Nutritive value of four by-product meals as potential protein sources in diets for *Octopus maya*

Valor nutritivo de cuatro harinas de subproductos como fuentes potenciales de proteína en dietas para el pulpo *Octopus maya*

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Méndez Aguilar F. D., M. A. Olvera Novoa, S. Rodríguez Morales and C. Rosas Vázquez. 2014. Nutritive value of four by-product meals as potential protein sources in diets for *Octopus maya*. *Hidrobiológica* 24 (1): 69-77.

ABSTRACT

The nutritional value of four meals made from animal by-products of squid (SBM), tuna (TBM), poultry (PBM) and pork (POBM), and their potential use in diets for *Octopus maya* were evaluated. Lyophilized crab-meat meal (CRM) (*Callinectes sapidus*) was used as a reference for the nutritional requirements of the octopus. CRM had the highest crude protein (CP) content (847.2 g kg^{-1}) and the lowest lipid content (27.5 g kg^{-1}). SBM and PBM had more than 700 g kg^{-1} CP content, while TBM and POBM had less than 600 g kg^{-1}. Lipid content varied between 75.3 (SBM) and 127.2 (PBM) g kg^{-1}. Energy content of CRM was 17.5 MJ kg^{-1}, and the maximum difference with respect to the by-product meals did not exceed 3 MJ kg^{-1}. Amino acid analysis showed that SBM and PBM had high content of Thr, Ser and Ala, which are the most important amino acids for the metabolism of *O. maya*. Fatty acid analysis showed that they also had a high content of PUFA. PBM showed deficiencies in EPA and DHA content, but had a high content of linoleic acid, which can be a precursor of C:20 and C:22. Based on this, SBM and PBM have nutritional qualities which could be adequate for use as primary protein sources in artificial diets for *O. maya*.

Key words: Artificial diets, by-product meals, crab, culture, octopus.

RESUMEN

Se evaluó el valor nutricional de cuatro harinas de subproductos de calamar (HSCA), atún (HSAT), ave (HSAV) y cerdo (HSCE), así como su uso potencial en dietas para *Octopus maya*. Como referencia de los requerimientos nutricionales del pulpo se usó harina de carne de jaiba (*Callinectes sapidus*) liofilizada (HCJA). HCJA tuvo el mayor contenido de proteína cruda (PC) (847.2 g kg^{-1}) y el menor contenido de lípidos (27.5 g kg^{-1}). HSCA y HSAV tuvieron un contenido de PC de más de 700 g kg^{-1}, mientras que HSAT y HSCE tuvieron menos de 600 g kg^{-1}. El contenido de lípidos varió entre 75.3 (HSCA) y 127.2 (HSAV) g kg^{-1}. El contenido de energía de HCJA fue de 17.5 MJ kg^{-1}, y la diferencia máxima con respecto a las harinas de subproductos no fue mayor de 3 MJ kg^{-1}. El análisis de aminoácidos mostró que HSCA y HSAV tuvieron un alto contenido de Thr, Ser y Ala, los cuales son los aminoácidos más importantes para el metabolismo de *O. maya*. El análisis de ácidos grasos mostró que tales harinas tuvieron también un alto contenido de PUFA. HSAV

mostró deficiencias en EPA y DHA; sin embargo, tuvo un alto contenido de ácido linoleico, el cual puede actuar como un precursor de C:20 y C:22. De acuerdo a esto, HSCA y HSAV tienen un perfil nutricional adecuado para su uso como fuentes primarias de proteína en dietas artificiales para *O. maya*.

Palabras clave: Cultivo, dietas artificiales, harinas de subproductos, jaiba, pulpo.

INTRODUCTION

Octopod aquaculture has been developed for decades (Uriarte *et al.,* 2011). Octopuses have rapid growth, adaptability to conditions of captivity and high price in the market, reason why they are considered appropriate for culture. Specially, those species without a 'paralarval' phase are more suitable, because they have an embryonic development that culminates in the appearance of a holobenthic juvenile, which facilitates feeding from early developmental stages (Domingues *et al.,* 2007).

Octopus maya (Voss & Solís, 1966) has holobenthic development, is easily maintained on artificial systems, accepts artificial diets immediately after hatching and has a high international market price; thus, this species is among the best candidates for commercial culture (Rosas *et al.,* 2007). At present, culture remains experimental due to the high cost of natural food typically provided (Baeza-Rojano *et al*., 2010); nevertheless, recent studies have shown the potential of artificial diets for commercial production (Aguila *et al.,* 2007; Domingues *et al.,* 2007; Martínez *et al*., 2014; Rosas *et al*., 2014).

