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Abstract

The amino acid composition and protein levels of three species of cephalopods (*Octopus vulgaris, Loligo gahi* and *Todarodes sagittatus),* the natural diets of common octopus (*O. vulgaris*) and different kind of meals were determined in order to optimize the content of these nutrients in artificial feeds. Arginine, leucine and lysine were the most abundant essential amino acids in cephalopods, while glutamate and aspartate represented the main non-essential amino acids. Arginine and leucine were the limiting amino acid in most samples, with maximum Chemical Score values for mussel (79-98%), squid (84%) and crustaceans (65-91%), medium for fish (41-70%) and minimum for meals (29-64%). Mussel, squid, crustaceans and fish showed a high essential amino acid index according to Oser (OI: 88-99%) suggesting a suitable amino acid balance. The protein from animal meals (fish and krill) covered all the essential amino acids except arginine and lysine in fish meal. The vegetable meal presented the worst amino acid balance (OI: 74-89%) with several deficiencies in essential amino acids, including arginine, threonine, lysine and methionine. Supplementation with arginine or leucine and protein complementation of crustaceans and bivalves with fish or animal meal are proposed as alternatives for improving the performance of protein in feed for cephalopods.

Keywords: Amino acid; Cephalopod; Feed composition; Feed formulation; Meal; Nutrition; Octopus

Abbreviations:

AA = Amino acid, AAR = Amino acid ratio, CS = Chemichal score, OI = Oser’s Index

Introduction

One of the main reasons for the lack of development in cephalopod aquaculture is that there are no feeds available that are palatable with a balanced nutritional composition for all stages of their growth (Vaz-Pires *et al*. 2004; Cerezo Valverde *et al.* 2008). The culture of the early life stages of cephalopods (including octopus, squid and cuttlefish) have depended on the supply of live prey to achieve acceptable growth and survival (Boletzky and Hanlon 1983; Baeza-Rojano *et al*. 2010). However, the culture of some species of octopus, such as *Octopus vulgaris*, are problematic and show high mortality rates during their planktonic life stage when live prey (Navarro and Villanueva 2003; Iglesias *et al*. 2007) or formulated diets (Villanueva *et al*. 2002) are used, emphasising our poor knowledge of their nutritional requirements. Nevertheless, in both juvenile and adult stages, the best results have been obtained with natural diets (Aguila *et al*. 2007; Domingues *et al*. 2008).

Whatever the case, the successful commercial ongrowing of any species needs a formulated diet, given the advantages of such compared with natural diets, and this is the case with seabream, seabass and turbot (Cho and Bureau, 2001; Davies *et al.* 2009). It is also necessary to know the correct nutritional composition of the feed to obtain good growth and feed efficiency. One starting point for optimising the nutritional composition of artificial octopus feeds could be to analyze the macro and micronutrients of major ingredients such as crabs, mussels and fish that are commonly used in the natural diets (Chapela *et al.* 2006; García García and Cerezo Valverde 2006; García García *et al.* 2009). In addition the ratios of the macro and micro nutrients in the tissues of several cephalopods species and in the raw materials may be used to develop an artificial diet.

In this respect, protein is the most expensive nutritional component for feed formulation in aquaculture. This is especially relevant considering that cephalopods are exclusively carnivorous species (Guerra and Nixon 1987) and the high protein/energy ratio needed to achieve maximum growth (up to 50 g protein/MJ in *Sepia officinalis* according to Lee, 1994). Such a high value can be explained by the predominance of amino acid metabolism and its use for energy purposes. Therefore, unlike in the case of fish, the substitution of proteins by fats or carbohydrates does not seem a good way of formulating feeds for cephalopods, although a minimum level of carbohydrates dry matter is necessary for producing extruded dry feeds with suitable physical properties (Thomas *et al*. 1998). For this reason, the quality of the protein or the amino acid balance may be the best measure of nutritional value for cephalopod diets. With such a procedure, it will be possible to obtain maximum growth and protein retention with the lowest possible percentage of proteins in the diet.

In recent years, researchers have developed feed formulations that have been found acceptable by octopus and have resulted in significant growth (Cerezo Valverde *et al*. 2008; Quintana *et al*. 2008; Rosas *et al*. 2008; Estefanell et al., 2011). However, the choice of major ingredients is extremely important in this respect. With this in mind, the coordinated project, JACUMAR (2007-2009), which is directed at optimising octopus ongrowing in Spain, has among its objectives the detailed biochemical analysis of different cephalopod species, their natural diets, waste products from the canning industry, and several different plant and animal meals as well. In this study, we evaluate the results obtained for the amino acids composition found in molluscs, crustaceans, fish and meals, selecting the most appropriate to elaborate cephalopod diets by reference to an index of nutritional quality.

Material and Methods

Collection and keeping of samples

Forty-two samples were gathered, including fish, crustaceans, molluscs and meals, from different participants in the National Plan entitled "Optimising the ongrowing of the octopus *Octopus vulgaris* 2007-2009". Some samples were collected both during summer and winter (Tables 1, 2 and 3). For molluscs, only the edible portion was selected, except in the case of *O. vulgaris* when, besides the whole animals, the gonad, digestive gland and muscle tissue were analysed. For fish, the filets, gonads and viscera were included, rejecting bony structures and fins. As for crustaceans, all the animals were emptied, including the meat from the claws, inside the shell, gills and gonads, rejecting only the skeletal structure. As an exception, the whole heads of *Penaeus* sp. from the canning industry were analysed, obtaining between 500 and 1000 g of sample per species, ensuring that it came from at least six different specimens. All the specimens were triturated to obtain a homogeneous mixture, which was vacuum-packed in 100 g portions and frozen -20º C until use (a maximum of three months until amino acid analysis).

Analytical methods and determination of amino acids

Prior to chemical analyses all samples were freeze-dried and then analysed according to AOAC (1997). Briefly, dry matter was obtained by drying (105±1ºC) to constant weight and crude protein (N x 6.25) was determined by the Kjeldhal method after acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden). All analyses were performed in triplicate. Following the method previously described by Bosch *et al.* (2006), amino acids of the samples were determined using a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) consisting of two pumps (Model 515, Waters), an auto sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. Aminobutyric acid (Sigma-Aldrich Co.) was added as an internal standard patron before hydrolysation. The amino acids were derivatised with 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-18 reverse-phase column Waters Acc. Tag (150 mm x 3.9 mm), and then transformed to methionine and cystine. It was not possible to differentiate the amino acid arginine of taurine (a non-protein nitrogen substance) because both compounds had the same retention time by the analytical technique used. In this regard, the nomenclature "Arg^{T}" (arginine plus taurine) has been indicated in tables. In any case, taurine was determined by an automatic amino acid analyzer (Biochrom 20®, Pharmacia Biotech, Cambridge, UK) in several samples (*O. vulgaris*: Id. 6, 7; *Carcinus maenas*: Id. 17; *Boops boops*: 20) using a cation exchange high performance column (200 x 4.6 mm column size; Pharmacia Biotech) and ninhydrin as derivative agent (Ultra Ninhydrin Reagent, Pharmacia Biotech). When the values of arginine include taurine has been clearly specified throughout the manuscript.

Data analysis

Crude protein is expressed as g kg^{-1} dry weight, with the mean and standard deviation shown for three replicates. Each replica came from the same homogeneous pool of specimens. The amino acid (AA) content is expressed as grams of AA kg^{-1} of protein and was obtained in a single sample from the pool. With the data obtained, the values of the following indices were calculated:

-Amino acid ratio (AAR, %) = $(\text{AA}_{\text{sample}})/(\text{AA}_{\text{reference}})*100$, where $\text{AA}_{\text{sample}}$ and $\text{AA}_{\text{reference}}$ are the amino acid contents in the test sample and whole *O. vulgaris*, which was taken as reference (mean values taking into account summer and winter samples). Amino acid ratios for arginine were calculated by subtracting the values of taurine in samples from octopus to avoid underestimation of the ratios.

-Chemichal score (CS, %): Minimum value from AARs calculated for essentials amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val).

-Limiting amino acid: This is the amino acid corresponding to CS in the test sample.

-Oser's Index (OI, %) was used as index of nutritional quality and obtained as the geometric mean ratio of amino acids in the samples to those detected in *O. vulgaris*, which were taken as reference, according to the formula:

$OI\ (\%) = (10^{\ (1/n*(\log(AAR1)+\log(AAR2)\ldots+\log(AARn))})$,

where AAR1, AAR2,…AARn are the ratios of essential amino acid and "n" the number of essential amino acids detected. When the ratio is above 100, this was taken as reference (Oser, 1951).

All the differences were analysed by one factor ANOVA and Tukey's test to establish homogenous groups, with the level of significance of $P<0.05$. A Neperian logarithmic transformation was made before the ANOVA to achieve homogeneity of variances.

