and high digestibility coefficients. However, its high cost motivated its reduction in aqua feeds, while maintaining the same protein levels from alternative protein sources.

Currently, in order to avoid the dependence on fishmeal, aquaculture diets should contain high amounts of alternative proteins that are more widely available and that have lower and more stable market prices than this marine resource. Alternative proteins include a large range of plant or animal proteins with competitive prices (Martínez-Llorens, Vidal, & Cerdá, 2012). Despite these economic advantages, previous results on Mediterranean yellowtail show that alternative proteins cannot totally replace fishmeal when they are used as the sole ingredient. When soybean is included to replace over 30% of the fishmeal, it resulted in poor growth and showed low nutrient efficiency (Tomás et al. 2005). Total fishmeal substitution presents some limitations in fish diets, such as the anti-nutrients that are particularly contained in vegetable proteins (Krogdahl et al. 2010), causing a protease enzyme activity decrease and, therefore, low protein digestibility (Monge-Ortiz et al. 2016). Another issue related to the use of alternative protein sources is the inadequate profile of amino acids (Cerezo et al. 2012), which results in poor fish growth and protein deficiencies. This forces the use of supplemental diets with synthetic amino acids, which increases the feed price. The best growth and nitrogen retention results when combination of protein sources are used, that include a combination of aminoacids in order to complement one source with limiting ones, that is the case of Sparus aurata (Kissil & Lupatsch, 2004; Sánchez-Lozano et al., 2009), and evidence suggests that for other species too, like Salmo salar (Espe, 2006) or Seriola dumerili (Monge-Ortiz et al, 2017). For this reason, the combination of animal proteins (such as defatted krill and poultry meal) with plant proteins in fish diets could be beneficial to palliate the deficiencies in amino acids of some protein sources, thus reducing the anti-nutrient compounds and avoiding supplementation with synthetic aminoacids (AAs), lack of palatability (Oliva-Teles 2012) and 'green liver' (Takagi et al. 2005). The adequate proportion to simulate the ideal amino acid profile in diets for the Mediterranean yellowtail will require studies on the digestibility and the protein quality of these ingredients.

Therefore, the evaluation of feed ingredients is crucial to nutritional research and feed development for aquaculture species (Glencross et al. 2007). The alternative protein sources and their inclusion levels need to be optimized in fish diets to make aquaculture production more efficient and cost-effective. Digestibility trials are essential to shed light on the potential of these ingredients for fish before their inclusion in diet formulations.

There are numerous factors involved in the digestibility performance of common feed ingredients for fish. Digestibility coefficients are also different for the same ingredient depending on the species under study (Davies et al. 2009). Apparent digestibility coefficient (ADC) values of feed ingredients are normally the result of differences in species, changes in the harvest/catch season of the raw materials and conditions under which they were processed. For that reason, studies on various marine fish species have been conducted (Lupatsch et al. 1997; Tibbets et al.2004; Booth et al. 2005; Tibbets et al. 2006; Davies et al. 2009) in order to assess the quality value of different ingredients.

Taking into account the relevance of digestibility estimation for diet formulation, the aim of this present study was to compare the apparent nutrient digestibility and protein quality of 12 different commercially available ingredients for Seriola dumerili.

Materials and Methods

Ingredients and Experimental Diets

Yellowtail (S. dumerili) juveniles were caught in the Mediterranean and transferred to the facilities of the Polytechnic University of Valencia, Spain.

Feed ingredients were tested for apparent protein digestibility, availability of digestible amino acids and protein quality in fishmeal, krill meal, squid meal, poultry meal (international feed number 5-03798), defatted krill meal (a product obtained by removing the fat with ethanol), soybean (Glycine max), wheat gluten (Triticum spp), camilina (Camelina sativa) (product given to our facilities by local farmers), pea (Pisum sativum), sunflower (Helianthus annuus) and fava bean (Vicia faba) meals (Table 1 and 3). Test diets were prepared by mixing a basal diet with one of the test ingredients (Table 2) in a ratio of 7 to 3, as described by Cho and Slinger (1979).

The different ingredients of the diets were weighed individually and mixed to form homogeneous dough, and were prepared by cooking-extrusion processing with a a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The conditions under which they were processed are as follows: a screw speed of 100 rpm, a temperature of 110 C, and a pressure of 40-50 atm. The experimental diets were assayed in quintuplicate. The fish were handfed once a day (8.00h), and fecal collection occurred 8 h later (16:00h). The pellet size used was 3mm during the experiment, increasing to 4mm in the final part of the experiment. The pellets were slowly distributed to give all fish time to eat. The uneaten diet was collected when fish had finished feeding.

Digestibility Assay

Fifteen yellowtails (175±2.8 g initial mean live weight) were randomly distributed in five experimental tanks (190 L fiberglass tanks, 88cm length, 62 cm diameter and 188 cm depth) of a marine semi-closed recirculating system designed on the basis of the Guelph system (the fecal material being collected in a settling column). Each tank was loaded with three fish. The velocity of the water flow was adjusted to minimize settling of the feces in the drainpipe and maximize feces recovery in the settling column.

There was a three-month adaptation period (adaptation to dry diets was a very long process, in which yellowtail were first fed anchovy and, later, a semi-moist diet—a mix of anchovy and fishmeal—before being fed the control diet). Once the fish had become accustomed to both the tanks and the dietary regime, collection of feces was initiated.

The fish were fed one meal a day at 10.00 h. The feed was offered only as long as the fish were actively feeding in order to avoid wastage. One hour after the meal, the drainpipe and the settling column were brushed out to remove uneaten feed particles and feces from the system to avoid mixing feed particles and feces in the recollection.

Feces were collected next day from the base of the settling column into a plastic container by gravity at 8.00 h. After feces collection, the fish were fed again at 10.00h, waiting two hours between feces recolection and feeding to avoid stress.