Octopuses require high levels of dietary protein because they use amino acids as their main energy substrate (George-Zamora *et al.,* 2011). They also have a low capacity to absorb lipids and carbohydrates (O'Dor *et al*., 1984), so their diet must meet these needs. In the wild, octopuses consume primarily crustaceans (Hanlon & Messenger, 1996), which have very high protein content (~850 g kg^{-1}) and low lipid content (~40 g kg^{-1}) (King *et al.,* 1990; Naczk *et al.,* 2004; Küçükgülmez *et al.,* 2006; Chen *et al.,* 2007). Under controlled conditions, this feed promotes the highest growth rates (Domingues *et al.,* 2007). Therefore, in order to replace natural food sources for octopus culture, several artificial diets with high protein content have been tested (Aguila *et al.,* 2007; Domingues *et al.,* 2007; Rosas *et al.,* 2007, 2012). These diets have been based on fish meal (FM) or fish protein concentrate (FPC). Both of these represent an excellent source of essential amino acids and fatty acids, especially polyunsaturated fatty acids (PUFA), which are required for the correct development of all cephalopods (Navarro & Villanueva, 2000). Nevertheless, the reduction on traditional fishing stocks and the consequent increase in price of FM have forced the aquaculture industry to find alternative protein sources (Tacon *et al*., 2006; Lara-Flores *et al.,* 2007). Recently, rendered by-products have been identified as one of those potential sources, because globally, livestock, fishing, and aquaculture industries generate a large quantity of wastes such as scraps meat, offal, bone, skin, etc. (Hardy *et al.,* 2005). These by-products can be used in the manufacture of high-quality meals, where depending on their origin, appear adequate for the replacement of FM in diets for fish (Abdel-Warith *et al.,* 2001; Liu *et al.,* 2004; Fasakin *et al.,* 2005; Rawles *et al.,* 2006) and crustaceans (Forster *et al.,* 2003; Hernández *et al.,* 2008; Samocha *et al.,* 2004). Although at the date there we have no references about the use of those ingredients in diets for octopus species, they seem to have a great potential because of their high nutritional value, their ready availability, and low cost. Accordingly, the aim of this study was to evaluate the potential use of by-product meals from the fishing and livestock industries as primary protein sources in artificial diets for *O. maya.* The proximate composition, energy content, soluble protein, amino acids profile and fatty acids profile were compared with those of crab meal (*Callinectes sapidus, Rathbun, 1896*) as a reference for the nutritional requirements of the octopus.

MATERIALS AND METHODS

Origin of the ingredients and sample handling. Adult specimens of the crab *C. sapidus* (10 females and 10 males) were caught on the coast of Sisal, Yucatán, Mexico, and transported to the laboratory of the Unidad Multidisciplinaria de Docencia e Investigación de la Universidad Nacional Autónoma de México, in the same locality. Crab meat obtained from breast (Bm) and claws (Cm), as well as visceral content including gills, hepatopancreas, and gonad (Vc) were analyzed individually. A portion was taken from each section of fresh tissue to determine the moisture. The rest was lyophilized for 48 h (Labconco FreeZone 2.5) for analyzes. Each sample of each section of the 20 crab specimens was analyzed independently to test whether their proximate composition varied between sex and between tissue types. Due to similarity between Bm and Cm observed during early assessment, they were mixed to form a muscle crab meal (CRM), which was used as the reference ingredient. Fishing by-product meals (squid SBM and tuna TBM by-product meals) were obtained from the national fishing industry while those from the livestock industry (poultry PBM and pork POBM by-product meals) were acquired from the National Renderers Association.

Proximate composition. Crude protein (CP, N × 6.25) and energy were determined using a carbon nitrogen analyser (CN) (*Thermo Quest FlashEA-1112 Series*). The rest of the analyses were carried out following standard methods (AOAC, 2000): lipid content

by the Soxhlet method (method AOAC 948.22), ash by calcinations in a muffle furnace at 600 °C (method AOAC 942.05), and nitrogen free extract (NFE) by difference. Moisture was determined using a moisture analyser (*Ohaus MB35*). In the crabs, the analyses were performed on different parts of each animal individually (N = 20), while in the meals, these were determined in triplicate. In addition, the content of soluble protein (SP) in the meals was determined and quantified in diluted homogenates (Bradford, 1976) using a Bio-Rad protein determination kit (Bio-rad@-500-0006). The absorbance of the samples was read at 595 nm in a micro-plate reader (model 550 Bio-rad).

Amino acids (AA) profile. Defatted samples of meals (20 mg) were hydrolyzed with 200 µl of HCl 6 N and phenol at 0.06% in a sealed vial and heated at 110 °C for 24 h, and the Waters AccQ-Tag ™ procedure was subsequently followed (George-Zamora *et al.*, 2011). The analysis was performed by High Pressure Liquid Chromatography (HPLC) in a Waters instrument equipped with a reverse phase column (3.9 × 150 mm) 4 µm Nova Pak C-18 and fluorescence detector (wavelength excitation and emission of 250 and 395 nm respectively). All analyses were performed in triplicate.