Results

Protein content

In molluscs, the highest protein values were detected in *O. vulgaris* (801.9-810.3 g kg^{-1}) and *L. gahi* (797.9 g kg^{-1}) and the lowest in *M. galloprovincialis* (634.5-651.2 g kg^{-1}), the difference being significant ($P<0.05$; Table 4). These values remained constant regardless of the season ($P>0.05$). In crustaceans, the protein content was significantly higher in *P. clarkii* (695.2 g kg^{-1}) compared to *Penaeus* sp. (570.1 g kg^{-1}) or *C. maenas* (543.0-607.1 g kg^{-1}; $P<0.05$). No differences were found in *C. maenas* between summer and winter samples from the same geographical area ($P>0.05$; Table 4). Fish from artisanal fisheries such as *B. boops* and *G. poutassou* had higher protein levels than the rest of the samples analyzed (922.1 and 919.0 g kg^{-1}, respectively; $P<0.05$; Table 5). In contrast, fish from by-catch of fish farms like *B. boops* (356.3-501.4 g kg^{-1}) and *S. aurata* (520.2 g kg^{-1}) showed the lowest values of all the fish analysed ($P<0.05$). *Mugil* sp., *S. pilchardus*, *T. trachurus* and *G. minutus* had intermediate values (567.2-884.6 g kg^{-1}). Significant seasonal variations were observed in protein levels. For example, protein levels were higher in *S. pilchardus* in winter than in summer (877.9 vs. 567.2 g kg^{-1}, respectively; $P<0.05$), but higher levels were observed in summer in *G. poutassou* and *T. trachurus* ($P<0.05$). In the meals, the highest protein content was found in the pea meal (785.1 g kg^{-1}) and fish meal (748.9 g kg^{-1}) and the lowest in sunflower (344.5 g kg^{-1}) and wheat (124.7 g kg^{-1}; $P<0.05$; Table 5). The soy (533.5 g kg^{-1}) and krill (559.3 g kg^{-1}) meals had intermediate values but with significant differences from the rest of the meals analysed ($P<0.05$).

Amino acid content

Arginine, lysine and leucine were the main essential amino acid in the molluscs, with contents that reached 156.7, 72.5 and 64.3 g AA kg^{-1} protein in whole *O. vulgaris*, respectively, with glutamate the main non-essential amino acid (from 104.6 in *M. galloprovincialis* to 145.0 g AA kg^{-1} protein in *T. sagittatus*; Table 6). The same amino acids predominated in all crustacean samples (91.4-128.2, 59.6-81.6, 58.0-75.0 and 115.3-165.8 g AA kg^{-1} protein for arginine -including taurine-, lysine, leucine and glutamate, respectively). The protein content of all the fish species was characterised by high lysine levels (88.0-109.4 g AA kg^{-1} protein in *G. poutassou* and *S. pilchardus* in winter, respectively; Table 7). The principal non-essential amino acid in all the fish species was glutamate (124.2-166.4 g AA kg^{-1} protein). In the sunflower, pea and fish meals, the main essential amino acid was arginine -including taurine-, (78.6, 92.2 and 78.0 g AA kg^{-1} protein, respectively) and in the soy, wheat and krill meals leucine (72.91, 64.2 and 83.2 g AA kg^{-1} protein, respectively; Table 8). The main non-essential amino acid in meals was glutamate (113.6-285.3 g AA kg^{-1} protein).

Protein quality evaluation

L. gahi was deficient in histidine, threonine and phenylalanine (AAR: 84, 93, 95%, respectively). *T. sagittatus* and one sample of *M. galloprovincialis* were deficient in arginine compared with the *O. vulgaris* protein (AAR between 63 and 84%, respectively; Fig. 1A). Furthermore, most of the samples of *M. galloprovincialis* had low levels of isoleucine (AAR: 84-102%) and leucine (ARR: 78-99%). None of the crustacean samples reached the arginine levels observed in *O. vulgaris* (AAR of between 65 and 91% in *P. clarkii* and *C. maenas*, respectively; Fig. 1B). In general, the rest of the essential amino acid levels were covered, with the exception of lysine in *Penaeus* sp. (AAR 87%) and slight deficiency in leucine, isoleucine and methionine (AAR>90%) in some samples of *C. maenas*. In fish there was a good amino acid profile with the exception of arginine (AAR from 41% in *B. boops* to 69% in *G. minutus*; Fig. 2). Similarly, all the meals were deficient in arginine (AAR from 29% in wheat meal to 63% in pea meal) but exceeded histidine, phenylalanine and valine level (Fig. 3). The animal meals covered the rest of the amino acids, except lysine in the fish meal (AAR 90%). The vegetal meals showed an even worse balance, with a lysine deficiency in sunflower, soy and wheat meals, methionine deficiency in all meals except sunflower and threonine deficiency in all of them without exception.

Therefore, the limiting amino acid in most of the samples was arginine, except in *L. gahi* where it was histidine (Fig. 4A), two samples of *M. galloprovincialis* where it was leucine (Fig. 4A), one sample of *C. maenas* (methionine; Fig. 4B), and sunflower meal (lysine; Fig. 4D). The Chemichal Score pointed to a gradient with the lowest values in fish (41-70%) and meals (29-64%), intermediate levels in crustaceans (65-91%) and the highest levels in molluscs (84-98%; Fig. 4). According to the Oser's Index, the best balanced protein as regards essential amino acids would be *M. galloprovincialis* (88-99%; Fig. 4A), squid *L. gahi* (96%; Fig. 4A) and all the crustacean samples (95-97%; Fig. 4B). The values in fish ranged from a minimum of 90% in *B. boops* from by-catch of fish farms and a maximum of 96% in *G. minutus* (Fig. 4C). The animal meals showed similar values to fish (92%), the worst results being observed in vegetal meals (74-89%; Fig. 4D).

Discussion

The most obvious difference between the proximal composition of cephalopods and other marine organisms is the high protein content and low lipid and mineral content of the former (Lee, 1994; Rosa *et al.* 2005; Ozogul *et al.* 2008; Cerezo Valverde *et al.* 2011). In the present study, protein levels reached between 800 and 810 g kg^{-1} dry weight in *O. vulgaris* and *L. gahi*. In other species, such as *Sepia officinalis* and *Loligo vulgaris*, these levels exceeded 820 g kg^{-1} (Zlatanos *et al.* 2006). Generally speaking, all the samples of bivalve molluscs (648-653 g kg^{-1}), crustaceans (543-695 g kg^{-1}), fish or krill meals (559-749 g kg^{-1}) and plant meals (124-785 g kg^{-1}) had lower protein content, with a few notable seasonal exceptions for fish samples. *B. boops*, *G. poutassou*, *S. pilchardus* and *G. minutus*, had protein contents ranging between 850 and 930 g kg^{-1}. These extremely high levels can be explained by their low fat content or the assay coinciding with the season of the year when such deposits were at their lowest. This phenomenon has been described in many species and is explained by the mobilisation of energy reserves during the time of least food availability and their accumulation if present in high amounts (Bandarra *et al.* 1997; Luzia *et al.* 2003; Pazos *et al.* 2003).

Similarly, the high lipid content in species from the by-catch of fish farms (e.g. *B. boops* or *S. aurata*; Cerezo Valverde *et al.* 2011) would have led to the very low protein content (350-520 g kg^{-1}), even in winter.

The results of this study also underline the marked genetic character of the amino acid composition of the samples, great similarity being observed within the same taxonomic group. As in the results obtained by Villanueva *et al*. (2004) and Rosa *et al*. (2004), the predominant amino acids in cephalopods were, in order, arginine, lysine and leucine (essential) and glutamic and aspartic acids (non-essential). The same pattern is preserved in bivalve molluscs and crustaceans, and may be the reason for the excellent growth recorded in the cephalopods when they are fed solely a crustacean-based diet (Cagnetta y Sublimi 2000; Aguado Giménez and García García 2002) or mixed diets containing crustaceans and fish or bivalves (García García and Cerezo Valverde 2006; Rodríguez *et al.* 2006; Biandolino *et al.* 2010; Prato *et al.* 2010). However, the high growth rates obtained with crustaceans are accompanied by high rates of ingestion and low feed efficiency and protein retention compared with the mixed or monodiets that include fish (García García and Cerezo Valverde 2006; Prato *et al.* 2010). In most of the fish species the predominant essential amino acids were lysine and leucine, with low levels of CS (41-70%) compared with crustaceans (65-91%), suggesting that the amino acid profile would not explain the greater protein retention observed in fish-based diets. Since the Chemichal Score is indicative of the maximum percentage of protein that may be retained for growth, these results coincide with the hypothesis proposed by García García and Cerezo Valverde (2006) concerning the existence of a nutritional factor present in fish but absent from crustaceans that would lead to better protein use. In this sense, cephalopods are exclusively carnivorous species (Guerra and Nixon, 1987) and rarely use carbohydrates or lipids as energy source (O'Dor *et al.* 1984; Lee 1994). The latter, in particular, are poorly assimilated by cephalopods in general (Sánchez *et al.* 2009; Seiça Neves *et al.* 2010). However, several recent studies have demonstrated efficient lipid dietary utilization (Estefanell *et al.* 2011) and a significant contribution of lipids and carbohydrates to the energy metabolism in octopus (García-Garrido *et al.* 2010; Morillo-Velarde *et al.* 2011). The low fat content detected in crustaceans compared with fish would explain the greater use of protein for energetic ends and lower retention of the same. Moreover, the amino acid profile of fish characterised by the marked deficiency in arginine, together with the lower ingestion rates compared with crustaceans may explain the lower growth obtained with a fish-based monodiets.

