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**Effect of two extruded diets with different
fish and squid meal ratio on growth,
digestibility and body composition of
Octopus vulgaris (Cuvier, 1797).**

TRABAJO FIN DE MÁSTER

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Effect of two extruded diets with different fish and squid meal ratio on growth, digestibility and body composition of *Octopus vulgaris* (Cuvier, 1797).

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Abstract

Growth and body composition of *Octopus vulgaris* fed with two formulated extruded diets based on different ratio of fish and squid meal (1:1, FMS and 3:1, 3FMS, fish meal:squid meal) and a natural diet based on crab (*Carcinus mediterraneus*) and bogue (*Boops boops*) were studied in present work. The animals showed a 100% survival rate in all cases. The best growth results were obtained in control group, where the animals showed a SGR $2.14 \pm 0.18\%$ BW/day, followed by the octopuses fed the FMS diet with $0.59 \pm 0.22\%$ BW/day, and finally, the group fed the 3FMS diet, $0.53 \pm 0.22\%$ BW/day. No significant differences were observed between groups FMS and 3FMS. The animals of control group showed the highest digestive gland index (6.18 ± 0.51), but no significant differences were shown between the three groups (5.33 ± 0.62 and 5.82 ± 0.62 in FMS and 3FMS respectively). Digestibility was not affected by the type of diet. Octopuses fed with extruded diets showed an ADC value of 86.9% and 89%, in FMS and 3FMS respectively. The two extruded diets showed no differences between them but differ with the Control diet in case of muscle and digestive gland, while present differences between two extruded diets in case of whole octopus. The results suggest that both shrimp meal and fish meal could be used for dry pelleted extruded diets, with good acceptance, but the correct formulation of extruded diets must be studied in order to obtain a growth similar to the natural one in octopus. The present study reports the first step in the search for an adequate dry pelleted extruded diet for *O. vulgaris*.

Keywords: *Octopus vulgaris*, growth rate, digestibility, extruded diet.

Resumen

En el presente trabajo se ha estudiado el crecimiento y la composición corporal del pulpo común (*Octopus vulgaris*), alimentado con dos dietas extrusionadas con diferente proporción de harina de pescado y calamar (1:1 y 3:1 de harina de pescado:harina de calamar), y una dieta natural a base de cangrejo (*Carcinus mediterraneus*) y boga (*Boops boops*). Los animales presentaron una supervivencia del 100% en todos los tratamientos. Los mejores resultados en el crecimiento se obtuvieron en el grupo control, en el que los animales mostraron un SGR de $2.14 \pm 0.18\%$ BW/día, seguido de los pulpos alimentados con la dieta FMS, con un crecimiento de $0.59 \pm 0.22\%$ BW/día, y por último, el grupo alimentado con la dieta 3FMS, con $0.53 \pm 0.22\%$ BW/día. No se observaron diferencias significativas entre los grupos FMS y 3FMS. Los animales del grupo control presentaron el mayor índice de glándula digestiva (6.18 ± 0.51), pero no se encontraron diferencias significativas entre las tres dietas (5.33 ± 0.62 y 5.82 ± 0.62 en FMS y 3FMS respectivamente). La digestibilidad no estuvo afectada por el tipo de dieta. Los pulpos alimentados con las dietas extrusionadas, mostraron un valor de 86.9% y 89%, en FMS y 3FMS respectivamente. Las dos dietas extrusionadas no mostraron diferencias entre ellas, pero sí respecto a la dieta control, en el caso del músculo y la glándula digestiva, mientras que si que se encontraron diferencias entre las dos dietas experimentales y la dieta control en el caso de los pulpos completos. Los resultados sugieren que la harina de calamar y la harina de pescado pueden ser utilizadas con los mismos resultados en las dietas secas extrusionadas, con buena aceptación, pero la correcta formulación de las dietas extrusionadas debe seguir siendo investigada para poder llegar a obtener crecimientos similares a los obtenidos con las dietas naturales en el caso del pulpo. El presente estudio representa los primeros pasos en la búsqueda de una dieta seca extrusionada para *O. vulgaris*.

Palabras clave: *Octopus vulgaris*, tasa de crecimiento, digestibilidad, dieta extrusionada.