Fatty acids (FA) profile. For analyses, fat from 100 mg of meal sample was extracted using the Soxhlet method. The fat obtained was saponified with a 20% KOH:Metanol (w:v) solution, and the free FA were recovered in hexane from the acidified saponifiable fraction (pH 1–2). The FA methyl esters (FAMEs) were obtained by esterification with BF_3 at 10% in methanol solution (boron/methanol tri-fluoride solution, Fluka, 15716) for 60 min at 80 °C. FAMEs were subsequently analyzed by Capillary Gas Chromatography in a Perkin Elmer Clarus 500 GC equipped with a Perkin Elmer Elite-WAX capillary column (film of 30 m × 0.25 mm × 0.25 µm, crossed link - PEG) and a flame ionization detector. Hydrogen was used to deliver the gas at a flow rate of 40 ml min^{-1}. The temperature injector and detector were programmed to 280 °C and 250 °C, respectively. Temperature of the column was programmed from 40 to 200 °C with an increase of 20 °C min^{-1} and from 200 to 250 °C with an increase of 2.5 °C min^{-1}. The individual FAMEs were identified by comparing the retention times with a reference standard (Supelco 37 Comp. FAME Mix, 47885-U). Results were reported as percentage area of the total FA identified. All the analyses were performed in triplicate.

Statistical analyses. Data from crab tissues were compared by two-way ANOVA ($p < 0.05$) to identify possible interactions between sex and kind of tissues, whereas data of meals were compared by one-way ANOVA ($p < 0.05$). Differences among samples were identified using a Tukey-Kramer test. For the analyses, amino acids (AA) were grouped as essential (EAA) and no essential (NEAA) amino acids, and fatty acids (FA) as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. In all the analyses homogeneity of variance (F test for equality of variances) and normality (Shapiro-Wilks test) of data was verified. All analyses were performed using the Info-Stat 1.1 Software.

RESULTS

Proximate composition. The proximate composition of *C. sapidus* did not show significant differences between sexes, except for the lipid content in Vc. Lipid content in Vc was higher in females, therefore, energy content was also higher ($p < 0.05$). Proximate compositions in the muscle tissues (Bm and Cm) were significantly different from those observed in Vc ($p < 0.05$). The greatest CP content was observed in the muscle tissues, with a mean of 847.2 ± 2.6 g kg^{-1}, compared with 537 ± 8.5 g kg^{-1} in Vc. In contrast, low values of lipids and ashes were found in muscle tissue (mean value of 27.5 ± 6.9 g kg^{-1} and 81.4 ± 3.2 g kg^{-1}, respectively), compared with those observed in Vc (280 ± 52.2 g kg^{-1} and 112 ± 0.6 g kg^{-1} respectively) (Table 1).

Crab meal used as reference ingredient (formed from the mixture of muscle tissues) showed higher CP and SP values (847.2 ± 2.6 g kg^{-1}, 67.6 ± 10.5 mg g^{-1}, respectively) than those obtained in the commercial ingredients ($p < 0.05$; Table 2). Among by-product meals, CP content was higher in PBM and SBM (737.7 ± 8.1 and 705.9 ± 20 g kg^{-1}, respectively), than in TBM and POBM (592.9 ± 13.2 g kg^{-1} and 560.9 ± 36.1 g kg^{-}1, respectively) ($p < 0.05$). On other hand, SP showed no differences among all by-product meals, averaging 2.2 ± 0.2 mg g^{-1}. The lower lipids content of all ingredients was observed in CRM, with 27.5 ± 6.9 g kg^{-1} ($p < 0.05$; Table 2). An intermediate value was observed in SBM (75.3 ± 2.1 g kg^{-1}), while the rest of the meals (TBM, PBM and POBM) averaged 126.5 ± 0.9 g kg^{-1} of lipids (Table 2). Energy content of ingredients oscillate between 14.5 ± 0.6 MJ kg^{-1} (POBM) and 18.5 ± 0.1 MJ kg^{-1} (PBM) (Table 2). Energy content in CRM was similar to SBM and PBM, but different from TBM and POBM ($p < 0.05$; Table 2).

Amino acids (AA) profile. The results of the AA profile are showed in Table 3. Total EAA content in CRM was 43.7 ± 0.3 g 100 g^{-1} protein and this value had no differences from either SBM or PBM. Low EAA levels were recorded in TBM and POBM with 35.9 ± 1 g 100 g^{-1} protein and 31.2 ± 0.7 g 100 g^{-1} protein, respectively ($p < 0.05$; Table 3). On the other hand, NEAA were more abundant in PBM and POBM (55.8 ± 0.5 and 55.4 ± 0.3 g^{-1} protein, respectively) than in the other ingredients (*p> 0.05*; Table 3). In all by-product meals, Leu was the most abundant EAA, while Glu was the most abundant into the NEAA (Table 3).