Therefore, the results of the present study suggest that the incorporation of fish protein in feeds destined for cephalopods should be supplemented with arginine to improve yields. When using primary materials from crustaceans two strategies might be followed: a) a moderate increase in lipids accompanied by supplementation with low levels of leucine, isoleucine and methionine, and a higher level of arginine; or b) complement proteins from crustaceans with proteins derived from fish - high leucine, isoleucine, lysine and methionine levels, but with moderate levels of fat -, incorporating both sources in the same feed, and supplemented with arginine. In our case, the use of *B. boops* from artisanal fisheries may be the best approach because of its high arginine levels, better overall amino acid balance, moderate fat content (Cerezo Valverde *et al.* 2011) and low market price (García García and García García 2011). Besides, bogue has a lower commercial value with a minimum demand for consumption.

Several researchers have attempted to add hydrolysed proteins or amino acids in crystalline form in artificial diets for cephalopods or added crystalline amino acids in culture water during the early stages of larval development. In the first case, the feeds resulted in low or negative growth rates in *S. officinalis* (Castro and Lee 1994;

Dominguez *et al.* 2005) and *O. maya* (Aguila *et al.* 2007) and moderate rates in *O. vulgaris* (Cerezo Valverde *et al*. in press), although this was largely due to the low degree of acceptability of the diets. Cerezo Valverde *et al*. (in press) observed that if the feed is cohesive and stable in water and it is accepted by the octopus the beneficial effects of supplementation with pure amino acids are evident and could be an effective tool to slow feeding animals. Domingues et al. (2005) also obtained best results with a diet representing the highest degree of amino acid supplementation in *Sepia officinalis*. Villanueva *et al*. (2004) observed the beneficial effect of adding a water solution of amino acids on octopus paralarval survival, although this did not translate into higher growth. Therefore, the role of dissolved amino acids in culture water remains uncertain.

In fish, the best growth and nitrogen retention results were obtained by complementing a deficient protein source by adding the limiting amino acid in the form of another protein source that contains it (Ketola 1982). The worst results obtained with diets supplemented with crystalline amino acids were attributed to their rapid absorption since optimal protein synthesis requires availability of all amino acids in the tissues simultaneously and in sufficient quantities (Schuhmacher *et al.* 1997). In the case of fish diet formulation, a combination of different raw materials is a good solution to alleviate the amino acid deficiencies of several protein sources (Kaushik *et al.* 2004; Sánchez-Lozano *et al.* 2009). However, all analyzed fish samples in the present study were deficient in arginine, meaning that dietary mixtures do not fully meet requirements for *O. vulgaris*. Furthermore, the problem of samples deficient in arginine is more pronounced than what is stated in this paper. Arginine ratios remained extremely low in fish, crustacean and meal samples despite the inclusion of taurine in the value of the arginine and taurine deduction in the whole octopus samples. According to our results and other authors taurine is particularly high in the tissues of molluscs and crustaceans

(Robertson *et al.* 1992; D'Aniello *et al.* 1995; Babarro and Fernández Reiriz 2006). Taurine values for *O. vulgaris* in the present study (64-75 AA kg^{-1} protein) were similar to those detected for *O. maya* (65-80 g AA kg^{-1} protein according to George-Zamora *et al.* 2011).

According to our results, *L. gahi* and *M. galloprovincialis* samples would better cover arginine requirements of octopus and displayed a balanced essential amino acids composition. However, in this respect, it should be noted that the ideal nutritional composition of a diet does not necessarily imply greater acceptability. Indeed, ongrowing experiments with diets containing *M. galloprovincialis* were associated with low ingestion and growth rates (López *et al.* 2009; Biandolino *et al.* 2010; Prato *et al.* 2010). There is a clear need for preliminary experiments with materials that improve acceptability before any new material is incorporated in diet formulation.

By far the worst balanced proteins were those contained in the vegetal meals. Both the wheat and soy meals should be supplemented with proteins rich in lysine, threonine, methionine and arginine. Similarly, while the pea meal should be supplemented with threonine, methionine and arginine, the sunflower meal should be supplemented with arginine and lysine. Of the plant meals analyzed, pea is the most suitable given its high protein content and best CS (63%) and OI (88%). Both the fish and krill meals offer similar benefits as the different species of fish taking into account the amino acid composition, CS or OI values. The fish meal has the advantage of a higher protein content and CS than krill although it has the disadvantage of needing lysine supplementation.

In conclusion, the suitability of molluscs and crustaceans for developing cephalopod meals is evident, although their protein quality indices could be improved with arginine or leucine supplementation and the joint use of protein from fish or krill meals. None of the vegetal meals assayed could on their own offers a good nutritional balance and would need supplementation or would have to be used alongside other raw materials. In the present study, the amino acid quality of feed for octopus was tested by amino acid ratios alone, however, in future studies, the digested essential amino acid estimation for each raw material should be taken into account for octopus diets formulation.

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References

Aguado Giménez F, García García B (2002) Growth and food intake models in *Octopus vulgaris* Cuvier (1797): influence of body weight, temperature, sex and diet. Aquacult. Int. 10: 361-377.

Aguila J, Cuzon G, Pascual C, Domingues PM, Gaxiola G, Sánchez A, Maldonado T, Rosas C (2007) The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: Digestive enzyme, blood metabolites, and energy balance. Aquaculture 273: 641-655.

AOAC (1997) Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists. Washington.

Babarro JMF, Fernández Reiriz MJ (2006) Variability of taurine concentrations in *Mytilus galloprovincialis* as a function of body size and specific tissue. Comp. Biochem. Physiol. 145B: 94-100.

Baeza-Rojano E, García S, Garrido D, Guerra-García JM, Domingues PM (2010) Use of Amphipods as alternative prey to culture cuttlefish (*Sepia officinalis*) hatchlings. Aquaculture 300: 243-246.

Bandarra NM, Batista I, Nunes ML, Empis JM, Christie WW (1997) Seasonal changes in Lipid Composition of Sardine (*Sardine pilchardus*). J. Food Sci. 62: 40-42.

Biandolino F, Portacci G, Prato E (2010) Influence of natural diet on growth and biochemical composition of *Octopus vulgaris* Cuvier, 1797. Aquacult. Int. 18: 1163-1175.

Boletzky SV, Hanlon RT (1983) A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. Mem. Natl. Mus. Victoria 44: 147-187.

Bosch L, Alegría A, Farré R (2006) Application of the 6-aminoquinolyl-N-hydroxysccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods. J. Chromatogr. B 831: 176–183.

Cagnetta P, Sublimi A (2000) Productive performance on the common octopus (*Octopus vulgaris* C.) when fed on a monodiet. CIHEAM, Cah. Options Méditerr. 47: 331-336.

Castro BG, Lee PG (1994) The effect of semi-purified diets on growth and condition of *Sepia officinalis* L. (Mollusca: Cephalopoda). Comp. Biochem. Physiol. 109: 1007-1016.

Cerezo Valverde J, Hernández MD, Aguado-Giménez F, Morillo-Velarde PS, García García B (in press) Performance of formulated diets with different level of lipids and glutamate supplementation in *Octopus vulgaris*. Aquac. Res.

Cerezo Valverde J, Hernández MD, Aguado-Giménez F, García García B (2008) Growth, feed efficiency, and condition of common octopus (*Octopus vulgaris*) fed on two formulated moist diets. Aquaculture 275: 266-273.

Cerezo Valverde J, Hernández MD, García-Garrido S, Rodríguez C, Estefanell J, Gairín JI, Rodríguez CJ, Tomás A, García García B (2011) Lipid classes from marine species and meals intended for cephalopod feeding. Aquacult. Int. 20: 71-89.

Chapela A, González AF, Dawe EG, Rocha F, Guerra A (2006) Growth of common octopus (*Octopus vulgaris*) in cages suspended from rafts. Sci. Mar. 70: 121-129.

Cho CY, Bureau DP (2001) A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquac. Res. 32: 349-360.

D'Aniello A, Nardi G., De Santis A., Vetere A, di Cosmo A, Marchelli R, Dossena A, Fisherl G (1995) Free L-amino acids and D-aspartate content in the nervous system of Cephalopoda. A comparative study. Comp. Biochem. Physiol. 112B: 661-666.

Davies SJ, Gouveia A, Laporte J, Woodgate SL, Nates S (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). Aquac. Res. 40: 1759-1769.