The water temperature was maintained around 20 C (21.5 ± 2.4 C) during the experimental period by a water conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were equipped with aeration and the level of dissolved oxygen was 6.6 ± 1.3 mg L^{-1}. Water salinity was 31.5 ± 4.1 g/L, pH 7.3 ± 0.4, NO_3^- (25–150 mg/L), and NO_2^- (0.05–0.5 mg/L), and the ammonium value was undetectable The photoperiod was maintained at 12-h light and 12-h dark by means of artificial daylight simulation. All these parameters were measured daily from Monday to Saturday. This parameters are optimal for S. dumerili in recirculation system, and are similar that in other Seriola experiments (Jover, 1999; Monge-Ortiz, 2018a, 2018b)

Over a six-month period, the fish groups were fed the experimental diets and feces were sampled from each tank. One tank was fed with the control diet over six months to determine the possible variations in digestibility throughout the experimental period. Experimental periods were 14 days long for each diet and each replicate. The last three days of the two-week period fish fasted in order to avoid mixing feces between diets. Feces were removed immediately to determine their amount and have enough for analysis following which fish continued to be fed the same diet for another week. The switching of dietary treatments was as follows: diets with fava bean, camilina, soybean and pea meal were tested from Week 1 to Week 2, from 7 to 8, from

13 to 14 and from 19 to 20 respectively; diets with sunflower, wheat, wheat gluten and defatted krill meal were assayed from Week 3 to Week 4, from 9 to 10, from 15 to 16, from 21 to 22 and from 25 to 26, respectively; and diets containing fish, krill, meat and squid meal were tested from Week 5 to 6, from 11 to 12, from 17 to 18, from 23 to 24, and from 27 to 28, respectively of the experimental trial.

The fecal material collected was dried to a constant weight in an oven at 60 C for 48 h prior to analysis and stored in airtight plastic containers until nutrient component and inert marker (acid insoluble ash, AIA) analysis. The AIA content of feeds and feces was determined by the method of Atkinson et al. (1984), to calculate the apparent digestibility coefficient, (ADC).

Apparent digestibility coefficients (ADC_{diet}, %) of each specific nutritional variable (protein (ADCp, %), lipid (ADCl, %), and AA) of the diets were calculated using the following formulae:

$$ADC_{diet} = 1 - \left(\frac{Marker_{diet} \times Nutrient_{faeces}}{Marker_{faeces} \times Nutrient_{diet}} \right)$$

In this equation, the terms $Marker_{diet}$ (g kg^{-1}) and $Marker_{faeces}$ (g kg^{-1}) represent the marker content of the diet and the feces, respectively, and $Nutrient_{diet}$ (g kg^{-1}) and $Nutrient_{faeces}$ (g kg^{-1}) are the nutritional parameters of concern (e.g. protein or energy) in the diet and the feces, respectively.

The apparent digestibility coefficients of dry matter, protein, lipid and amino acids for the test ingredients (30% replacement level) were calculated as follows:

$$ADCN_{ing} = \frac{[(a+b) \times ADCN_{test} - a \times ADCN_{ref}]}{b}$$

In the equation above, "*a*" is nutrient contribution of reference diet to nutrient content of test diet, "*b*" is nutrient contribution of test ingredients to nutrient content of combined diet, *"a + b"* is the level of nutrient in combined diet (%); $ADCN_{test}$ (%) = apparent digestibility coefficient of a nutrient in combined diet and $ADCN_{ref}$ (%) = apparent digestibility coefficient of a nutrient in reference diet (Forster 1999).

Chemical Analysis

Chemical analysis of the dietary ingredients was performed prior to diet formulation (Table 1). Diets and their ingredients were analyzed according to AOAC (1995) procedures: dry matter (105 C to constant weight), ash (incinerated at 550 C to constant weight), crude protein (N x 6.25), assessed by the Kjeldahl method after acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden), and crude lipid, extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator).

Following the aforementioned method by Bosch et al. (2006), the amino acid content of the fish carcasses and the diets were established using a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) with two pumps (Model 515, Waters), an auto-sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. Before hydrolysation, alpha aminobutyric acid was added as an internal standard. The derivatization of amino acids was made by using AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteic acid after oxidation with performic acid. Amino acids were separated with a C-17 reverse-phase column Waters Acc. Tag (150 mm x 3.9 mm) and subsequently transformed to Met and Cys.

Protein Quality

The values of the following indices were calculated:

- Amino acid ratio (AAR, %) = $(AA_{sample})/(AA_{reference})*100$, where AA_{sample} and $AA_{reference}$ are the digestible amino acid contents in the test sample and whole S. dumerili (Fig. 1), the one taken as reference (average values of 20 samples of fish weighing 100 to 500g).
- Chemical score (CS, %): minimum value from AARs calculated for digestible essentials amino acids (EAA: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val).
- Limiting amino acid: the digestible amino acid corresponding to CS in the test sample.
- Oser's Index (OI, %): used as index of nutritional quality and achieved as the geometric mean ratio of digestible amino acids in samples of the ones detected in Seriola dumerili, which were taken as a reference according to the following formula:

$$OI\ (\%) = (10^{\,(1/n*(\log(AAR_1)+\log(AAR_2)\ldots+\log(AAR_n)))}),$$

in which AAR_1, AAR_2,…AAR_n are the ratios of digestible essential amino acids, and "n" is the number of detected digestible essential amino acids. When the ratio lay above 100, this was taken as reference (Oser 1951).

Statistical Analysis

Digestibility coefficients were evaluated using one-way analysis of variance (ANOVA). ACD data were $\log_{10}$ transformed to meet the assumptions of statistical tests (normality, linearity and homosdasticity). The Newman-Keuls test was used to assess specific differences among diets at a level of P<0.05 (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

Results

Proximate Composition and Amino Acid Content

The proximate composition of the 12 test feed ingredients are reported in Table 1. The greatest protein values were observed in animal meals; fishmeal (713.2 g kg^{-}1) wheat gluten (810 g kg-1), squid meal (718.8 g kg-1) and extracted krill meal (723.1 g kg-1), while the lowest were found in vegetable meas like pea (215.9 g kg-1), fava bean (236.6 g kg-1) and sunflower meal (291.3 g kg-1).

Vegetal meals presented the poorest amino acid balance, with serious deficiencies in EAA, such as in arginine, threonine, lysine and methionine (Table 3). Arginine, lysine and leucine were the predominant essential amino acids in animal ingredients, of which fish and defatted krill meal had a relatively similar content. The principal non-essential amino acids present in animal and vegetal meals were aspartate and glutamine.

