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Effect of two artificial wet diets agglutinated with gelatin on feed and growth performance of common octopus (*Octopus vulgaris*) sub-adults

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ABSTRACT

Acceptance and effect on growth of two artificial wet diets for common octopus sub-adults were studied. These artificial wet diets were compounded of frozen shrimp or squid, both agglutinated with commercial gelatin. The diet based on frozen squid (SQ) promoted higher growth and conversion efficiency than the diet based on frozen shrimp (SH); while these rearing parameters of the SQ diet were not significantly lower than the ones obtained with the control diet (frozen squid). Feeding results indicate that gelatin did not reduce the palatability of the SQ diet, compared to the control diet. In conclusion, the good results obtained with the SQ diet support the hypothesis that this kind of wet artificial diet (a natural diet plus an agglutinant) can possibly be a base paste for other prepared diets.

1. Introduction

The common octopus (*Octopus vulgaris*) is potential specie for aquaculture diversification because of its high market price and growth rate in culture conditions, as well as great demand throughout different regions of the world. The commercial ongrowing of this specie is only being done in Galicia, Northwest of Spain, and maximum annual production obtained in 1998 and 1999 was less than 33.0 t (FAO, 2001, 2002). Moreover, the annual production decreased to 14.6 t in 2001 (García García et al., 2004). This limited commercial production was a consequence of the availability of juveniles captured from the wild, since massive production of captive common octopus juveniles has not been achieved. The major bottleneck is the high mortality observed in the paralarvae stage (Iglesias et al., 2006, 2007).

Furthermore, irrespective of the paralarvae mortality, commercial production is still limited due to the lack of an artificial diet that promotes acceptable growth and survival for this specie or other cephalopods (Vaz-Pires et al., 2004; Domingues et al., 2005, 2006, 2007a). Ongrowing and maintenance of juveniles and adult cephalopods is currently done with natural prey such as live, fresh or frozen fishes, crustaceans and molluscs. A profitable and supportable long term industrial culture of common octopus depends on the development of a satisfactory artificial diet. This artificial diet might increase the profitability and minimize the high economical risk associated with the octopus industry in Galicia (García García et al., 2004). According to Domingues et al. (2005, 2006), if prepared diets could successfully replace natural prey, cost reductions of up to 80% could be obtained, particularly when commercial production methods are used. Also, these formulated diets would allow accurate studying of the nutritional requirements of common octopus.

Preliminary experiments to develop artificial diets for cephalopods have been carried out with either moist and dry pellets (Castro, 1990; Lee et al., 1991; Castro et al., 1993; Castro and Lee, 1994), or surimi (fish myofibrillar protein concentrate) (Castro et al., 1993; Castro and Lee, 1994; Domingues, 1999), observing that large juveniles and adult *Sepia officinalis* readily accepted prepared diets. Octopuses (*Octopus bimaculoides*) have also been used to develop artificial diets (Lee et al., 1991) and it was suggested that moisture content of these diets may be an important property that affects ingestion. Later, it has been observed in *S. officinalis* that heat treatments had a negative effect on the nutritional quality of the diets, unlike a freeze-dried process (Domingues et al., 2007b). Current studies on the development of artificial diets for octopuses like *O. vulgaris* (Cerezo Valverde et al., 2008) and *Octopus maya* (Aguila et al., 2007; Domingues et al., 2007a; Rosas et al., 2007, 2008) indicate that textural, attractant and nutritional properties of the octopus diets must be further developed.

Initially, artificial diets to be developed for octopuses must have organoleptic properties similar to the natural food that they capture in the wild (crustacean, mollusc and fish), particularly to improve palatability. Also, these artificial diets must have textural properties to keep it agglutinated until ingestion. This requires that the diet must

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be able to be manipulated by the octopuses and sustain moderate lixiviation resulting from the contact with seawater.

Granulation and extrusion processes used for fish feeds are not successful for octopus diets, since they become disaggregated by the animals during manipulation prior to ingestion (García García and Cerezo Valverde, 2006). Due to the necessity of obtaining a final product with high stability in the water, agglutinant products are usually included in artificial diets used for feeding fish. For cephalopods, Cerezo Valverde et al. (2008) used alginate as an agglutinant of bogue (*Boops boops*) and prawn (*Hymenopenaeus muelleri*), to prepare a diet for *O. vulgaris*. Nevertheless, the diet did not promote optimal growth. However, for *O. maya*, an artificial wet diet based on crab muscle tissue agglutinated with gelatin delivered adequate growth and survival, compared to crab muscle tissue (Quintana and Rosas, 2007; Rosas et al., 2008). These studies showed that the use of agglutinant additives in the preparation of artificial diets kept them aggregated and support adequate palatability and ingestion in very early juveniles. These results support our current hypothesis, which is to determine if this type of diet could also be adequate for *O. vulgaris* sub-adults. Until a dry artificial feed for cephalopods can be produced, artificial wet diets agglutinated with gelatin could be used to initiate studies on the nutritional requirements for these species.