Domingues PM, Dimarco FP, Andrade JP, Lee PG (2005) Effect of artificial diets on growth, survival and condition of adult cuttlefish, *Sepia officinalis* Linnaeus, 1758. Aquacult. Int. 13: 423-440.

Domingues PM, Ferreira A, Marquez L, Andrade JP, López N, Rosas C (2008) Growth, absorption and assimilation efficiency by mature cuttlefish (*Sepia officinalis*) fed with alternative and artificial diets. Aquacult. Int. 3: 215-229.

Estefanell J, Roo J, Alfonso JM, Fernández-Palacios H, Izquierdo M, Socorro J (2011) Efficient utilization of dietary lipids in *Octopus vulgaris* (Cuvier 1797) fed fresh and agglutinated moist diets based on aquaculture by-products and low price trash species. Aquac. Res. doi: 10.1111/j.1365-2109.2011.03014.x

García García B, Cerezo Valverde J (2006) Optimal proportions of crabs and fish in diet for common octopus (*Ocotpus vulgaris*) ongrowing. Aquaculture 253: 502-511.

García García B, Cerezo Valverde J, Aguado-Giménez F, García García J (2009) Growth and mortality of common octopus *Octopus vulgaris* reared at different stocking densities in Mediterranean offshore cages. Aquacult. Res. 40: 1202-1212.

García García J, García García B (2011) Econometric model of viability/profitability of octopus (*Octopus vulgaris*) ongrowing in sea cages. Aquacult. Int. doi: 10.1007/s10499-011-9432-1.

García-Garrido S, Hachero-Cruzado I, Garrido D, Rosas C, Domingues PM (2010) Lipid composition of mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. Aquacult. Int. 18: 1223-1241.

George-Zamora A., Viana MT, Rodríguez S, Espinoza G, Rosas C (2011) Amino acid mobilization and growth of juvenile *Octopus maya* (Mollusca:Cephalopoda) under inanition and re-feeding. Aquaculture 314: 215-220.

Guerra A, Nixon M (1987) Crab and mollusc shell drilling by *Octopus vulgaris* (Mollusca: Cephalopoda) in the Ria de Vigo (north-west Spain). J. Zool., Lond. 211: 515-523.

Iglesias J, Sánchez FJ, Bersano JGF, Carrasco JF, Dhont J, Fuentes L, Linares F, Muñoz JL, Okumura S, Roo FJ, van der Meeren T, Vidal EAG, Villanueva R (2007) Rearing of *Octopus vulgaris* paralarvae: Present status, bottlenecks and trends. Aquaculture 266: 1-15.

Kaushik SJ, Covès D, Dutto G, Blanc D (2004) Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchux labrax*. Aquaculture 230: 391–404.

Ketola HG (1982) Amino acid nutrition of fishes: requirements and supplementation of diets. Comp. Biochem. Physiol. 73: 17-24.

Lee PG (1994) Metabolic substrates in cephalopods. In: Pörtner HO, O'Dor RK, MacMillan DL (Eds.), Physiology of Cephalopod Mollusc. Lyfestyle and Performance Adaptations. Gordon and Breach Publishers, Basel, Switzerland, pp.

López M, Rodríguez C, Carrasco JF (2009) Engorde de juveniles de pulpo (*Octopus vulgaris* Cuvier, 1797) con distintas dietas naturales y artificiales. In: Beaz D, Villarroel M, Cárdenas S (Eds.). Book of abstracts. XII Congreso Nacional de Acuicultura, Madrid, 24-26 Nov. pp. 170-171.

Luzia LA, Sampaio GR, Castellucci CMN, Torres EAFS (2003) The influence of season on the lipid profiles of five commercially important species of Brazilian fish. Food Chem. 83: 93-97.

Morillo-Velarde PS, Cerezo Valverde J, Serra Llinares RM, García García B (2011) Energetic contribution of carbohydrates during starvation in common octopus (*Octopus vulgaris*). J. Molluscan Stud. 77: 318-320.

Navarro JC, Villanueva R. (2003) The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. Aquaculture 219: 613-631.

O'Dor R.K, Mangold K, Boucher-Rodoni R, Wells MJ, Wells J (1984) Nutrient Absorption, Storage and Remobilization in *Octopus vulgaris.* Mar. Behav. Physiol. 11: 239-258.

Oser BL (1951) Method for integrating essential amino acid content in the nutritional evaluation of protein. J. Am. Diet. Assoc. 27: 396-402.

OzogulY, Duysak O, Ozogul F, Özkütük AL, Türeli C (2008) Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. Food Chem. 108: 847-852.

Pazos AJ, Sánchez JL, Román G, Pérez-Parallé, Abad M (2003) Seasonal changes in lipid classes and fatty acid composition in the digestive gland of *Pecten maximus*. Comp. Biochem. Physiol. 134B: 367-380.

Prato E, Portacci G, Biandolino F (2010) Effect of diet on growth performance, feed efficiency and nutritional composition of *Octopus vulgaris*. Aquaculture 309: 203-211.

Quintana D, Domingues PM, García S (2008) Effect of two artificial wet diets agglutinated with gelatin on feed and growth performance of common octopus (*Octopus vulgaris*) sub-adults. Aquaculture 280, 161-164.

Robertson JD, Cowey CB, Leaf G (1992) The free amino acids in muscle of three marine invertebrates *Nephrops norvegicus* (L.), *Limulus polyphemus* (L.) and *Eledone cirrhosa* (Lamarck). Comp. Biochem. Physiol. 101A: 545-548.

Rodríguez C, Carrasco JF, Arronte JC, Rodríguez M (2006) Common octopus (*Octopus vulgaris* Cuvier, 1797) juvenile ongrowing in floating cages. Aquaculture 254: 293-300.

Rosa R, Costa PR, Nunes M.L (2004) Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). Mar. Biol. 145: 563-574.

Rosa R, Pereira J, Nunes ML (2005) Biochemical composition of cephalopods with different life strategies, with special reference to a giant squid, *Architeuthis* sp. Mar. Biol. 146: 739-751.

Rosas C, Tut J, Baeza J, Sánchez A, Sosa V, Pascual C, Arena L, Domingues PM, Cuzon G (2008) Effect of type of binder on growth, digestibility, and energetic balance of *Octopus maya*. Aquaculture 275: 291-297.

Sánchez M, Hernández MD, Cerezo Valverde J, García García B (2009) Protein and lipid digestibility in common octopus (*Octopus vulgaris*). In: Cephalopod International Advisory Council Symposium (CIAC'09). September 3rd-11th 2009, Vigo, Spain. p. 86.

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

Schuhmacher A, Wax C, Gropp JM (1997) Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. Aquaculture 151: 15-28.

Seiça Neves MM, Cerezo Valverde J, García García B (2010) Digestibility of a formulated diet with alginate as binder in octopus (*Octopus vulgaris*). In: EAS Aquaculture Europe 2010. Book of abstracts. Porto, Portugal. 5-8 Oct. 2010, 500-501.

Thomas M, van Vliet T, van der Poel AFB (1998) Physical quality of pelleted animal feed 3. Contribution of feedstuff components. Anim. Feed Sci. Technol. 70: 59-78.

Vaz-Pires P, Seixas P, Barbosa A (2004) Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. Aquaculture 238: 221-238.

Villanueva R, Koueta N, Riba J, Boucaud-Camou E (2002) Growth and proteolytic activity of *Octopus vulgaris* paralarvae with different food ratios during first feeding, using Artemia nauplii and compound diets. Aquaculture 205: 269-286.

Villanueva R, Riba J, Ruíz-Capillas C, González AV, Baeta M (2004) Amino acid composition of early stages of cephalopods and effect of amino acid dietary treatments on *Ocotpus vulgaris* paralarvae. Aquaculture 242: 455-478.

Zlatanos S, Laskaridis K, Feist C, Sagredos A (2006) Proximate composition, fatty acid analysis and protein digestibility-corrected amino acid score of three Mediterranean cephalopods. Mol. Nutr. Food Res. 50: 967-970.

Table 1. Samples used to determine amino acids in molluscs and crustaceans.