Digestibility

The ADCs of dry matter for yellowtail ranged from 71.88 to 89.53% for animal ingredients, and from 65.89 to 79.98% for plant products. The higer protein ADCs appeared in fishmeal (91.3%), wheat gluten (87%), defatted krill meal (86.7%), squid meal (85.2%) and poultry meal (85.0%); mid-range in krill meal (75.8%), soybean meal (73.2%) and sunflower and pea meal (63%); and low in camilina (48.5%) and fava bean meal (54.9%).

Apparent digestibility coefficients of lipids in all the treatments were above 88%, except in the case of pea meal (83.5%).

For the animal ingredientss, the availabilities of amino acids (Table 5) in fish, squid, meat and defatted krill meal were generally higher than those in krill meal. Among all the plant ingredients, the availabilities of amino acids in wheat gluten were the highest, followed by soybean-, pea- and sunflower meal. Camilina and bean meal presented the lowest AAs ADC.

Protein Quality Evaluation

Defatted krill meal was the most balanced digestible protein ingredient (Figs. 1, 2). It was only deficient in histidine, threonine and valine (AAR: 70 (CS), 86 and 87%). Fishmeal was slightly deficient in isoleucine, leucine, lysine, phenylalanine and valine (AAR: 90, 94, 95, 97 and 88 %, respectively). Krill meal was deficient in histidine, lysine and valine (AAR inferior to 90%). Squid and poultry meals presented 60 to 85% ARRs in all the digestible amino acids, except for arginine, which was the most balanced of them all, while isoleucine was the least balanced (AAR<50%). With respect to the vegetal ingredients, wheat gluten showed the lowest CS (13.98% for lysine), followed by sunflower, which was found to be deficient in Met (CS: 17.97%), and camilina meal (poor in methionine, CS 30.8%). Pea meal proved to be a balanced amino acid ingredient (its CS, Met, was above 50%).

Oser's Index (OI, Fig. 2) calculated for protein meals was positively related with CS, presenting the lowest values of OI those presented the lowest CS, such in the case of wheat meal and sunflower meal (50.41% OI, 13.89% CS and 67.96% OI, 17.97% CS respectively). On the contrary, defatted krill and fishmeal (92 to 96 %) showed the most balanced protein with regard to essential amino acids, whereas all the other ingredients presented lower levels (50-78%).

Discussion

The results obtained in the present study confirmed the very high digestibility of certain animal proteins for carnivorous marine fish (Lupatsch et al., 1997; Tibbets et al. 2006). ADCs of animal proteins were close to those obtained for fishmeal concerning crude protein digestibility, and that fact is probably the result of their very high protein concentrations. Only krill meal ADCp was lower than the other animal protein sources, probably because of the unusually high amount of fat of the krill meal sample used in this study (and consequent low protein level). This was the main difference between the krill meal and the defatted krill meal, which has a lower fat and a higher protein level, making it a higher quality product for yellowtail diets. Tibbetts (2006) obtained similar results with shrimp, with low ADC, due to the high ash content of the shrimp meal.

Protein ADCs of fishmeal assessed in yellowtail are also similar to the ones encountered in turbot (Psetta maxima), seabass (Dicentrarchus labrax), sea bream (Sparus aurata), Atlantic cod (Gadus morhua), red drum (Sciaenops ocellatus), Australian snapper (Pagrus auratus) and Atlantic halibut (Hippoglossus hippoglossus), at 91 to 96% (Gaylord and Gatlin 1996; Lupatsch et al. 1997; Gomes da Silva and Oliva-Teles 1998; Burel et al. 2000; Peach 2005; Tibbetts et al. 2006; Davies et al. 2009; Booth et al. 2010).

Protein ADCs were high for poultry meal (85%). Animal by-product meals can vary greatly in their proximate composition, depending on several factors (raw material source and freshness, production processes and storage) and, due to its very nature, the values reported for protein ADCs are also highly variable in studies on fish. In many fish species, poultry meal has received positive results in diets (ADCp 85.5% in sea bass, 79 to 80% in sea bream, 78.4% in turbot, and 80% in Atlantic cod), and is regarded as the most effective animal protein (Lupatsch et al. 1997; Tibbetts et al. 2006; Davies et al. 2009).

The ADCp of krill hydrolysate found in Atlantic halibut (Peach 2005) and Atlantic cod (Tibbetts et al. 2006) was nearly 100%, very different indeed to the krill ADCp in yellowtail (76%), but that krill meal was obtained through finely grinding whole krill that had previously been freeze-dried (*Euphausia superba*) and, therefore, the proximate composition was fairly different from the one found in commercially produced krill meals like the one used in the present work. Those discrepancies are explained by the differences in product quality, as in the case of the defatted krill meal (full-fat krill meal) with a higher protein level.

There are numerous studies on digestibility of soybean meal on various fish species. The protein ADC, however, varied a great deal (76 to 98%). Yellowtail is within this range, although on the lower side, i.e. among the species that present a lower soybean protein digestibility. Other authors observed a decrease in protein digestibility (Robaina et al. 1995; Refstie et al. 1999), mainly attributed to the presence of phytate.

Protein ADC was high for wheat gluten meal (87%), but inferior to that reported for other marine fish (100 %), like seabass (Robaina et al. 1999) and Atlantic cod (Tibbetts et al. 2006). The high palatability and digestibility, together with the absence of anti-nutritional factors, make wheat gluten meal, supplemented with some amino acids (lysine, methionine and arginine), a suitable fishmeal replacement candidate in yellowtail diets.

Pea meal had a middle ADCp. This ingredient appears to have some potential for its use in marine fish diets (Sánchez-Lozano et al. 2009, 2011); nonetheless it should be pre-extruded in order to boost the digestibility of non-protein ingredients.

Lipid ADC results indicate that lipids from both animal and plant sources were well digested by *Seriola dumerili*. There were only slight differences in this parameter between soybean meal and most of the raw materials. Several studies have concluded that dietary soybean meal decreases the lipid digestibility in salmonids (Romarheim et al. 2008). This fact must be attributed to the bile acid level, which is reduced by dietary soybean meal (Romarheim et al. 2006; Yamamoto et al. 2007).