1. Introduction

The octopus (*O. vulgaris*) is a benthic, neritic species occurring from the coast line and living in depths from 0 to 200m (Vaz-Pires *et al.*, 2004). It has a worldwide distribution in tropical and subtropical waters of the Atlantic, Indian and Pacific oceans, as well as in the Mediterranean Sea (Mangold, 1983; Quetglas *et al.*, 1998). They are found in a variety of habitats, such as rocks, coral reefs and grass (FAO, 1984).

Throughout its distribution range, this species is known to undertake limited seasonal migrations, usually overwintering in deeper waters and occurring in shallower waters during summer. Their natural diet consist mainly of crustaceans (mostly brachyuran of the genera *Portunus*, *Dromia*, *Calappa* and *Pagurus*), shellfish, fish (which includes species *Pagellus*, *Dentex*, *Boops* and a few soleidae and triglids) and a small percentage of other cephalopods (Pereiro & Bravo de Laguna, 1979).

Currently only are traded a few species of cephalopods a large scale. The squid (*Loligo vulgaris*) is approximately 73% of the catches of cephalopods worldwide, followed by cuttlefish (*Sepia officinalis*) with 15% and common octopus (*Octopus vulgaris*) with 8% (FAO 2012).

The need to diversify marine aquaculture products that have saturated the market, such as gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), and represent the highest percentage of production in the Spanish market, has stimulated the development of producing new species (Biandolino *et al.*, 2010).

An ideal candidate for this diversification is the common octopus (*O. vulgaris*), which is a species that has drawn great attention to it in recent years (Iglesias *et al.*, 2000; Navarro & Villanueva, 2000; Vaz-Pires *et al.*, 2004; Almansa *et al.*, 2006; Biandolino *et al.*, 2010), as it has great potential as an aquaculture product, a great appreciation for the consumer and an attractive selling price to consider its production (Prato *et al.*, 2010; Garcia *et al.*, 2011). It has a high nutritional value for human diet because of its high levels of n3 PUFA (Petza *et al.*, 2011) and a rapid growth rate, about 13% of body weight per day with natural food (Vaz-Pires *et al.*, 2004). It also adapts quickly and easily to life in captivity, and has a great resistance to transportation and stress, as well as a high fertility rate, which makes it a good species for aquaculture (Villanueva, 1995). However, it also presents some problems for industrial production, such as low larval viability and absence of artificial feeding, which has good nutritional qualities and is efficient for on growing the species (Vaz-Pires *et al.*, 2004; Domingues *et al.*, 2007; Quintana *et al.*, 2008; Garcia *et al.*, 2011).

The first works on nutrition with this species took place with natural diets, since the objective was to adapt it to captivity (Aguado & García García, 2002; García García & Cerezo Valverde, 2006). But this type of diet also represents a problem. Even though by

means of its spectacular growth of octopus has been achieved -of the order of a kilo a month- the increase in the costs of production, the waste excess and the limited availability of this type of food in the market to ensure the production of the species make it necessary to search for an artificial diet (MISPECES, 2012).

The first artificial diets tested on octopus (*O. vulgaris*) were the so-called wet diets (Cerezo Valverde *et al.*, 2008; Quintana *et al.*, 2008; Estefanell *et al.*, 2011; Garcia *et al.*, 2011; García-Garrido *et al.*, 2011), which achieved growth from almost zero in the order of $0.22 \pm 0.12\%$ BW/day in Cerezo Valverde *et al.* (2008), to the one obtained by García-Garrido *et al.* (2011), which reached $8.9 \pm 5.8\%$ BW/day. Despite the improvements made in wet diets, they show some disadvantages, just as natural food, such as the high cost of storage, since they should be kept in cold storage to avoid spoilage and pathogenic developments due to the large quantity of water that these diets have, and difficulty in manufacture, since it is a process purely made by hand.

Therefore, it is necessary to obtain a dry diet that is accepted by octopuses, and which grows similarly to natural food. Thus, the use of dry diets presents a great number of advantages, such as the regular supplies and its composition (MISPECES, 2012), the fact that it allows us to preserve the quality of the product for a long time, and its easy transportation and storage, which involve less manipulation, thus reducing infectious diseases (Mazón *et al.*, 2007). For these reasons, the optimal development of artificial diets can provide a great improvement in the on growing of the species.