Id.	Group Species	Common Name	Sample	Sampling period	Location (Spain)	n*	Fresh weight±SD (g)
	MOLLUSCS						
1	*Loligo gahi*	Squid	Edible portion	Feb-08	Andalucía (S)	28	40.6 ± 6.4
2	*Mytilus galloprovincialis*	Mussel	Edible portion (boiled)[a]	Jul-07	Galicia (NW)	90	4-9 (without shell)
3	*Mytilus galloprovincialis*	Mussel	Edible portion (boiled)[a]	Feb-08	Galicia (NW)	90	4-9 (without shell)
4	*Mytilus galloprovincialis*	Mussel	Edible portion	Feb-08	Asturias (N)	40	48.6±0.4
5	*Mytilus galloprovincialis*	Mussel	Edible portion	Jun-08	Asturias (N)	40	40-50
6	*Octopus vulgaris*	Common octopus	Whole animal	Jul-07	Murcia (SE)	6	1005.0 ± 291.9
7	*Octopus vulgaris*	Common octopus	Whole animal	Mar-08	Murcia (SE)	6	868.0 ± 46.6
8	*Octopus vulgaris*	Common octopus	Muscle	Jul-07	Murcia (SE)	10	1448.9 ± 337.6
9	*Octopus vulgaris*	Common octopus	Muscle	Mar-08	Murcia (SE)	10	771.7 ± 138.2
10	*Octopus vulgaris*	Common octopus	Digestive gland	Jul-07	Murcia (SE)	24	1369.0 ± 316.9
11	*Octopus vulgaris*	Common octopus	Digestive gland	Mar-08	Murcia (SE)	36	967.2 ± 394.4
12	*Octopus vulgaris*	Common octopus	Gonad	Mar-08	Murcia (SE)	36	967.2 ± 394.4
13	*Todarodes sagittatus*	Sea-arrow	Mantle, arms and fins[a]	Jul-07	Galicia (NW)	100	100-150
	CRUSTACEANS						
14	*Carcinus maenas*	Common shore crab	Edible portion	Feb-08	Asturias (N)	24	50-60
15	*Carcinus maenas*	Common shore crab	Edible portion	Jun-08	Asturias (N)	30	50-60
16	*Carcinus maenas*	Common shore crab	Edible portion	Jul-07	Murcia (SE)	86	41.6 ± 10.4
17	*Carcinus maenas*	Common shore crab	Edible portion	Mar-08	Murcia (SE)	178	39.0 ± 14.0
18	*Penaeus sp.*	Prawn	Heads[a]	Jul-07	Galicia (NW)	150	9.0 ± 2.1
19	*Procambarus clarkii*	Red crayfish	Edible portion	feb-08	Andalucía (S)	268	11.4 ± 1.8

[a] From the canning industry. *Number of specimens used to obtain a homogeneous sample.

Table 2. Samples used to determine amino acids in fish.

Id.	Group Species	Common Name	Sample	Sampling period	Location (Spain)	n*	Fresh weight±SD (g)
	FISH						
20	*Boops boops*	Bogue[a]	Edible portion	Feb-08	Murcia (SE)	118	20.6 ± 11.3
21	*Boops boops*	Bogue[b]	Edible portion	Feb-08	Murcia (SE)	11	177.4 ± 67.6
22	*Boops boops*	Bogue[a]	Edible portion	Jul-07	Canary Islands	20	92.0 ± 23.0
23	*Boops boops*	Bogue[b]	Edible portion	Jul-07	Canary Islands	6	333.0 ± 34.0
24	*Gadus poutassou*	Blue whiting[a]	Edible portion	Jul-07	Galicia (NW)	25	36.8±7.5
25	*Gadus poutassou*	Blue whiting[a]	Edible portion	Feb-08	Galicia (NW)	25	30-50
26	*Gadus poutassou*	Blue whiting[a]	Edible portion	Feb-08	Asturias (N)	10	103.2±4.2
27	*Gadus poutassou*	Blue whiting[a]	Edible portion	Jun-08	Asturias (N)	10	90-110
28	*Mugil sp.*	Mullet[a]	Edible portion	Jun-07	Cataluña (NE)	6	300-2000
29	*Mugil sp.*	Mullet[a]	Edible portion	Ene-08	Cataluña (NE)	6	300-2000
30	*Sardina pilchardus*	Sardine[a]	Edible portion	Jul-07	Murcia (SE)	32	49.9 ± 11.3
31	*Sardina pilchardus*	Sardine[a]	Edible portion	Feb-08	Murcia (SE)	135	20.4 ± 5.7
32	*Sparus aurata*	Seabream[b]	Edible portion	Feb-08	Canary Islands	6	421.0 ± 76.0
33	*Trachurus trachurus*	Scad[a]	Edible portion	Feb-08	Asturias (N)	10	112.9±1.7
34	*Trachurus trachurus*	Scad[a]	Edible portion	Jun-08	Asturias (N)	10	100-120
35	*Gadus minutus*	Poor cod[a]	Edible portion	Jun-07	Cataluña (NE)	75	15-30
36	*Gadus minutus*	Poor cod[a]	Edible portion	Ene-08	Cataluña (NE)	75	15-30

[a] From artisanal fisheries; [b] From the by-catch of fish farms. *Number of specimens used to obtain a homogeneous sample.

Table 3. Samples used to determine amino acids in meals.

Id.	Group	Company
	PLANT MEALS	
37	Sunflower	Piensos y Cereales Desco S.L., Valencia, Spain.
38	Pea (75% protein)	Dibaq-Diproteg, S.A., Segovia, Spain.
39	Soybean	COCERVA, Náquera, Valencia, Spain.
40	Wheat	Piensos y Cereales Desco S.L., Valencia, Spain.
	ANIMAL MEALS	
41	Krill	Sopropeche, Barcelona, Spain.
42	Fish	COCERVA, Náquera, Valencia, Spain.

Table 4. Moisture (g kg^{-1} fresh weight) and crude protein in molluscs and crustaceans (g kg^{-1} dry weight).

Id.	**Group/Species**	**Moisture**	**Crude protein**
	MOLLUSCS		
1	*L. gahi*	802.7±0.9^{c}	797.9±05.1^{ab}
2	*M. galloprovincialis*	759.1±0.6^{e}	651.2±21.9^{efg}
3	*M. galloprovincialis*	762.0±2.6^{e}	648.6±3.0^{efg}
4	*M. galloprovincialis*	798.0±1.9^{c}	652.9±5.2^{efg}
5	*M. galloprovincialis*	799.7±1.4^{c}	634.5±3.0^{fg}
6	*O. vulgaris*	798.5±2.3^{c}	810.3±17.6^{a}
7	*O. vulgaris*	780.4±6.2^{d}	801.9±23.6^{ab}
8	*O. vulgaris* (muscle)	832.8±6.8^{b}	778.6±57.2^{abc}
9	*O. vulgaris* (muscle)	798.2±2.7^{c}	780.7±36.8^{ab}
10	*O. vulgaris* (digestive gland)	682.0±1.6^{g}	738.5±4.3b^{cd}
11	*O. vulgaris* (digestive gland)	694.1±1.8^{f}	619.5±3.5^{g}
12	*O. vulgaris* (gonad)	720.2±4.2^{d}	712.6±21.9^{cde}
13	*T. sagittatus*	880.2±2.2^{a}	693.6±13.7^{def}
	CRUSTACEANS		
14	*C. maenas*	737.0±10.4^{d}	543.0±19.3^{c}
15	*C. maenas*	788.4±1.7^{c}	584.5±3.0^{bc}
16	*C. maenas*	801.5±2.3^{bc}	571.6±2.1^{bc}
17	*C. maenas*	819.8±1.9^{a}	607.1±2.7^{b}
18	*Penaeus sp.*	805.4±6.7^{ab}	570.1±29.8^{bc}
19	*P. clarkii*	813.5±4.6^{ab}	695.2±21.6^{a}

Different superscripts indicate significant differences ($P<0.05$) in the moisture or protein content between samples of the same group.

Table 5. Moisture (g kg^{-1} fresh weight) and crude protein in fish and meals (g kg^{-1} dry weight).

Id.	**Group/Species**	**Moisture**	**Crude protein**
	FISH		
20	*B. boops*	802.6±5.4^{a}	922.1±33.6^{a}
21	*B. boops*	627.9±2.7^{j}	501.4±5.4^{h}
22	*B. boops*	759.0±2.0^{de}	854.7±2.0^{bc}
23	*B. boops*	526.0±3.0^{k}	356.3±2.2i
24	*G. poutassou*	775.4±1.6^{bcd}	919.0±17.3^{a}
25	*G. poutassou*	736.0±5.3^{f}	727.8±4.0^{ef}
26	*G. poutassou*	720.5±2.4^{g}	804.4±13.7^{cd}
27	*G. poutassou*	763.4±2.3^{cde}	811.3±1.3^{cd}
28	*Mugil sp.*	761.7±4.3^{cde}	759.1±2.0^{de}
29	*Mugil sp.*	755.6±4.0^{e}	735.3±3.1^{ef}
30	*S. pilchardus*	657.7±7.9^{i}	567.2±18.5^{g}
31	*S. pilchardus*	777.3±6.9^{bc}	877.9±30.0^{ab}
32	*S. aurata*	681.3±3.0^{h}	520.6±1.6^{hg}
33	*T. trachurus*	709.6±8.4^{g}	677.8±28.1^{f}
34	*T. trachurus*	762.4±4.1^{de}	732.4±2.4^{e}
35	*G. minutus*	775.0±5.7^{bcde}	871.0±4.0^{ab}
36	*G. minutus*	787.0±7.2^{ab}	884.6±2.5^{ab}
	MEALS		
37	Sunflower	93.0±1.5^{b}	344.5±10.0^{c}
38	Pea	89.0±2.2^{b}	785.1±4.0^{a}
39	Soybean	100.0±2.3^{a}	533.5±9.1^{b}
40	Wheat	103.0±0.9^{a}	124.7±4.4^{d}
41	Krill	63.3±4.3^{c}	559.3±2.9^{b}
42	Fish	89.1±2.2^{b}	748.9±33.1^{a}

Different superscripts indicate significant differences ($P<0.05$) in the moisture and protein content between samples of the same group.