Regarding the availability of AAs, it should be noted that the digestibility of each AA within a feed ingredient is variable. This variability, nonetheless, increases in those ingredients with a lower protein ADC (AAs ADC of camilina or fava bean meals were between 38 and 66%). In general, apparent digestibility coefficients of AAs reflect the apparent CP digestibility coefficients of some test ingredients. In some of them, however, there are differences, like in wheat gluten, in which apparent digestibility coefficients of AAs were lower than ADCp. On the contrary, krill meal, pea meal or poultry meal presented higher AA ADCs. Thus, amino acid availabilities cannot always be estimated from the ingredient ADCp. Fishmeal and defatted krill meal are the most balanced digestible amino acid ingredients and have one advantage: they benefit from having a higher protein content and CS than krill. However, the defatted krill meal also has one shortcoming: the need for histidine supplementation. Regarding other animal sources, squid meal was the most balanced amino acid in arginine and poultry meal protein quality was similar to camilina and soybean meal.

Despite the high protein digestibility observed in present work for some protein meals, the biological value of the protein should also be taken into account before diet formulation to include them in fish diets. For example, wheat gluten digestibility resulted very high, however it shows a low OI and the lowest CS (in this case Lysine) and therefore it cannot replace the fishmeal in high dietary levels for Mediterranean yellowtail feeding.

It has been observed in studies performed with sea bream (Lupatsch et al. 1997) that, when using ACDp values obtained for individual ingredients to calculate the ACDp for compounds diets, both values were very similar, and consequently, protein digestibility can be considered to be additive. Therefore, ACDp results obtained in this present experiment, along with the protein quality assessments, should be taken into account for formulating diets for Mediterranean yellowtail. This is a useful tool to simulate the ideal protein in diets because, by combining different ingredients, the inclusion of synthetic amino acids could be avoided or diminished, which would reduce the cost of fish diets and improve protein digestion.

In conclusion, animal sources are evidently suitable as protein ingredients in yellowtail diets, although the protein quality indices present in them could be enhanced through some essential amino acid supplementation. None of the vegetal meals assayed could offer a good nutritional balance on their own and, therefore, they would either need to be supplemented or need to be used together with other raw materials.

Acknowledgments

Project financed by the "Ministerio de Ciencia e Innovación" (reference AGL2011-30547-C03). We would also thank IEO (Instituto Español de Oceanografía), Centro Oceanográfico de Murcia (España) and Dr. Fernando de la Gándara for providing us the fish used in the present study.

Literature Cited

AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists 16th Edition (16th ed.)AOAC Inc., Arlington, Virginia, USA

Atkinson J.L., Hilton J.W. and S.J. Slinger. 1984. Evaluation of acid insoluble ash as an indicator of feed digestibility in rainbow trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences, 41:1384–1386

Booth M.A., Allan G.L. and A.J. Anderson. 2005. Investigation of the nutritional requirements of Australian snapper Pagrus auratus (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research, 36:378–390

Booth M.A., Allan G.L. and I. Pirozzi. 2010. Estimation of digestible protein and energy requirements of yellowtail kingfish Seriola lalandi using a factorial approach. Aquaculture, 307:247-259.

Bosch L., Alegria A. and R. Farré. 2006. Application of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), reagent to the RP-HPLC determination of amino acids in infant foods. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 831:176–178.

Bureau, D.P., Harris, A.M. and Cho, C.Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). Aquaculture, 180: 345-358.

Burel C., Boujard T., Tulli F. and S.J. Kaushik. 2000. Digestibility of extruded peas, extruded lupin and rapeseed meal in rainbow trout (Oncorhynchus mykiss) and turbot (Psetta maxima). Aquaculture, 188:285–298

Cerezo Valverde J., Martínez-Llorens S., Tomás Vidal A., Jover M., Rodríguez C., Estefanell J., Gairín J.I., Domingues P.M., Rodríguez C.J. and B. García. 2012. Amino acids composition and protein quality evaluation of marine species and meals for feed formulations in cephalopods. Aquaculture International, 21:413-433

Cho C. Y. and S.J. Slinger. 1979. Apparent digestibility measurement in feedstuffs for rainbow trout, Pages 239-247 in J. Halver, K. Tiews (Eds.), Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, vol. 2 Heenemann, Berlin.

Davies J., Gouveia A., Laporte J., Woodgate S. and S. Nates. 2009. Nutrient digestibility profile of premium (category III grade) animal protein by-products for temperate marine fish species (European sea bass, gilthead sea bream and turbot). Aquaculture Research, 40:1759-1769.

Forster I. 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquaculture Nutrition, 5:143–145.

Gaylord T.G. and D.M. Gatlin. 1996. Determination of digestibility coefficients of various feedstuffs for red drum (Sciaenops ocellatus). Aquaculture, 139:303–314.

Glencross B.D., Booth M. and G.L. Allan. 2007. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition, 13:17–34.

Gomes da Silva J. and A. Oliva-Teles. 1998. Apparent digestibility coefficients of feedstuffs in seabass (Dicentrarchus labrax) juveniles. Aquatic Living Resources, 11:187–191.

426 **Jover M., García-Gómez A., Tomás A., De la Gándara F. and L. Pérez.** 1999. Growth of the Mediterranean yellowtail (Seriola dumerili) fed extruded diets containing different levels of protein and lipid. Aquaculture, 179:25-33.

429 **Krogdahl Å., Penn M., Thorsen J., Refstie S. and A.M. Bakke.** 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquaculture Research, 41:333–344.

432 **Lupatsch I., Kissil G., Sklan, D. and E. Pfeffer.** 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, Sparus aurata L. Aquaculture Nutrition, 3:81–89.

435 **Martínez-Llorens, S., Vidal, A. T. and M.J. Cerdá.** 2012. A new tool for determining the optimum fish meal and vegetable meals in diets for maximizing the economic profitability of gilthead sea bream (Sparus aurata, L.) feeding. Aquaculture Research, 43:1697–1709.

439 **Masumoto T., Itoh Y., Ruchimat T., Hosokawa H. and S. Shimeno.** 1998. Dietary amino acids budget for juvenile yellowtail (Seriola quinqueradiata). Bulletin of Marine Science and Fisheries, 18:33-37.