Nevertheless, a dry diet must have a number of special qualities when referring to octopus. It has to submit specific textural and organoleptic characteristics, which correspond to their feeding mechanisms and the structure of their digestive system (Petza *et al.*, 2011).

The first problem in designing an artificial diet for octopus is the acceptance, so that the animal finds it palatable and eats it completely (García & Cerezo Valverde, 2006; Cerezo Valverde *et al.*, 2008; Quintana *et al.*, 2008; Querol *et al.*, 2012). The lack of acceptance is caused, among other reasons, by the diets palatability, since octopuses have a large number of chemoreceptors in the tentacles, which allows them to differentiate flavors in a very precise way. Therefore, it seems logical that the diets of this species contain some sort of flavor enhancer to promote food acceptability (Cerezo Valverde *et al.*, 2008; Domingues *et al.*, 2007), with the aim of producing more palatable diets for octopuses.

Another problem to keep in mind in the manufacture of an extruded diet for octopus is stability. Granulated extruded diets designed for fish are not appropriate in the case of octopuses, because they disintegrate rapidly before consumption when they are being manipulated by the animal (Cerezo Valverde *et al.*, 2008). The feed should be stable

enough so that it does not disintegrate in the water before consumption, or when being manipulated, since octopuses do not eat the food immediately (Petza *et al.*, 2011). In several experiments gelatin and alginate were used to improve feed stability in water and to create a gummy texture that is acceptable to octopuses, both *O. vulgaris* (Cerezo Valverde *et al.*, 2008; Garcia *et al.*, 2011), and *Octopus maya* (Rosas *et al.*, 2008).

For all the mentioned reasons, setting the nutritional requirements for the growth of common octopus (*O. vulgaris*) has been complicated (Petza *et al.*, 2011), because the main objective is to find a form of dry feed that is consumed by this species and with which a growth similar to that obtained with natural food can be obtained.

A dry feed “base” with a stable structure in water has been recently produced thanks to gelatin and maltodextrin. It has had a good acceptance by octopus, because it contains a strong flavoring, like the case of egg yolk (Querol *et al.*, 2012). Parting from this formula which uses extruded feed, a way to improve the nutritional composition of diets to enhance growth has been opened.

Due to their natural diet, proteins are the main energy source for the octopus. For this reason, in this study it had been tested two protein sources, which were, fish meal, protein source for excellence in the manufacture of feed for aquatic animals, and squid meal, chosen for its amino acid profile, rich in arginine. Arginine is one of the essential amino acid for cephalopods, deficient in most commonly used raw materials in the manufacture of aquatic feeds, including fish meal, so that the squid meal is adequate to fill this deficiency, thus preventing the need for supplementation with this amino acid. (Cerezo Valverde *et al.*, 2012).

Therefore, the objective of this study is to evaluate two artificial dry diets made with fish and squid meal in an on growing experiment, in order to assess its influence on growth, digestibility and body composition of the octopus.

2. Materials and methods

2.1. Animals

The octopuses (*O. vulgaris*) were caught using drag nets in Tarragona (Spain) and were transported to the facilities of IRTA (Institut de Recerca i Tecnologia Agroalimentària). Before the experiment started, the octopuses were kept in a recirculation seawater system for ten days. During this period of acclimation, the water temperature was 17-19°C, and no mortality was observed. The specimens were fed with crab (*Carcinus mediterraneus*).

2.2. Experimental design

During 34 days, eighteen specimens distributed in three groups (six per group) were placed in individual 250l rectangular fiberglass tanks. Each one containing PVC tubes as shelters. Two groups were fed with the experimental diets, FMS and 3FMS, and the control group, fed with crab (*C. mediterraneus*) and bogue (*Boops boops*). The octopuses were fed daily at 9:00h and at 15:00h and, the uneaten food was collected at 14:00 h and at 20:00h, respectively.

Octopuses were weighted individually every 15 days. The octopuses were introduced individually into a mesh, and weighed, and then were returned to their tank.

The feces of the three groups were used to calculate the digestibility of diets. They were recollected during the last 10 days of the experiment with a scoop net, water was removed from them and they were frozen until the analysis.