Fatty acids (FA) profile. The FA profile of the meals is shown in Table 4. Saturated fatty acids (SFA) were the most abundant FAs in all meals, with maximum values in CRM (52.9% ± 1.1) and POBM (52.4% ± 2.3) ($p < 0.05$). The lowest and highest MUFA values were observed in CRM and PBM, with a mean of 23.8% ± 0.4 and 47.9% ± 0.3, respectively ($p < 0.05$). The less abundant class of all FAs were PUFA, with values between 2.3% ± 0.4 (POBM) and 23.2%

Table 1. Proximate composition of the different sections of the crab *C. sapidus* (g kg^{-1} dry matter). Energy MJ kg^{-1}

	Breast meat (Bm)		Claw meat (Cm)		Visceral content (VC)	
	Male	Female	Male	Female	Male	Female
Moisture	838.5^{a} ± 1.1	836.9^{a} ± 2.8	835.1^{a} ± 1.4	835.9^{a} ± 4.4	812.2^{b} ± 4.7	813.8^{b} ± 11.2
Crude Protein	847.5^{b} ± 20.7	846.7^{b} ± 19.5	844.1^{b} ± 22.0	850.4^{b} ± 29.9	531.0^{a} ± 7.50	543.0^{a} ± 12.3
Lipids	30.2^{a} ± 3.5	35.9^{a} ± 9.3	22.6^{a} ± 5.0	21.1^{a} ± 1.8	243.0^{b} ± 43.8	316.8^{c} ± 41.5
Ash	77.2^{a} ± 4.6	83.7^{a} ± 6.5	80.7^{a} ± 4.9	84.0^{a} ± 3.4	111.7^{b} ± 6.4	112.5^{b} ± 3.0
NFE	45.1	33.7	52.6	44.5	114	27.7
Energy	17.07^{b} ± 0.9	17.40^{b} ± 0.7	17.38^{b} ± 0.51	17.73^{b} ± 1.22	13.00^{a} ± 0.65	20.63^{c} ± 0.19

Values are means ± SD (n = 20). NFE Nitrogen-free extract. Values with different superscripts are significantly different ($p<0.05$).

± 0.7 (CRM). The total PUFA content of fishery origin by-product meals (CRM, SBM and TBM) was significantly higher than the observed in livestock origin by-product meals (PBM and POBM) ($p < 0.05$, Table 4). In PUFA, the highest level of eicosapentanoic acid (EPA, *C20:5n3*) was recorded in CRM (9.2% ± 0.2) while the highest level of docosahexanoic acid (DHA, *C22:6n3*) was recorded in TBM (12.3% ± 0.7) ($p < 0.05$, Table 4). It is interesting to note that very low values of EPA and DHA were registered in PBM and POBM ($p < 0.05$, Table 4).

DISCUSSION

Proximate composition. Proximate composition of the blue crab *C. sapidus* was very similar to that found for other crab species. Elevated levels of protein (830 to 890 g kg^{-1} on dry basis) and low levels of lipids (10 to 50 g kg^{-1} on dry basis) have been reported for *C. sapidus* on the Mediterranean (Küçükgülmez *et al*., 2006), and for other species such as *Carcinus maenas* (Linnaeus, 1758) in Canada (Naczk *et al.,* 2004), *Cancer magister* (Dana, 1852) at the northwest coasts of EUA (King *et al*., 1990), or *Eriocheir sinensis* (Edwards, 1853) in China (Cheng *et al*., 2007). Also, high levels of lipids in the visceral mass are reported for *Portunus pelagicus* (Linaeus, 1758) (345 g kg^{-1}; Wu *et al*., 2010) and *E. sinensis* (584 g kg^{-1}; Chen *et al.,* 2007). Interestingly, different populations of crustaceans maintain a very similar proximate composition worldwide, which could be considered as a reference in studies related to nutritional requirements of cephalopods, because of their preference for these prey. In contrast, the nutritional quality of the by-product meals directly depends on the material used for its manufacture. Still, results found in this study are similar to those observed for these kinds of by-product meals, according to the reports from other authors (Hertrampf & Piedad-Pascual, 2000; Civera *et al*., 2006; Uyan *et al.,* 2006; Hernández-González, 2008).

Several by-product meals include high contents of bones and cartilage in its composition, which can increase the ash content and thus affect its quality when these exceed 150 g kg^{-1} (Akiyama *et al*., 1989; Ricque *et al*., 1998). This is because elevated ash content can affect digestibility, as observed in shrimp and fish (Akiyama *et al*., 1989). For *Octopus maya*, is known that a reduction in the digestibility can limit the nutrients absorption and in consequence the growth rate (Rosas *et al*., 2012). For this reason, TBM and POBM which exceeded an ash content of 230 g kg^{-1}, could not be recommended to made octopus diets.