Table 6. Amino acid content expressed as grams of AA kg^{-1}of protein in molluscs and crustaceans.

	Molluscs													Crustaceans					
*Id.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Essential amino acids (g AA kg^{-1} protein)																			
Arg^{T}	148.6	138.9	118.1	184.0	156.5	231.0	189.0	230.9	201.6	145.0	147.6	223.6	89.0	128.2	108.1	112.0	111.7	113.8	91.4
His	16.0	21.0	17.5	19.4	15.8	21.3	17.1	18.0	17.7	24.5	21.9	21.0	19.5	23.8	27.6	26.8	24.7	30.9	21.8
Ile	36.0	37.8	35.6	34.9	31.2	36.5	37.5	33.2	36.7	44.0	42.2	37.3	43.0	34.7	39.2	34.7	36.9	40.2	42.1
Leu	65.4	63.5	57.6	55.3	50.5	63.8	64.3	60.0	64.1	69.2	64.1	61.3	76.6	59.9	64.9	58.0	60.6	66.1	75.0
Lys	81.0	74.9	87.6	64.9	73.0	51.7	72.5	54.6	66.9	65.2	72.7	74.3	73.5	59.6	78.4	63.3	67.9	54.4	81.6
Met	24.7	19.5	16.3	15.1	17.3	15.3	18.2	15.4	16.9	18.7	18.0	16.5	20.4	15.3	25.9	16.6	51.0	19.2	20.0
Phe	30.0	33.8	29.3	51.3	26.5	36.4	26.5	30.2	27.4	41.0	36.8	31.5	30.9	36.4	35.8	37.0	34.1	46.3	37.4
Thr	38.4	53.3	45.2	53.0	33.9	44.9	37.6	41.3	40.2	52.2	44.2	42.0	44.8	44.7	42.3	43.5	44.2	42.6	42.6
Val	35.9	43.5	39.3	38.8	31.0	35.2	36.4	32.8	35.9	43.2	42.9	39.5	41.0	38.9	42.0	40.2	42.8	47.5	45.2
Non essetial amino acids (g AA kg^{-1} protein)																			
Ala	50.1	50.1	51.6	47.6	44.0	43.6	51.0	44.0	49.2	44.1	45.3	42.1	63.5	48.3	41.6	54.9	53.7	53.9	68.2
Asp	106.1	81.6	103.5	87.5	89.9	75.8	102.4	76.6	101.0	84.3	102.9	92.1	102.4	82.5	99.4	86.7	97.9	90.4	113.4
Cys	22.2	42.0	21.3	28.2	23.2	18.5	21.4	17.6	20.2	39.5	44.8	30.8	19.0	21.5	23.5	23.3	24.0	26.2	20.5
Gly	48.2	78.7	76.8	93.3	79.2	69.6	62.7	57.6	70.1	52.0	45.3	43.6	86.5	71.5	55.1	54.2	62.1	97.3	56.4
Glu	143.4	104.6	125.4	109.2	118.3	110.0	142.4	110.5	140.0	112.0	120.0	117.5	145.0	115.3	146.0	114.9	125.2	115.7	165.8
Pro	51.5	36.9	37.9	32.6	35.9	37.7	35.8	35.4	37.1	32.5	32.5	33.8	50.7	38.6	55.9	47.9	51.8	43.3	35.6
Ser	41.2	55.5	59.5	47.0	44.4	44.7	46.2	40.7	47.7	48.1	49.0	49.9	44.3	40.7	45.3	38.3	40.9	45.4	43.2
Tyr	24.1	35.0	26.6	25.3	25.9	34.2	23.9	32.1	24.8	32.7	31.1	29.4	25.9	34.3	32.6	35.9	29.6	34.9	29.5
Tau	n.a.	n.a.	n.a.	n.a.	n.a.	74.3	64.7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	64.0	n.a.	n.a.

*Id.: 1-*L. gahi*; 2, 3, 4, 5-*M. galloprovincialis*; 6, 7-*O. vulgaris* (whole); 8, 9-*O. vulgaris* (muscle); 10, 11-*O. vulgaris* (digestive gland); 12-*O. vulgaris* (gonad); 13-*T. sagittatus*; 14, 15, 16, 17-*C. maenas*; 18-*Penaeus* sp.; 19-*P. clarkia*; ^{T}Including taurine; n.a. = not analysed.

Table 7. Amino acid content expressed as grams of AA kg^{-1} of protein in fishes.

*Id.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Essential amino acids (g AA kg^{-1} protein)																	
Arg^{T}	83.6	57.7	69.2	66.2	70.3	71.4	73.6	65.8	74.8	79.3	79.6	76.8	78.8	79.4	73.5	81.0	97.7
His	31.5	51.4	28.6	53.6	19.2	17.5	22.8	18.3	23.6	31.5	52.4	36.8	26.1	32.4	28.6	19.6	22.9
Ile	44.1	40.8	42.3	42.0	42.9	38.4	43.4	42.5	44.8	44.1	41.4	44.7	41.8	40.7	39.5	47.8	41.5
Leu	80.3	75.0	77.3	76.9	81.9	71.5	80.0	76.6	79.5	78.2	74.9	79.3	75.7	73.9	69.4	83.4	75.9
Lys	100.1	100.1	103.7	103.3	95.7	93.5	88.0	100.6	94.9	91.3	78.0	109.4	104.3	90.5	89.2	94.5	85.5
Met	28.0	25.6	28.6	27.1	27.9	18.2	26.8	28.5	26.5	28.8	28.3	30.5	29.9	26.8	25.6	27.4	28.7
Phe	33.9	31.6	34.3	35.4	37.8	33.0	42.9	35.6	32.9	38.9	45.7	35.4	34.6	35.8	35.4	37.2	46.7
Thr	47.7	41.1	46.5	44.2	45.0	41.9	47.9	37.9	45.2	48.9	47.6	45.5	45.6	44.7	38.0	45.9	47.5
Val	50.2	48.1	47.7	47.9	49.4	44.3	47.9	42.7	52.5	49.2	48.8	51.8	48.7	46.3	40.4	53.8	46.7
Non essetial amino acids (g AA kg^{-1} protein)																	
Ala	62.7	60.6	63.6	61.7	65.5	70.4	60.1	60.7	66.8	59.4	56.6	65.6	63.8	60.3	55.5	67.9	60.1
Asp	115.0	112.0	112.7	110.8	107.4	112.4	97.4	110.2	108.3	93.2	85.8	119.9	112.9	99.9	91.9	101.8	93.0
Cys	28.2	26.7	21.3	17.6	22.7	12.7	20.7	20.8	21.4	24.0	21.4	20.0	23.7	18.6	16.7	20.5	23.3
Gly	51.3	45.7	48.0	47.6	50.4	71.9	44.0	56.3	51.3	55.3	56.2	51.8	57.4	56.5	61.4	45.2	50.0
Glu	166.2	153.9	161.9	159.0	152.0	161.8	141.7	166.4	150.0	134.0	124.2	166.1	159.6	142.7	138.1	147.1	142.3
Pro	34.2	29.6	30.5	29.1	30.2	38.6	33.8	38.8	32.9	33.0	33.3	35.1	33.4	33.6	32.3	30.5	25.9
Ser	48.0	40.4	42.5	43.8	42.9	48.0	42.6	42.4	41.0	43.8	42.3	45.5	44.8	39.5	39.6	40.4	43.5
Tyr	29.3	26.8	30.4	30.2	30.3	21.1	42.6	26.1	25.3	35.2	35.9	28.7	29.8	26.5	23.7	29.5	38.6
Tau	23.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

*Id.: 20, 21, 22, 23-*B.boops*; 24, 25, 26, 27-*G. poutassou*; 28, 29-*Mugil* sp.; 30, 31-*S. pilchardus*; 32-*S. aurata*; 33, 34-*T. trachurus*; 35, 36-*G. minutus*. ^{T}Including taurine; n.a. = not analysed.

Table 8. Amino acid content expressed as grams of AA kg^{-1} of protein in plant and animal meals.