2.3. Diets

The extruded diets under study had a diameter of 1cm, and 4-5 cm of length, and were prepared at the Institute of Animal Science and Technology of the Universitat Politècnica de València, using a semi-industrial extruder; Clextral BC45, under conditions of 50 ATM and 100 °C. The difference between diets was the fish- squid meal ratio, 3FMS diet contained the triple of fish meal than FMS diet (Table 1). A 2% of Taurine was also added to the composition of the diet in order to promote muscle growth.

Table 1. Composition of experimental diets (%).

	Maltodextrin	Gelatin	Fish meal	Squid meal	Yolk	Taurine
FMS	9.8	19.6	19.6	19.6	29.4	2
3FMS	9.8	19.6	29.4	9.8	29.4	2

Maltodextrin: PRAL S.A., Gelatin: PILARICA S.A., Fish meal: COCERVA S.C., Yolk: Pasteurised egg yolk powder (IGRECA SAS), Squid meal: Max Nollert®, Taurine: Sigma-Aldrich logistik.

Extruded diets nutrients are shown in Table 2, around 60% crude protein and 21% crude lipid in dry matter, respect natural diet. Natural composition was 57% crude protein and 11% crude lipid.

Table 2. Proximate composition (expressed as percentage of wet weight) of experimental diets.

	Control	FMS	3FMS
Moisture (%)	78.23	18.61	13.43
Crude protein (% ww)	12.46	49.52	53.38
Crude lipid (% ww)	2.36	16.43	18.56
Ash (% ww)	4.02	15.34	16.04

2.4. Biochemical analysis

At the end of the feeding trial half of the octopuses of each treatment were completely analyzed, as well as the muscle and digestive gland of the another nine animals, which were analyzed separately.

The digestive gland was extracted and homogenized, and, the tentacles (muscle) and complete octopus were filleted, minced into small pieces and mixed until a homogenous sample was obtained.

Diets, digestive glands, tentacles and whole fish, were analysed according to AOAC (1990) procedures: dry matter (105 °C to constant weight), ash (incinerated at 550 °C to

constant weight), and crude protein ($N \times 6.25$) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganäs, Sweden) and crude lipid extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator).

The apparent digestible coefficient of the two artificial diets was determined by Acid Insoluble Ash method (Atkinson *et al.*, 1984). The ash was boiled HCl for 5 mins. and filtered through a low fiber filter (Whatman n°42). Filters were burned in a muffle furnace at 500°C during 16h.

2.5. Parameters calculated

The following indices were calculated:

Weight gain: $WG = W_f - W_i$; Absolute growth rate: $AGR = (W_f - W_i)/t$; Specific growth rate: $SGR = (\ln W_f - \ln W_i)$; Digestive gland index: $DGI = (DGW/W_f) \times 100$; where W_f = final weight in g; W_i = initial weight in g; t = time in days; DGW = digestive gland weight.

Apparent digestibility: $ACD(\%) = 100 - [100 \times (AIA_{feed}/AIA_{faces}) \times (\text{nutrient}_{faces}/\text{nutrients}_{feed})]$, where $AIA(\%) = (\text{ash of the sample}/\text{dry weight of the sample}) \times 100$; AIA = Acid insoluble ash.

2.6. Statistical Analysis

The effect of the different diets on the above indices was analyzed by one-way ANOVA and the significance of the differences in mean values was analyzed by Neuman-Keuls test. The statistical assays were carried out with the Statgraphics (Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA) and significance is indicated by P-values of less than 5%.

3. Results

The next figure shows the weight evolution of the octopuses fed the three experimental diets. Animals which were fed artificial diets had the same behavior, and the growth of the control group was much higher.

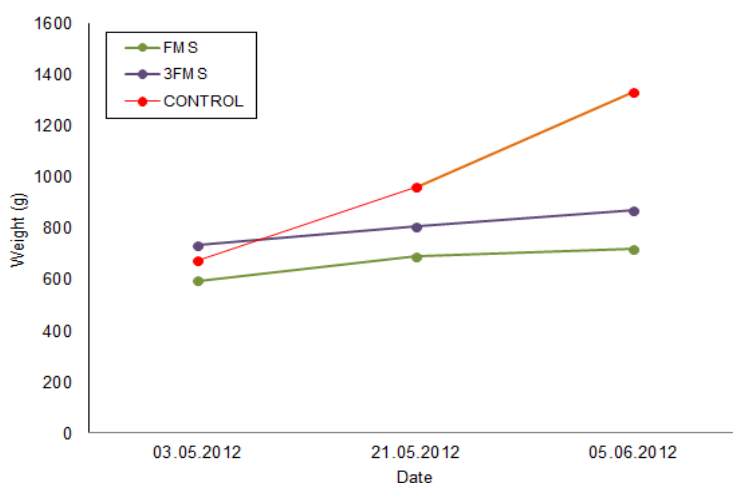


Figure 1. Evolution of weights.