In cephalopods, it has been suggested than high lipid values (<215 g kg^{-1}) may affect growth rates, either because this inter-

Table 2. Proximate composition (g kg^{-1} dry matter), soluble protein (mg g^{-1}) and energy (MJ kg^{-1}) of the different by-product meals and crab meal.

	CRM	SBM	TBM	PBM	POBM
Moisture	56.2^{a} ± 2.00	67.4^{c} ± 0.50	73.6^{d} ± 0.20	62.9^{b} ± 0.70	65.7^{b,c} ± 0.70
Crude Protein	847.2^{c} ± 2.60	705.9^{b} ± 20.00	592.9^{a} ± 13.20	737.7^{b} ± 8.10	560.9^{a} ± 36.10
Lipids	27.5^{a} ± 6.90	75.3^{b} ± 2.10	125.5^{c} ± 2.00	127.2^{c} ± 0.90	126.7^{c} ± 0.00
Ash	81.4^{b} ± 3.20	61.7^{a} ± 2.60	234.7^{d} ± 4.20	115.0^{c} ± 5.70	305.0^{e} ± 6.20
NFE	43.9	157.1	46.9	20.1	7.4
Soluble Protein	67.56^{b} ± 10.50	1.89^{a} ± 0.23	2.38^{a} ± 0.98	2.4^{a} ± 0.23	2.23^{a} ± 0.10
Energy	17.54^{c,d} ± 0.54	16.97^{b,c} ± 0.29	15.99^{b} ± 0.33	18.46^{d} ± 0.11	14.49^{a} ± 0.63

Values are means ± SD (n = 3). NFE Nitrogen-free extract. Values with different superscripts are significantly different *($p<0.05$).* CRM: Crab meal; SBM: Squid meal; TBM: Tuna meal; PBM: Poultry meal; POBM: Pork meal.

Table 3. Amino acids profile (g 100 g^{-1} protein) of the different by-product meals and crab meal.

	CRM	SBM	TBM	PBM	POBM
Hys	2.23 ± 0.07	4.17 ± 0.18	2.98 ± 0.48	2.41 ± 0.14	1.57 ± 0.01
Thr	5.35 ± 0.00	4.85 ± 0.61	3.74 ± 0.16	6.86 ± 0.00	4.32 ± 0.09
Arg	7.37 ± 0.00	5.04 ± 0.93	3.43 ± 0.05	5.49 ± 0.00	4.79 ± 0.03
Val	3.59 ± 0.05	3.99 ± 0.09	3.81 ± 0.45	4.93 ± 0.00	3.22 ± 0.03
Met	2.73 ± 0.03	2.74 ± 0.02	2.59 ± 0.16	2.06 ± 0.08	1.49 ± 0.00
Lys	7.95 ± 0.09	6.57 ± 0.54	5.60 ± 0.29	6.23 ± 0.06	5.02 ± 0.00
Ile	3.85 ± 0.06	3.38 ± 0.39	2.37 ± 0.11	4.49 ± 0.03	2.55 ± 0.14
Leu	7.12 ± 0.08	7.35 ± 0.15	7.45 ± 0.34	7.62 ± 0.10	5.96 ± 0.00
Phe	3.53 ± 0.07	3.36 ± 0.21	3.93 ± 0.20	3.98 ± 0.07	2.71 ± 0.06
Total EAA	$43.72^{c,d}$ ± 0.34	41.45^{c} ± 1.41	35.91^{b} ± 1.04	44.07^{d} ± 0.49	31.23^{a} ± 0.72
Asp	9.46 ± 0.16	9.74 ± 0.08	7.31 ± 0.44	8.40 ± 0.05	7.25 ± 0.02
Ser	3.83 ± 0.09	4.33 ± 0.64	3.13 ± 0.10	4.53 ± 0.07	3.00 ± 0.06
Glu	13.97 ± 0.11	13.16 ± 0.61	12.90 ± 0.85	17.80 ± 0.00	12.26 ± 0.00
Gly	8.81 ± 0.13	5.86 ± 0.25	7.66 ± 0.31	9.49 ± 0.12	15.28 ± 0.03
Ala	5.91 ± 0.12	6.45 ± 1.19	6.78 ± 0.24	6.97 ± 0.07	8.35 ± 0.03
Pro	4.28 ± 0.19	3.92 ± 0.00	4.21 ± 0.23	6.09 ± 0.32	7.67 ± 0.13
Tyr	3.40 ± 0.06	3.22 ± 0.02	2.36 ± 0.11	2.60 ± 0.04	1.58 ± 0.02
Total NEAA	49.64^{b} ± 0.50	$46.69^{a,b}$ ± 2.52	44.36^{a} ± 1.05	55.88^{c} ± 0.48	55.39^{c} ± 0.28