442 **Mazzola, A., Favaloro, E. and G.L. Sarà.** 2000. Cultivation of the Mediterranean amberjack, Seriola dumerili (Risso, 1810), in submerged cages in the Western Mediterranean Sea. Aquaculture, 181:257-268.

445 **Monge-Ortiz R., Martínez-Llorens S., Márquez L., Moyano F.J., Jover-Cerdá M. and A. Tomás-Vidal.** 2016. Potential use of high levels of vegetal proteins in diets for market-sized gilthead sea bream (Sparus aurata). Archives of Animal Nutrition, 70 http://dx.doi.org/10.1080/1745039X.2016.1141743

Monge-Ortiz R., Tomás-Vidal A., Rodriguez-Barreto D., Martínez-Llorens S., Pérez J.A., Jover-Cerdá M., Lorenzo A. 2018. Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (Seriola dumerili) juveniles: Effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. Aquculture Nutrition. 24:605-615.

Monge-Ortiz R., Tomás-Vidal A., Gallardo-Álvarez F.J., Estruch G., Godoy-Olmos S., Jover-Cerdá M., Martínez-Llorens S. 2018. Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for Seriola dumerili: Effects on performance and nutrient efficiency. Aquaculture Nutrition, 24:1163-1174.

Oliva-Teles A. 2012. Nutrition and health of aquaculture fish. Journal of Fish Diseases, 35:83–108.

Oser B.L. 1951. Methods for integrating essential amino acid content in the nutritional evaluation of protein. Journal of the American Dietetic Association, 27:396–402.

Peach R.W. 2005. Protein and energy utilization by juvenile Atlantic halibut (Hippoglossus hippoglossus). M.Sc. Thesis. Dalhousie University, Halifax/Nova Scotia Agricultural College, Truro, Nova Scotia.

Refstie S., Svihus B., Shearer K.D. and Storebakken, T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. Animal Feed Science and Technology, 79:331–345.

Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D. and H. Fernández-Palacios. 1995. Soybean and lupin meals as protein sources in diets for gilthead sea bream (Sparus aurata): nutritional and histological implications. Aquaculture, 130:219–233.

Robaina L., Corraze G., Aguirre P., Blanc D., Melcion J.P. and S. Kaushik. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (Dicentrarchus labrax) fed pelleted or extruded diets with or without wheat gluten. Aquaculture, 179:45-56.

Romarheim O.H., Skrede A., Gao Y., Krogdahl Å., Denstadli V., Lilleeng E. and T. Storebakken. 2006. Comparison of white flakes and toasted soybean meal partly replacing fishmeal as protein source in extruded feed for rainbow trout (Oncorhynchus mykiss). Aquaculture, 256:354–364.

Romarheim O.H., Skrede A., Penn M., Mydland L.T., Krogdahl A. and T. Storebakken. 2008. Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (Oncorhynchus mykiss) fed diets containing defatted soybean meal. Aquaculture, 274:329–338

Sánchez-Lozano N.B., Martínez-Llorens S., Tomás-Vidal A. and M. Jover Cerdá. 2009. Effect of high-level fishmeal replacement by pea and rice concentrate protein on growth, nutrient utilization and fillet quality in gilthead sea bream (Sparus aurata, L.). Aquaculture, 298:83–89.

Sánchez-Lozano N., Martínez-Llorens S., Tomás-Vidal A. and M. Jover-Cerdá. 2011. Amino acid retention of gilthead sea bream (Sparus aurata, L.) fed with pea protein concentrate. Aquaculture Nutrition, 17:e604–e614.

Tibbetts S.M., Lall S.P. and J.E. Milley. 2004. Apparent digestibility of common feed ingredients by juvenile haddock, Melanogrammus aeglefinus. L. Aquaculture Research, 35:643-651.

Tibbetts S.M., Milley J.E. and S.P. Lall. 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, Gadus morhua (Linnaeus, 1758). Aquaculture, 261:1314–1327.

Tomás A., De la Gándara F., García-Gómez A., Pérez, L. and M. Jover. 2005. Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, Seriola dumerili. Aquaculture Nutrition, 11:333–340.

Tomás A., De la Gándara F., García-Gómez A. and M.J. Cerdá. 2008. Effect of the protein/energy ratio on the growth of Mediterranean yellowtail (Seriola dumerili). Aquaculture Research, 39:1141–1148.

Takagi S., Murata H., Goto T., Ichiki T., Munasinghe D.M., Endo M., Matsumoto T., Sakurai A., Hatate H., Yoshida T., Sakai T., Yamashita H., Ukawa M. and T. Kuramoto. 2005. The green liver syndrome is caused by taurine deficiency in Yellowtail, Seriola quinqueradiata fed diets without fishmeal. Aquaculture Science, 53:279-290.

Yamamoto T., Suzuki N., Furuita H., Sugita T., Tanaka N. and T. Goto. 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout Oncorhynchus mykiss. Fisheries Science, 73:123–131

TABLE 1. Proximate composition of ingredients used in the digestibility trial (expressed as *g kg* $^{-1}$ of dry matter basis, dm).

Ingredients[a] *(g kg*$^{-1}$*)*	Dry matter	Crude Protein	Crude lipid	Ash	N-free extract
Plant protein ingredients					
Fava bean meal	890	237	12	33	718
Camilina meal	918	391	45	61	503
Soybean meal	882	499	22	71	408
Pea meal	866	216	10	39	736
Sunflower meal	896	291	15	67	627
Wheat meal	890	116	15	18	851
Wheat gluten	933	810	9	9	173
Animal protein ingredients					
Defatted krill meal	878	723	24	102	151
Fish meal	903	713	93	168	26
Krill meal	888	561	225	104	111
Poultry meal	970	531	153	269	47

Squid meal	928	719	30	110	142

[a] Wheat (Triticum spp), Sunflower (Helianthus annuus L.), pea (Pisum sativum L.), fava bean (Vicia faba L.) and soybean meal (Glycine max L. Merr.) were provided by DESCO, Museros, Valencia (Spain); Defatted krill meal was provided by LUDAN RENEWABLE ENERGY, Valencia (Spain); Wheat gluten and Camilina (Camelina sativa, L. Crantz) were provided by DADELOS AGRÍCOLA, Valencia (Spain); Poultry meal was provided by VALGRA S.A., Beniparrell, Valencia (Spain); Krill meal was provided by AKER SEAFOODS ANTATARCTIC, S. A. Lysaker (Norway); Squid meal was provided by MAX NOLLERT, Utrecht (Netherlands).