	Plant meals				*Animal meals*	
Id.	37 (Sunflower)	38 (Pea)	39 (Soybean)	40 (Wheat)	41 (Krill)	42 (Fish)
Essential amino acids (g AA kg^{-1} protein)						
Arg[T]	78.6	92.2	68.5	42.3	73.9	78.0
His	27.9	29.5	26.0	23.8	22.4	33.6
Ile	43.3	47.8	46.2	35.4	56.9	44.7
Leu	62.0	87.4	72.9	64.2	83.2	71.5
Lys	27.5	68.2	58.1	30.5	67.3	56.0
Met	18.4	10.7	9.6	11.9	29.6	25.8
Phe	59.3	59.3	54.8	49.3	53.0	62.2
Thr	37.2	34.2	37.2	30.2	48.9	45.4
Val	49.2	50.3	45.3	43.6	55.7	48.8
Non essetial amino acids (g AA kg^{-1} protein)						
Ala	40.5	40.8	40.6	37.5	58.0	56.2
Asp	81.8	117.7	115.3	52.7	105.5	79.9
Cys	41.7	24.7	20.2	37.0	6.7	17.7
Gly	65.2	27.4	42.5	44.5	48.7	67.7
Glu	177.9	163.3	181.8	285.3	131.7	113.6
Pro	84.6	42.5	71.1	107.3	40.3	89.1
Ser	44.2	51.8	50.5	46.8	43.3	37.7
Tyr	24.9	20.5	30.1	13.1	47.8	45.5

[T] Including taurine.

Figure 1(A-B). Amino acid ratios (%) for essential amino acids in molluscs (A: 1-*L. gahi*; 2, 3, 4, 5-*M. galloprovincialis*; 13-*T.sagittatus*) and crustaceans (B: 14, 15, 16, 17-*C. maenas*; 18-*Penaeus* sp.; 19-*P. clarkii*).

Figure 2(A-B). Amino acid ratios (%) for essential amino acids in fish (A: 20, 21, 22, 23-*B.boops*; 24, 25, 26, 27-*G. poutassou*. B: 28, 29-*Mugil* sp.; 30, 31-*S. pilchardus*; 32-*S. aurata*; 33, 34-*T. trachurus*; 35, 36-*G. minutus*.).

Figure 3. Amino acid ratios (%) for essential amino acids in vegetal and animal meals (37-Sunflower; 38-Pea; 39-Soybean; 40-Wheat; 41-Krill; 42-Fish).

Figure 4(A-D). Oser's index (OI), Chemical Score (CS) and limiting amino acid in mollusc (A: 1-*L. gahi*; 2, 3, 4, 5-*M. galloprovincialis*; 13-*T.sagittatus*), crustacean (B: 14, 15, 16, 17-*C. maenas*; 18-*Penaeus* sp.; 19-*P. clarkii*), fish (C: 20, 21, 22, 23-*B.boops*; 24, 25, 26, 27-*G. poutassou*. B: 28, 29-*Mugil* sp.; 30, 31-*S. pilchardus*; 32-*S. aurata*; 33, 34-*T. trachurus*; 35, 36-*G. minutus*) and meal (D: 37-Sunflower; 38-Pea; 39-Soybean; 40-Wheat; 41-Krill; 42-Fish) samples. *Arginine is the limiting amino acid for all fish samples.

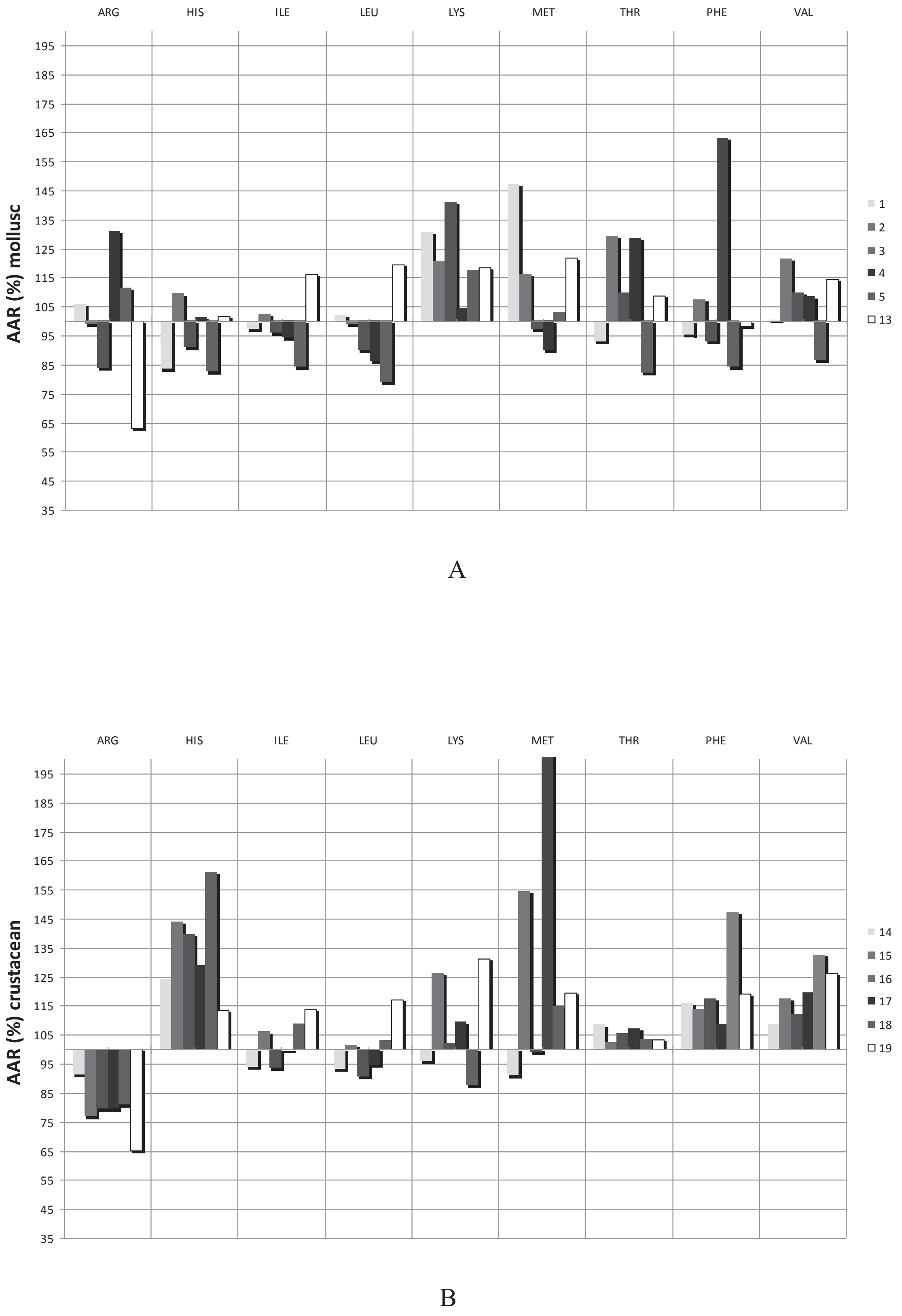
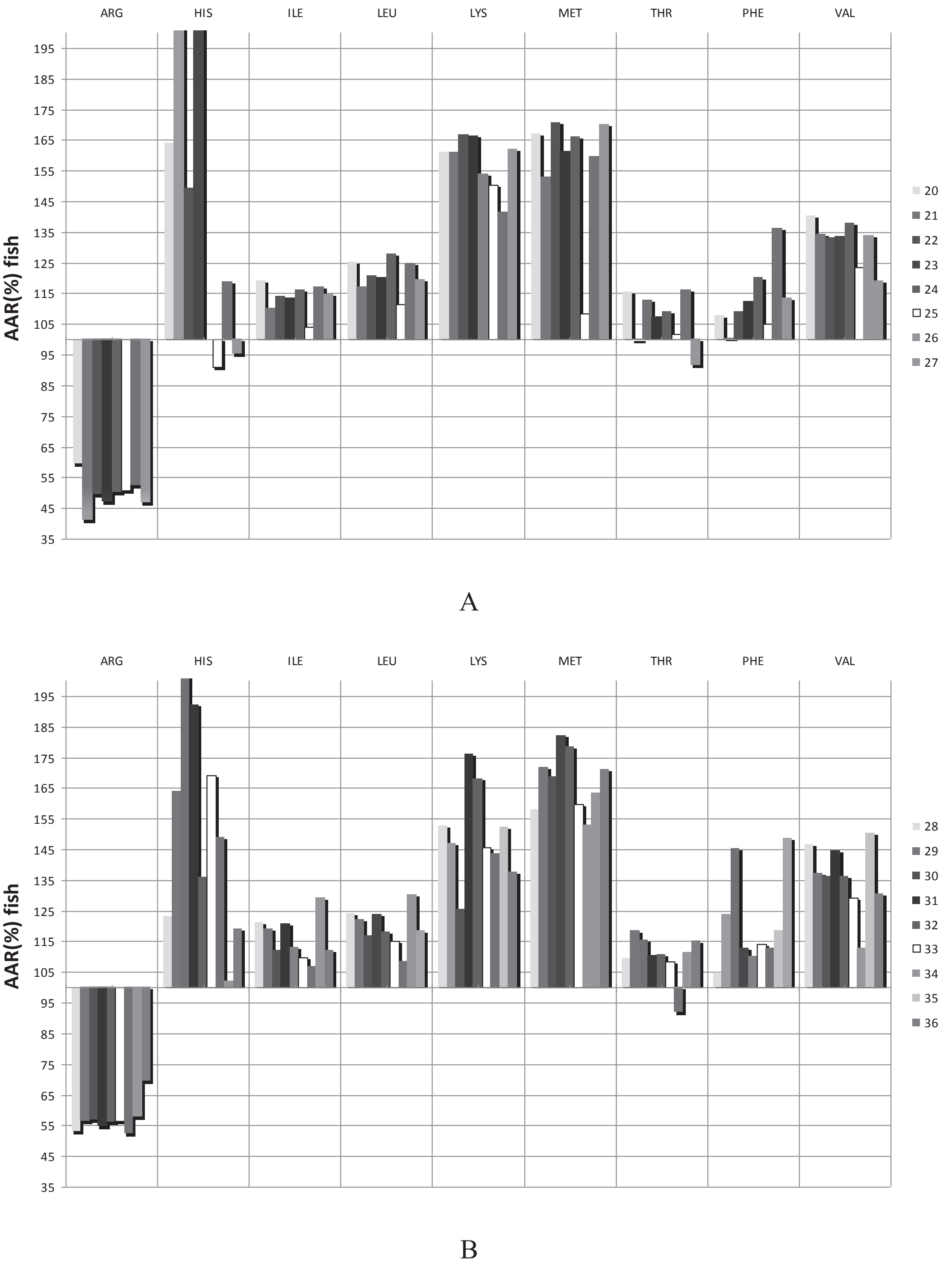