Values are means ± SD (n = 3). Values with different superscripts are significantly different ($p < 0.05$). CRM: Crab meal; SBM: Squid meal; TBM: Tuna meal; PBM: Poultry meal; POBM: Pork meal. EAA: Essential amino acids. Hys: Hystidine; Thr: Threonine; Arg: Arginine; Val: Valine; Met: Methionine; Lys: Lysine; Ile: Isoleucine; Leu: Leucine; Phe: Phenylalanine. NEAA: No essential amino acids. Asp: Asparagine; Ser: Serine; Glu: Glutamine; Gly: Glycine; Ala: Alanine; Pro: Proline; Tyr: Tyrosine.

feres with the absorption of proteins or because of the low capacity of these organisms to metabolize lipids (Domingues *et al.,* 2007; García-García & Cerezo-Valverde, 2006). In this sense, Petza *et al* (2011) showed that, *O. vulgaris* (Lamarck, 1798) fed with a sardine diet with 590 CP and 340 g kg^{-1} lipids, have protein retention efficiency even better than a shrimp diet (900 CP and 50 g kg^{-1} lipids). Also, sardines promoted better feeding efficiency and a similar or even higher growth rate than other natural or artificial diets, demonstrating that the assimilation and use of lipids by the octopuses could be more related to the quality of fatty acids than their quantity (Petza *et al.,* 2011).

The low SP content observed in by-product meals are related to the manufacturing process. In these commercial meals, the raw materials are washed, cooked, pressed, and dried by heat in order to stabilize them. This process allows the myofibrillar protein to be concentrate, but could also provoke the loss of soluble nutrients. Meanwhile, lyophilization used to obtain CRM, allows all sarcoplasmatic protein and other soluble components such as pigments, amines, vitamins, and enzymes to be retained. For finfish, the SP is related to the digestibility and attractiveness of formulated diets and its total or partial loss can decrease the quality of those diets (Montero & Gómez-Guillén, 1998). In artificial diets for *O. maya*, it has been observed that the FPC can have a beneficial effect, possibly due to its high content of di-peptides and free AAs (Aguila *et al.,* 2007; Martínez *et al*., 2014); nevertheless, no evidence suggests that SP is strictly required in cephalopod diets.

Amino acids (AA) profile. The AA profile of *C. sapidus* was very close to that found by other authors (Küçükgülmez *et al.,* 2006; Aguila *et al.,* 2007), as well as those of the commercial meals (Hernández-González, 2008). This suggests a constant quality that could be appropriate to formulate balanced diets.

In cephalopod diets, not only the quantity but also the quality of protein is an essential factor to achieve adequate growth rates of organisms (Aguila *et al.,* 2007; Domingues *et al.,* 2009). This is especially true in octopuses, because they have low lipid reserves and during fasting conditions they mobilize certain AAs as an energy source to attempt to maintain homeostasis (Villanueva *et al.,* 2004; Solórzano *et al.,* 2009; George-Zamora *et al.,* 2011). In *O. vulgaris*, Leu, Lys and Arg represent half of the EAA required during early life stages (Villanueva *et al.,* 2004), whereas Thr has been considered as a limiting AA in cephalopod diets, because they are used as substrate to produce energy during gluconeogenesis

Table 4. Fatty acids profile (g/100 g fatty acid detected) of the different by-product meals and crab meal.