At the end of the experiment the fish survival was not affected by treatment (100%). Significant differences in fish growth (live weight, AGR and SGR) were observed (Table 3), as animals fed with Control diet showed significantly better growth indices.

Regarding biometric parameters, digestive gland index (DGI) values were higher in control (6.18%) than in FMS (5.33%) and 3FMS (5.82%), although these differences were not significant (Table 3).

Table 3. Mean values \pm S.D. for growth and biometric parameters of each experimental group (N = 6).

	Control	FMS	3 FMS
Wi (g)	675.83 \pm 82.78	596.25 \pm 101.39	735 \pm 101.39
Wf (g)	1335 \pm 79.31 ^a	720 \pm 97.14 ^b	870 \pm 97.14 ^b
WG (g)	659.17 \pm 37.79 ^a	123.75 \pm 46.29 ^b	135 \pm 46.29 ^b
AGR (g/day)	19.97 \pm 1.14 ^a	3.75 \pm 1.4 ^b	4.09 \pm 1.4 ^b
SGR (% BW/day)	2.14 \pm 0.18 ^a	0.59 \pm 0.22 ^b	0.53 \pm 0.22 ^b
DGI (%)	6.18 \pm 0.51	5.33 \pm 0.62	5.82 \pm 0.62

Values on the same line and different superscripts are significantly different. Wi, initial weight; Wf, final weight; WG, weight gain; AGR, absolute growth rate; SGR, specific growth rate; DGI, digestive gland index.

Table 4. Proximate composition (expressed as percentage of wet weight) (N=3) of muscle, digestive gland and whole octopus.

	Initial	Control	FMS	3FMS
Muscle (Tentacle)				
Moisture (%)	81.40	80.17±0.59	81.52±0.59	81.40±0.59
Crude protein (% ww)	15.71	16.56±0.40 ^a	14.76±0.40 ^b	14.27±0.40 ^b
Crude lipid (% ww)	0.14	0.47±0.09	0.50±0.09	0.52±0.09
Ash (% ww)	2.20	1.95±0.11	2.22±0.11	1.92±0.11
Digestive gland				
Moisture (%)	70.26	61.70±0.60 ^a	57.83±0.60 ^b	57.50±0.60 ^b
Crude protein (% ww)	18.74	23.77±0.72 ^a	15.95±0.72 ^b	16.20±0.72 ^b
Crude lipid (% ww)	2.09	9.81±1.05 ^a	20.86±1.05 ^b	19.36±1.05 ^b
Ash (% ww)	2.20	1.63±0.06	1.40±0.06	1.46±0.06
Whole octopus				
Moisture (%)		78.36±0.58 ^a	78.92±0.58 ^a	82.15±0.58 ^b
Crude protein (% ww)		17.54±0.40 ^a	15.86±0.40 ^b	13.80±0.40 ^c
Crude lipid (% ww)		1.33±0.26	1.70±0.26	0.99±0.26
Ash (% ww)		2.01±0.08	1.93±0.08	1.74±0.08

Values on the same line and different superscripts are significantly different. The initial composition of the whole octopus was not calculated because the octopuses captured were insufficient for performing this analysis.

The main differences in the nutritional composition of octopus fed with the experimental diets were shown in Table 4. Moisture muscle content in the three diets, ranged from 80 to 82% with no significance differences. In the case of the digestive gland moisture, there are no differences between the two extruded diets, but was significantly lower than control group (61.70% compared with 57.83 and 57.5% in FMS and 3FMS respectively), and in case of the whole octopus moisture, was significantly higher in 3FMS diet than in Control and FMS diet.

The crude protein of the muscle, ranged from 14 to 17%; the digestive gland protein, ranged from 15% to 24%, and whole octopus protein, ranged from 13 to 18%, with significantly lower values in control group. There were no differences between the two diets in muscle and digestive gland, but the two experimental diets were significantly different in whole octopus.