TABLE 2. Formulation and apparent digestibility coefficients of the experimental diets.

		Plant protein ingredients							Animal protein ingredients					
Test ingredients (g/kg)	Control	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal
Fava bean meal		300										
Camilina meal			300									
Soybean meal				300								
Pea meal					300							
Sunflower meal						300						
Wheat meal	240	168	168	168	168	168	168	168	168	168	168	168
Wheat gluten							300					
Defatted krill meal								300				
Fish meal	665	465.5	465.5	465.5	465.5	465.5	465.5	465.5	765.5	465.5	465.5	465.5
Krill meal										300		

Poultry meal											300	
Squid meal												300
Fish oil	75	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Multivitamin and mineral mix	20	14	14	14	14	14	14	14	14	14	14	14
Crude Protein (% w.w.)	46.2	37.4	41.3	40.3	35.8	37.4	55.2	56.0	53.0	45.4	51.3	49.5
Crude Lipids (% w.w.)	12.2	9.0	10.1	8.8	8.7	9.0	9.0	13.0	13.9	13.8	14.8	10.9
AIA (*g kg* $^{-1}$ dry matter)	2.2	2.2	3.1	3.1	2.8	1.6	1.8	2.0	3.5	3.4	3.6	3.0
Dry matter ADC (%)	84.8	72.1	76.4	75.8	76.5	78.9	84.1	78.6	83.9	76.6	80.2	81.0
Protein ADC (%)	96.9	85.8	83.6	90.4	88.5	90.2	94.5	93.5	97.5	90.0	93.8	96.1
Lipid ADC (%)	98.5	96.2	97.5	94.9	96.1	97.2	98.2	96.6	98.2	96.8	98.1	96.7

522 TABLE 3. Amino acid composition of ingredients (expressed as *g kg* $^{-1}$ of dry matter basis, dm).

	Plant protein ingredients							Animal protein ingredients				
	Fava bean meal	Camilina meal	soybean meal	Pea meal	Sunflower meal	Wheat meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal
EAA[a] *(g kg* $^{-1}$*)*												
Arginine	17.7	36.7	32.3	15.2	27.7	4.6	25.7	53.9	60.5	42.2	37.2	63.3
Histidine	6.6	10.3	12.5	5.0	8.4	2.4	14.5	13.0	29.1	12.8	10.8	12.0
Isoleucine	9.2	15.1	20.5	8.5	14.7	3.6	30.1	34.2	32.2	32.5	10.1	15.2
Leucine	18.1	28.6	37.2	15.4	24.7	6.7	57.9	57.5	55.5	47.4	26.2	32.6
Lysine	17.0	17.9	30.5	16.6	13.7	3.2	12.1	58.0	56.9	38.4	25.6	33.8
Methionine	2.8	3.2	8.1	3.1	1.8	6.3	8.8	17.5	20.2	16.8	7.5	8.4
Phenylalanine	9.8	19.1	23.0	9.6	16.2	4.5	43.1	33.2	30.8	30.3	15.8	15.5
Threonine	8.3	16.9	17.5	7.5	13.0	3.2	19.5	31.1	34.2	27.8	16.8	18.0

Valine	10.1	22.4	20.3	9.2	18.0	4.7	32.6	35.6	37.6	31.7	17.6	23.6
NEAA[b] (g kg $^{-1}$)												
Alanine	9.8	18.8	19.0	8.3	14.8	3.5	20.0	41.4	45.8	33.1	36.8	52.6
Aspartate	25.9	39.4	57.7	23.6	43.6	5.4	22.3	81.0	65.1	60.3	50.8	46.0
Cystine	2.1	4.5	4.1	2.0	2.9	7.1	11.2	3.5	6.6	3.8	7.3	35.8
Glutamine	41.3	76.8	94.1	36.7	74.9	28.9	319.8	110.5	92.2	75.2	69.9	102.6
Glycine	10.2	23.3	18.6	8.4	20.2	4.7	24.5	30.6	48.3	27.8	75.4	106.9
Proline	8.9	22.7	21.7	7.4	16.0	9.4	108.2	23.8	29.7	23.0	42.7	53.6
Serine	12.1	17.3	24.2	9.6	15.4	4.9	36.7	29.1	29.9	24.8	22.8	22.9
Tyrosine	4.2	9.7	12.4	3.5	6.7	1.3	22.9	29.3	23.0	27.3	11.2	11.8
Ratio EAA/NEAA	0.87	0.80	0.80	0.91	0.71	0.60	0.43	0.96	1.05	1.02	0.53	0.51

[a]EAA: Essential amino acids.

[b]NEAA: Non-essential amino acids.

TABLE 4. Apparent digestibility coefficients (ADCs) for dry matter, crude protein and crude lipid in test ingredient consumed by yellowtail.

	Plant protein ingredients						*Animal protein ingredients*					
ADC (%)	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal	SEM[a]
Dry matter	42.2^{d}	58.8^{c}	54.9^{c}	57.2^{c}	65.7^{c}	82.2^{ab}	68.0^{c}	87.2^{a}	57.7^{c}	70.2^{bc}	70.1^{bc}	3.4
Protein	54.9^{d}	48.5^{e}	73.2^{c}	63.0^{d}	62.9^{d}	87^{b}	86.7^{b}	91.3^{a}	75.8^{c}	85.0^{b}	85.2^{b}	1.3
Lipid	90.2^{ab}	91.3^{ab}	91.1^{ab}	83.5^{c}	94.0^{a}	90.8^{ab}	88.4^{b}	94.2^{a}	92.0^{ab}	93.9^{a}	88.3^{b}	1.2

[a] Standard error of the mean. Means of quintuplicate groups. Data Log Different letters in the same line denote significant differences ($P < 0.05$). Newman–Keuls test.

TABLE 5. Apparent digestibility coefficients (ADCs) of amino acids in test ingredient consumed by yellowtail.