Kind of FA	CRM	SBM	TBM	PBM	POBM
C8:0	0.04 ± 0.02	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.06 ± 0.01
C10:0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.30 ± 0.07
C11:0	0.07 ± 0.02	0.09 ± 0.03	0.04 ± 0.00	0.09 ± 0.00	0.12 ± 0.00
C12:0	0.22 ± 0.03	0.14 ± 0.11	0.14 ± 0.00	0.17 ± 0.02	0.29 ± 0.06
C13:0	0.19 ± 0.06	0.24 ± 0.01	0.10 ± 0.03	0.10 ± 0.00	0.15 ± 0.02
C14:0	2.31 ± 0.06	7.03 ± 0.66	4.82 ± 0.11	1.04 ± 0.00	3.50 ± 0.46
C15:0	1.32 ± 0.03	1.04 ± 0.03	1.42 ± 0.05	0.14 ± 0.01	0.35 ± 0.32
C16:0	25.49 ± 0.80	20.22 ± 1.51	27.05 ± 1.21	26.27 ± 0.71	24.55 ± 2.42
C17:0	2.49 ± 0.03	1.06 ± 0.01	1.69 ± 0.09	0.19 ± 0.01	1.39 ± 0.20
C18:0	11.96 ± 0.56	5.77 ± 0.73	7.79 ± 0.31	7.88 ± 0.46	20.98 ± 0.89
C20:0	0.65 ± 0.02	0.37 ± 0.08	0.51 ± 0.01	0.40 ± 0.06	0.51 ± 0.08
C21:0	7.02 ± 0.13	1.10 ± 0.09	2.04 ± 0.25	0.54 ± 0.04	0.03 ± 0.01
C22:0	0.40 ± 0.01	0.32 ± 0.05	0.38 ± 0.02	0.07 ± 0.00	0.05 ± 0.02
C23:0	0.70 ± 0.07	0.12 ± 0.04	0.42 ± 0.01	0.06 ± 0.05	0.03 ± 0.02
C24:0	0.10 ± 0.01	0.13 ± 0.03	0.29 ± 0.00	0.01 ± 0.01	0.06 ± 0.01
Total SFA	52.99^{c} ± 1.07	37.69^{a} ± 3.07	46.71^{b} ± 1.49	37.02^{a} ± 1.14	52.37^{c} ± 2.31
C14:1	0.08 ± 0.00	0.10 ± 0.01	0.14 ± 0.02	0.20 ± 0.01	0.51 ± 0.06
C15:1	0.06 ± 0.02	0.11 ± 0.02	0.03 ± 0.00	0.08 ± 0.01	0.10 ± 0.03
C16:1	6.56 ± 0.13	6.57 ± 0.19	8.69 ± 0.00	6.81 ± 0.29	2.70 ± 0.36
C17:1	0.18 ± 0.07	0.39 ± 0.37	0.10 ± 0.07	0.15 ± 0.00	0.55 ± 0.10
C18:1n9c/t	15.38 ± 0.23	24.36 ± 1.32	22.33 ± 0.40	40.20 ± 0.03	37.42 ± 0.46
C20:1n9	1.35 ± 0.09	8.36 ± 0.45	1.64 ± 0.07	0.34 ± 0.01	0.30 ± 0.03
C22:1n9	0.14 ± 0.00	1.28 ± 0.03	0.28 ± 0.00	0.09 ± 0.01	0.03 ± 0.01
C24:1n9	0.04 ± 0.03	0.51 ± 0.01	0.73 ± 0.05	ND	ND
Total MUFA	23.78^{a} ± 0.36	41.67^{c} ± 1.23	33.95^{b} ± 0.48	47.87^{d} ± 0.26	41.6^{c} ± 1.04
C18:2n6c	5.68 ± 0.24	1.73 ± 0.01	1.70 ± 0.11	13.92 ± 0.63	1.73 ± 0.39
C18:2n6t	0.18 ± 0.06	ND	0.32 ± 0.00	0.13 ± 0.13	0.17 ± 0.02
C18:3n6	0.24 ± 0.03	0.12 ± 0.01	0.16 ± 0.02	0.17 ± 0.00	0.01 ± 0.01
C18:3n3	1.16 ± 0.04	0.66 ± 0.06	0.39 ± 0.20	0.54 ± 0.02	0.20 ± 0.02
C20:2	0.97 ± 0.15	0.31 ± 0.02	0.58 ± 0.26	0.13 ± 0.01	0.03 ± 0.00
C20:3n6	0.37 ± 0.02	0.17 ± 0.00	0.21 ± 0.00	0.14 ± 0.00	0.06 ± 0.00
C20:3n3	0.03 ± 0.01	ND	0.01 ± 0.01	ND	ND
C20:4n6 (AA)	0.11 ± 0.00	0.16 ± 0.03	0.12 ± 0.01	ND	ND
C20:5n3 (EPA)	9.18 ± 0.16	5.38 ± 0.48	3.41 ± 0.22	0.03 ± 0.03	0.04 ± 0.01
C22:2	0.04 ± 0.03	0.22 ± 0.02	0.11 ± 0.04	ND	ND
C22:6n3 (DHA)	5.28 ± 0.04	11.88 ± 1.24	12.32 ± 0.70	0.05 ± 0.05	0.02 ± 0.00
Total PUFA	23.23^{d} ± 0.71	$20.63^{c,d}$ ± 1.85	19.33^{c} ± 1.02	15.11^{b} ± 0.88	2.26^{a} ± 0.43
Total n3	15.64 ± 0.24	17.92 ± 1.78	16.13 ± 1.12	0.63 ± 0.10	0.26 ± 0.03
Total n6	6.57 ± 0.35	2.18 ± 0.03	2.51 ± 0.11	14.35 ± 0.76	1.97 ± 0.40
n3/n6	2.38 ± 0.09	8.21 ± 0.70	6.41 ± 0.15	0.04 ± 0.00	0.13 ± 0.01
DHA/EPA	0.57 ± 0.01	2.21 ± 0.03	3.61 ± 0.03	1.69 ± 0.00	0.55 ± 0.11

Values are means ± SD (n = 3). Values with different superscripts are significantly different ($p< 0.05$). ND are not detected fatty acids. CRM: Crab meal; SBM: Squid meal; TBM: Tuna meal; PBM: Poultry meal; POBM: Pork meal.