ARG
HIS
ILE
LEU
LYS
MET
THR
PHE
VAL
AAR (%) mollusc
195
185
175
165
155
145
135
125
115
105
95
85
75
65
55
45
35
1
2
3
4
5
13
A
AAR (%) crustacean
14
15
16
17
18
19
B

Figure 1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HIS
ILE
LEU
LYS
MET
THR
PHE
VAL
AAR(%) fish
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185
175
165
155
145
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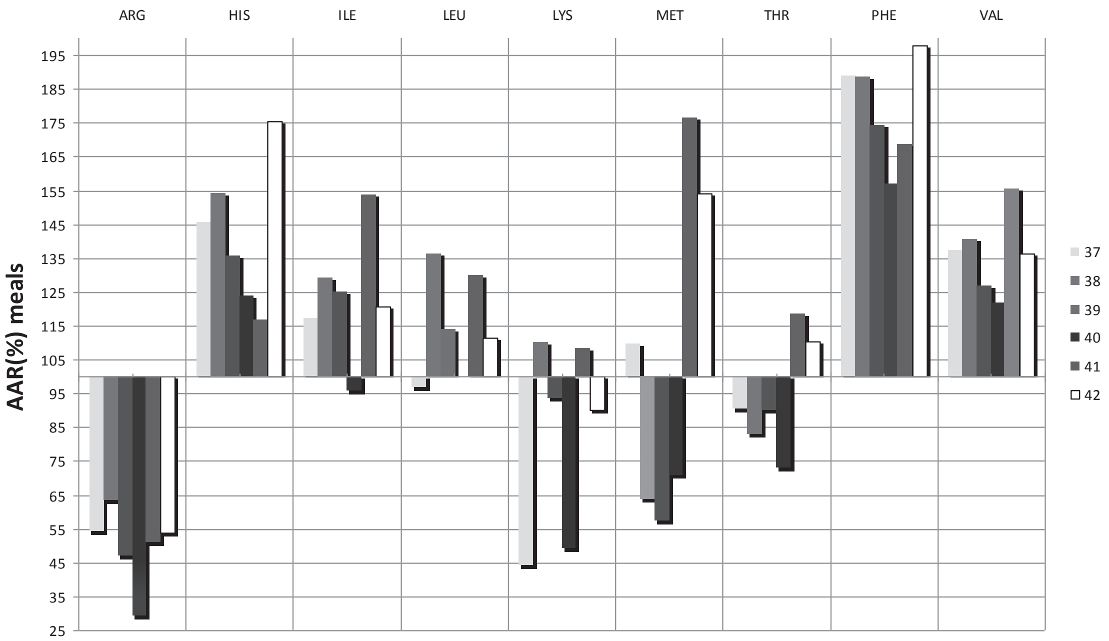
115
105
95
85
75
65
55
45
35
20
21
22
23
24
25
26
27
A
28
29
30
31
32
33
34
35
36
B

Figure 2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HIS
ILE
LEU
LYS
MET
THR
PHE
VAL
AAR(%) meals
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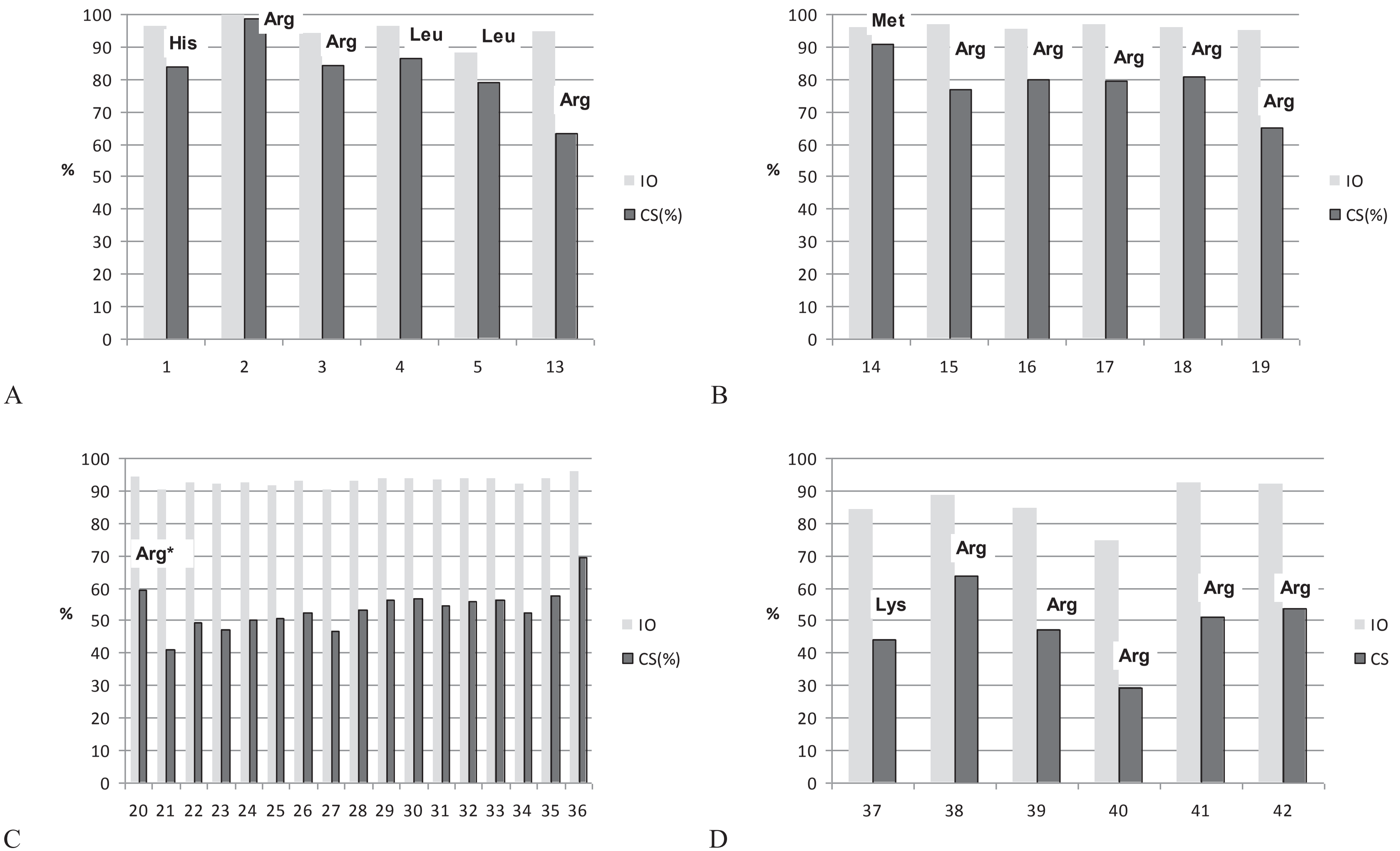
185
175
165
155
145
135
125
115
105
95
85
75
65
55
45
35
25
37
38
39
40
41
42

Figure 3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%
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80
70
60
50
40
30
20
10
0
1
2
3
4
5
13
His
Arg
Arg
Leu
Leu
Arg
IO
CS(%)
B
%
14
15
16
17
18
19
Met
Arg
Arg
Arg
Arg
Arg
IO
CS(%)
C
%
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
Arg*
IO
CS(%)
D
%
37
38
39
40
41
42
Lys
Arg
Arg
Arg
Arg
Arg
IO
CS

Figure 4