	Plant protein ingredients						*Animal protein ingredients*					
ADC (%)	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Defatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal	SEM[a]
EAA[b]												
Arginine	41.0^{g}	37.7^{h}	68.8^{f}	79.2^{d}	72.3^{e}	84.1^{c}	89.1^{a}	90.0^{a}	86.6^{b}	82.7^{c}	90.3^{a}	0.8
Histidine	47.4^{e}	42.9^{ef}	85.4^{b}	77.0^{cd}	81.0^{bc}	77.6^{cd}	90.2^{a}	90.3^{a}	81.8^{bc}	91.5^{a}	92.1^{a}	1.5
Isoleucine	53.6^{e}	39.7^{f}	68.8^{d}	64.4^{d}	69.3^{d}	78.0^{c}	89.0^{a}	91.0^{a}	80.7^{bc}	85.8^{ab}	85.5^{ab}	1.5
Leucine	51.5^{f}	46.7^{g}	72.6^{d}	63.4^{e}	70.6^{d}	79.4^{c}	85.4b	87.6^{ab}	77.0^{c}	91.4^{a}	90.9^{a}	1.2
Lysine	64.2^{d}	41.2^{e}	79.0^{bc}	69.9^{cd}	78.7^{bc}	70.1^{cd}	91.6^{ab}	93.7^{a}	78.1^{bc}	83.8^{ab}	90.7^{ab}	2.5
Methionine	61.4^{f}	65.5^{e}	47.4^{g}	66.5^{e}	63.3^{ef}	93.5^{ab}	86.6^{c}	92.0^{b}	72.3^{d}	95.4^{a}	88.2c	0.9
Phenylalanine	57.1^{f}	57.1^{f}	65.1^{e}	78.1^{d}	81.0^{d}	85.2^{c}	87.0^{bc}	93.6^{a}	80.7^{d}	89.3^{b}	80.4^{d}	1.0
Threonine	45.8^{d}	50.2^{d}	62.6^{c}	64.1^{c}	67.8^{c}	79.7^{b}	69.2^{c}	90.0^{a}	84.1^{ab}	90.8^{a}	93.1^{a}	2.3

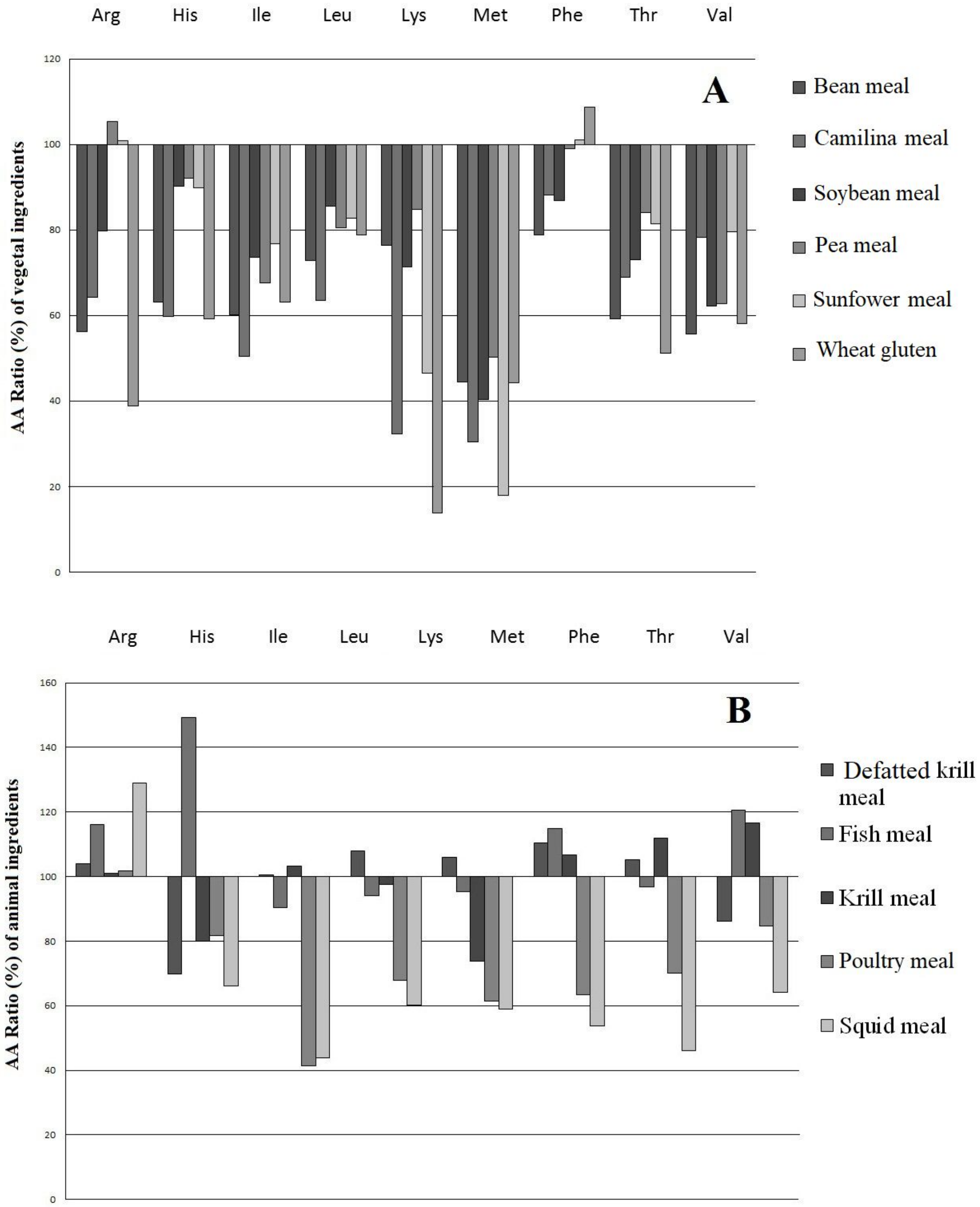
Valine	53.1^{c}	55.2^{c}	69.7^{bc}	63.2^{bc}	70.7^{bc}	76.3^{ab}	86.5^{a}	92.0^{a}	88.0^{a}	90.4^{a}	90.8^{a}	4.3
NEAA[c]												
Alanine	61.6^{e}	49.2^{f}	69.4^{d}	59.9^{e}	73.3^{cd}	79.4^{bc}	85.2^{ab}	91.7^{a}	79.8^{bc}	91.2^{a}	91.3^{a}	2.2
Aspartate	40.4^{d}	63.5^{c}	73.6^{bc}	72.7^{c}	70^{c}	80.0^{ab}	84.7^{a}	91.7^{a}	82.7^{a}	88.8^{a}	86.7^{a}	2.6
Cystine	57.7^{c}	60.6^{c}	35.6^{d}	75.2^{abc}	62.9^{bc}	95.3^{a}	82.5^{abc}	91.0^{ab}	67.6^{bc}	75.7^{abc}	94.8^{a}	6.4
Glutamine	62.6^{c}	45.9^{d}	84.6^{a}	64.3^{c}	74.2^{b}	85.1^{a}	88.0^{a}	93.3^{a}	65.9^{c}	84.9^{a}	87.9^{a}	2.7
Glycine	48.6^{c}	42.2^{d}	68.7^{b}	69.0^{b}	69.3^{b}	88.9^{a}	88.4^{a}	92.3^{a}	71.8^{b}	88.8^{a}	95.1^{a}	2.0
Proline	55.5^{d}	46.6^{e}	66.2^{c}	73.8^{bc}	68.3^{c}	88.4^{a}	82.6^{ab}	90.3^{a}	83.4^{ab}	85.3^{ab}	87.9^{a}	3.0
Serine	55.1^{e}	49.9^{f}	69.2^{c}	71.1^{c}	64.5^{d}	81.0^{b}	90.1^{a}	86.3^{ab}	78.9^{b}	83.6^{ab}	91.0^{a}	2.2
Tyrosine	60.8^{f}	53.9^{g}	66.4^{e}	70.5^{d}	75.3^{c}	85.1^{b}	88.4^{a}	89.3^{a}	84.7^{bc}	84.4^{a}	84.7^{a}	0.8