(Akagi & Ohmori, 2004). George-Zamora *et al* (2011) determined that in *O. maya*, Thr, Ser, and Ala, represent the main AAs that are used as metabolic fuel during starvation periods. In this study, all analyzed by-product meals presented high Thr, Ser, and Ala levels, similar to or even greater than those found in the muscle of *O. maya* (3.7, 3.7, and 4.1 g 100 g^{-1} protein for the same AA respectively) (Domingues *et al.,* 2007). However, it is interesting to note that SBM and PBM appear to meet nearly all the principal AA requirements for octopuses. They have a higher Leu content than CRM, as well as the highest values of Lys, Arg, Thr, and Ser of all by-product meals. Also, they have the highest EAA content of all commercial meals, which does not differ from that of CRM. For this reason, SBM and PBM could be considered as appropriated protein sources for *O. maya* diets.

Fatty acid (FA) profile. The FA profile of aqua feeds is important since they (and especially PUFAs), play diverse roles in the physiological and biochemical processes within aquatic animals (Izquierdo, 1996; Halver & Hardy, 2002; Glencross, 2009). For example, in cephalopods, PUFA constituted more than 50% of all FA content, since DHA and EPA are required in large quantities, primarily during the first development stages. It was observed in *O. vulgaris*, *S. officinalis* and *Loligo vulgaris*, that DHA represent between 21 and 33% of their FA composition, while EPA ranges between 13 and 15% (Navarro & Villanueva, 2000). In addition to their concentration, the DHA/EPA ratio is also important for the correct development of most marine species. In cephalopods, high DHA/EPA ratios have been observed in both hatchling cuttlefish (2.3) and octopus eggs (2.6), which reflect their natural FA profile (Navarro & Villanueva, 2000). In the present study, CRM had the highest PUFA content, wherein DHA and EPA were the most abundant FAs in that ingredient. However, similarly high PUFA contents were also found in SBM and TBM, which had more than twice DHA content in comparison with CRM. Although these differences imply a significant increase of the DHA/EPA ratio (and therefore a deviation from the reference profile), in other marine species (fish, for example), an increase in the DHA/EPA ratio can improve the growth rates (Izquierdo, 1996). In the by-product meals of livestock origin, only PBM showed a high content of PUFA, but it was no derived neither DHA nor EPA. The use of poultry oil has been tested in diets for other species with specific DHA and EPA requirements (as salmon or trout) with no adverse effects observed on growth rate, feeding intake, feeding efficiency, or survival of fish (Glencross, 2009). These trials demonstrated that the tissue fatty acids of the fish were reflective of their diet, except for levels of DHA and EPA, which appeared to be regulated at constant levels. Salmonids are known to have the capacity to meet their EFA requirements from linoleic and linolenic acids, since they are capable of use as precursors to elongate C:20 and C:22 FA. Presence of similar elongases and desaturases has been reported for other species of marine fish or freshwater (Agaba *et al.,* 2005), and recently, also for octopus (Monroig *et al.,* 2012). Interestingly, analysis of the PBM showed that about 14% of FAs identified correspond to the linoleic acid (C18:2n6c), suggesting that this can represent a potential source of EFA for *O. maya*.

In summary, all analyzed by-product meals in this study had a high CP content (>500 g kg^{-1}) and adequate lipid values (<215 g kg^{-1}). However, SBM and PBM had the highest CP values (>700 g kg^{-1}) but also a low ash content (<150 g kg^{-1}). Also, these by-product meals showed the highest values of all key AA used by *O. maya*. Although only the fishery by-product meals had a high DHA and EPA content, PBM was rich in linoleic acid, which represent a precursor of those essential fatty acids. Still, the lack of certain EFA in formulated diets is usually corrected by the addition of either fish oil or other rich compounds in this FA, which allows the use of good quality ingredients despite of deficiencies. On this basis, SBM and PBM could represent a viable option for use as low-cost ingredients in artificial diets *O. maya,* which also are a source of AA and EFA good quality. Further studies should evaluate digestibility of the sole ingredients or formulated diets, as well as the growth response in trials with live animals.

ACKNOWLEDGEMENTS

The present study was partially financed by DGAPA-UNAM project IN212012, and CONACyT CB-2010-01: 150810 to C. Rosas. Thanks are given to CONACyT and CINVESTAV-IPN Mérida for financial support to F. D. Méndez-Aguilar. Thanks are also given to Dra. Elsa Noreña and Dra. Laura Espinosa, for their help on the laboratory analysis; and to Dr. Pedro Gallardo and PhD Becky Williams for their comments and help on translating of this manuscript.

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Recibido: 28 de octubre del 2013.

Aceptado: 18 de marzo del 2014.