Regarding lipid content, animals fed Control diet did not show significant differences with the two artificial diets in the case of muscle and whole octopus, but in the case of digestive gland, the animals fed with Control diet showed lower values of lipid content (9.81% compared 20.86 and 19.36% respectively) than the FMS and 3FMS diets.

The percentage of ash in the three groups did not show significant differences between the different diets, and no differences between muscle, digestive gland and whole octopus.

Apparent digestibility coefficient (ADC) was not affected by the type of diet (Fig. 2). Octopuses fed with artificial diets FMS and 3FMS showed a similarly data 86.9% and 89% ADC of dry matter respectively. Control diet ADC was not calculated due to conservation problems of natural food samples.

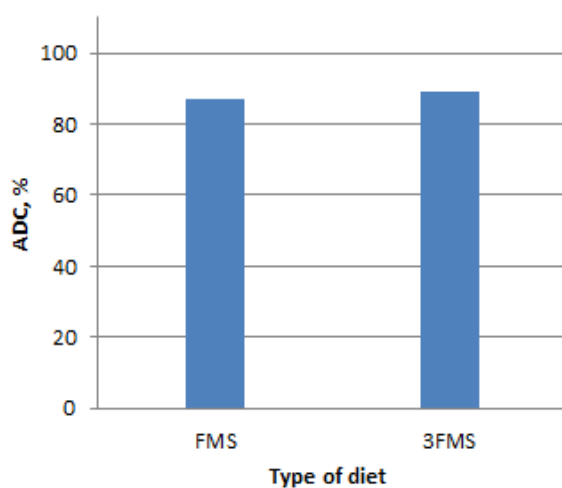


Figure 2. ADC (Apparent digestibility coefficient) of experimental diets.

4. Discussion

This test took place during only 34 days, due to a sharp increase in the water temperature of the bay, and because the test was conducted in an open recirculating system. Within days there was an important drop in intake, so it was decided to end the feeding test.

Prepared diets presented high levels of protein and similar to natural food (about 60% protein by dry weight). They also presented total lipid levels of around 21%. This amount of lipids is greater than that the one present in natural food (10.84%) which triggered a lower growth of animals fed 3FMS and FMS, since it has been shown that the lipids interfere with the absorption of proteins, concluding that diets with high amounts of lipids promote high growth (Domingues *et al.*, 2007) and also the high lipid diets, producing damage to the nidamental glands (García García & Aguado Giménez, 2002).

Referring to growth, the average weight gain was 123 and 135g, and was obtained for 34 days with specific growth rates (SGR) of $0.59 \pm 0.22\%$ BW/day and $0.53 \pm 0.22\%$ BW/day with FMS and 3FMS diets respectively. No significant differences in octopus growth between the experimental diets, however, both of them were lower than the Control diet ($2.14 \pm 0.18\%$ BW/day).

If we compare specific growth rates with other on growing test conducted by *O. maya* on growing, we can see either similar results or slightly negative ones with dry diets, as seen in Domingues *et al.* (2007) due to a very poor absorption of the dry diet because of its incorrect formulation, and with moist diets, as in Rosas *et al.* (2007). A growth exceeding the one in this study can also be observed in the previous ones, as is the case of Rosas *et al.* (2008), with moist diets based on crab paste with a SGR of $1.91 \pm 0.14\%$ BW/day and an initial weight for $350 \pm 20\text{g}$, due to improvements in the agglutination of diets and the use of gelatin to increase the digestibility. Another example is Águila *et al.* (2007), also using moist diets with different concentrations of fishmeal, which obtained a growth of 0.86% BW/day, both of them have a lower weight to the present study.

In previous fattening studies with *O. vulgaris*, lower growths than the ones obtained in this study have been observed. Such is the case of wet diet use by García *et al.* (2011) with $0.21 \pm 0.28\%$ BW/day. Specific growth rates higher to the ones obtained in this present study are found in Cerezo Valverde *et al.* (2008), who obtained a SGR of $0.71 \pm 0.22\%$ BW/day. Both of them had a similar weight than present study.

The on growing of *O. vulgaris* has also been tested (García-Garrido *et al.* 2011), feeds were made of fish hydrolyzate mixture and squid in different proportions, which also tested binding behaviors: alginate and gelatin, obtaining a higher SGR of this study

($0.89 \pm 5.8\%$ BW/day), with a lower weight than the present study, which manifestate a good performance of squid meal in octopus feed.