[a] Standard error of the mean. Means of quintuplicate groups. Different letters in the same line denote significant differences ($P < 0.05$). Newman–Keuls test

[b] EAA: Essential amino acids.

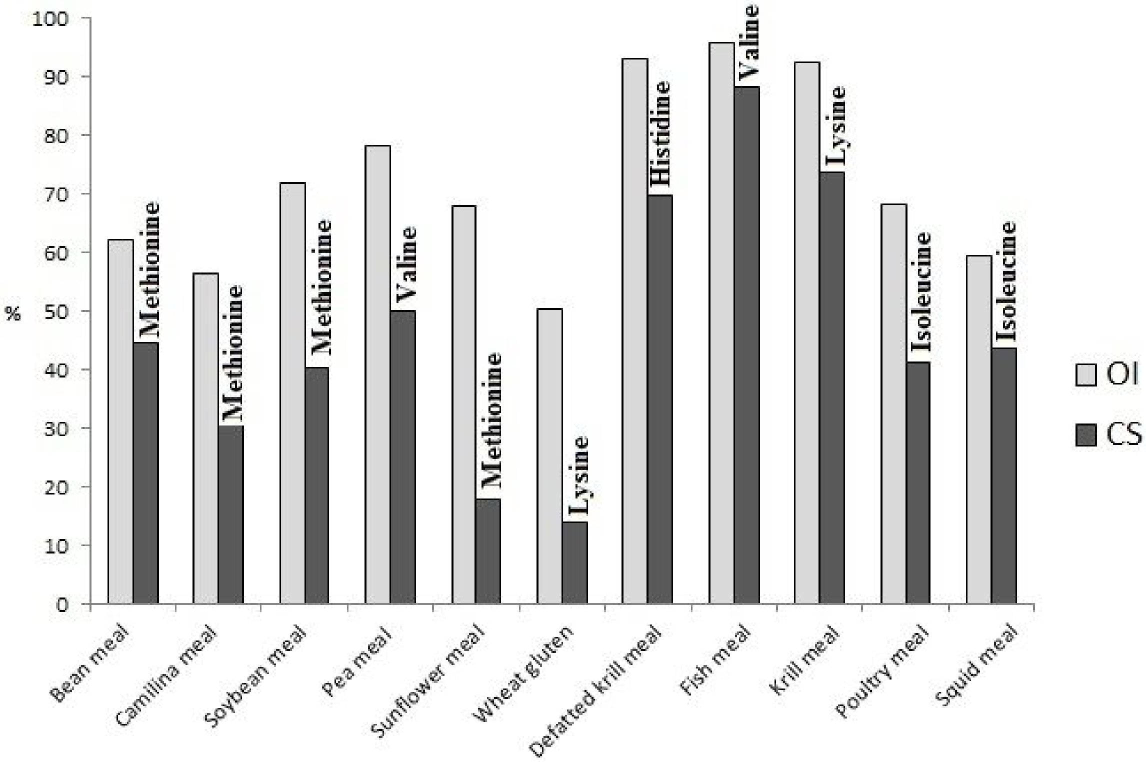
[c] NEAA: Non-essential amino acids.

FIGURE 1. Amino acid ratios (%) for essential digestible amino acids in vegetable ingredients (A) and in animal ingredients (B). EAA of whole body of Seriola dumerili were used to estimate this index as $AA_{reference}$ (expressed in g/100 g of protein): Arg (7.14), His (2.65), Iso (4.76), Leu (7.50), Lys (8.14), Met (2.37), Phe (4.32), Thr (3.85), Val (5.60).


Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Val
A
AA Ratio (%) of vegetal ingredients
Bean meal
Camilina meal
Soybean meal
Pea meal
Sunfower meal
Wheat gluten
B
AA Ratio (%) of animal ingredients
Defatted krill meal
Fish meal
Krill meal
Poultry meal
Squid meal

33

FIGURE 2. Oser´s index (OI), Chemical Score (CS) and limiting amino acid in test ingredients for digestible amino acids.


100
90
80
70
60
50
40
30
20
10
0
%
Methionine
Methionine
Methionine
Valine
Methionine
Lysine
Histidine
Valine
Lysine
Isoleucine
Isoleucine
Bean meal
Camilina meal
Soybean meal
Pea meal
Sunflower meal
Wheat gluten
Defatted krill meal
Fish meal
Krill meal
Poultry meal
Squid meal
OI
CS