There were no significant differences in rates of digestive gland (DGI) which are positively correlated with animal size, although in the case of the Control diet, this was slightly higher as seen in previous studies (Cerezo Valverde *et al.*, 2008).

Both feeds have a high digestibility of dry matter, 86.9% and 89% in FMS and 3FMS respectively. This high digestibility may be due to the use of gelatin in the manufacturing of diets, since to previous studies have shown that gelatin does not affect digestibility (Rosas *et al.*, 2007), unlike other binders, such as alginate, this might be due to the fact that it is a polysaccharide obtained from seaweed, which can not be digested by the octopus, due to the lack of or low activity of amylases (Águila *et al.*, 2007).

This digestibility data could not be compared to the control group due to a problem in the storage of the sample. However, when compared to previous studies with the same raw materials as the ones fed to the control group, we observe that it was lower, with 96.3% in the case of crab and 94.5% in the case of bogue (Mazón *et al.*, 2007). The same happened in another test with *O. vulgaris* where the dry matter digestibility of the bogue was 98.37% and the crab, 94.6% (Hernandez *et al.*, 2004). In addition, in other studies with *Octopus maya* (Rosas *et al.*, 2008) and *Enteroctopus megalocyathus* (Farias *et al.*, 2010), we can see that the digestibility of natural feed obtained in these tests was lower than that obtained in the present work, 82% and 71% respectively, which is even lower than the digestibility of extruded diets.

Despite the good results, the possible higher digestibility of the Control diet in this trial, could promote the absorption of nutrients, thus increasing the differences in weight gains recorded between treatments.

The results of the analysis of the octopus proximal composition showed, in the case of the study of muscle (tentacles), that statistical differences between artificial diets and the Control diet appeared only in the case of the protein, and yet the values were very similar, 14 and 16% respectively. As for total lipid levels, moisture and ash, the values were similar between treatments and also similar to the results obtained in the initial octopus analysis.

In a similar study by Cerezo Valverde *et al.* (2008), no significant differences were observed between the Control diet and the experimental regarding lipids and proteins as the results were similar to those obtained in the present study.

However, these differences that are not manifested in muscle composition can be seen in the digestive gland, in the moisture and the protein and lipids, which doubled the

levels observed in the control group when compared to extruded feed. The data obtained is consistent with Cerezo Valverde *et al.* (2008), which also demonstrated differences in lipid and protein composition between digestive glands of octopus fed the Control and experimental diets.

The presence of approximately twice the lipids in the digestive gland of octopuses fed with the experimental diets compared to those fed the Control diet could be due to the diets 3FMS and FMS. They have twice the amount of lipids than Control diet, and they are stored in the gland. Contrary seen in a previous study, as the amount of lipids present in the gland were much lower than those fed the Control diet (Cerezo Valverde *et al.*, 2008), this difference may be due that the feed in that study had a three times lower lipid content than the one used for the present study.

In a comparison between lipid glands with the initial data octopuses, it can be observed that all vary significantly in moisture, protein and lipid content, both with respect to artificial diets and with the Control diet. With this we conclude, that the feed for the octopus during development, is going to affect the composition of the glands notably more than the muscle ones.

Finally, if we compare the composition of the whole octopus, observing lipid levels and ash, statistical differences among the three treatments cannot be found, not exceeding in any of the two parameters the 2% of body composition. However in terms of protein levels and moisture, and despite the diets being similar, they present statistical differences, being 3FMS diet the one that has the highest moisture level, and the lowest value of protein. This difference could be due to a better digestibility of natural food by octopuses in the control group, consistent with the observations made in previous studies (Aguado Giménez & García García, 2002; García García & Cerezo Valverde, 2006).

With everything that has been observed, it can be concluded that the use of fish meal with squid meal can be used in the manufacture of dry diets for octopus, since they were not rejected by the animal, they did not cause a drastic decline in digestibility, and octopus muscle composition was not changed (although there was a fattening of the digestive gland), and growth occurred.

Both artificial diets produced similar growth, as proportions of fishmeal studied regarding squid meal did not seem to have a determining influence on it.

5. Acknowledgements

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6. References

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