The objective of the present study is to determine preliminary aspects of the above described artificial diets for *O. vulgaris* sub-adults that could be used to develop diets for other cephalopods, and to obtain data on individual common octopus ongrowing in isolated tanks, fed with wet artificial diets. For this, the acceptance and effects of frozen shrimp and squid agglutinated with commercial gelatin on growth of sub-adult octopuses were analysed.

2. Materials and methods

2.1. Animals

Octopuses (*O. vulgaris*) were captured in the coastal waters of Huelva (South of Spain) on February 2007, using artisan bottom trawl nets, and brought to the IFAPA “Centro Agua del Pino” research facilities (Cartaya, Spain). A total of 30 animals were individually placed in 30 cylindroconic tanks of 80 L seawater. Before the start of the experiment, these octopuses were acclimated for 9 days and fed with frozen squid (*Loligo gahi*). After this period, octopuses were weighted (486.6 ± 104.0 g of average weight).

2.2. Diets

Three diets were fed to the octopuses (10 replicates per diet): 1) frozen squid (*L. gahi*) as the control diet, 2) squid paste (*L. gahi*) agglutinated with gelatin (SQ) and 3) shrimp paste (*Palaemonetes varians*) agglutinated with gelatin (SH). Diet 1 was used as control because squid compared to crab (usually employed as control diet) did not promote differences in growth of common octopus (Cagnetta and Sublimi, 1999). Also, we obtained the same results in our research facilities (unpublished data). Diets 2 and 3 were prepared with whole frozen squid, including viscera, head and arms, and whole shrimp, including cephalothorax and carapace, respectively, that were triturated; the resultant paste was manually agglutinated with 10% gelatin (Panreac® Aditio 80–100 Blooms). Afterwards, these agglutinated diets were separated into individual spherical portions (10–50 g) and frozen until use. Diets were prepared every 2 days. Moisture content of the diets was determined by drying 10 samples at 105 °C until constant weight according to an AOAC (1980) method. Moisture values were of 79.2 ± 0.2, 72.5 ± 1.6 and 75.0 ± 0.7% for the control, SQ and SH diets, respectively.

2.3. Feeding and experimental conditions

The feeding trial was performed for 39 days following initial sampling. A daily ration of 10% (wet weight diet/wet weight animals) was fed to each octopus once a day (at 12:00 h, approximately). The uneaten diet was removed from each tank each morning and dried at 105 °C until constant weight, to subtract the moisture content and improve accuracy of ingestion results. When calculations of feeding rates and conversion efficiencies were performed, the dry weights of the uneaten remains were recalculated into wet weight remains (since the moisture of each diet was known) in order to obtain these values in a wet weight basis (wet weight diet/octopus wet weight).

Water temperature was of 19.9 ± 1.0 °C and a natural winter photoperiod (10 h light:14 h dark) was used. Water flow was adjusted in each tank to maintain oxygen concentration close to saturation levels.

2.4. Growth and feed intake

All octopuses were weighted every 10 days. For each sampling interval and for the entire feeding trial, the following data were calculated: 1) Instantaneous growth rate (IGR) ($\%BWd^{-1}$) = ((lnW2 − lnW1) / t) × 100, where W2 and W1 are the final and initial wet weights of the octopuses, respectively, ln the natural logarithm, t the number of days of the feeding trial and $\%BWd^{-1}$ means percentage of the body weight per day; 2) feeding rate (FR) ($\%BWd^{-1}$) = (FI / average $W(t)$) × 100, where FI is feed intake and average $W(t)$ is the average wet weight of the octopuses for the time period (t); 3) conversion efficiency (FC) = ((W2 − W1) / FI) × 100, where W2 − W1 is the weight gained by the octopuses during the time period.

2.5. Statistics

Results are presented as means ± S.D. (standard deviations). The data were tested for normal distribution with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of variances with the Levene's test and, when necessary, transformations (square root, logarithmic, etc.) were performed. For data expressed as percentage, arcsin transformation (Fowler et al., 1998) was directly applied. Differences in conversion efficiency, feed intake and growth among the three diets were analysed with one-way ANOVA (Zar, 1999). When differences were found, a Tukey multiple comparison test was performed. Statistical analysis was carried out with Spss 9.0 and significance is taken to be indicated by P-values of less than 5%.

3. Results

3.1. Growth

Significantly (P < 0.05) lower final weights were found in the SH fed group, compared to the control and SQ fed groups (Table 1). Weight gain (data not showed) presented the same trend, being higher for the control (415.2 ± 99.2) and SQ (328.9 ± 168.0) groups compared to SH group (114.7 ± 61.2). For both, final and total weight gain, no significant differences were found between the control and SQ groups.

Comparing octopus weight for each sampling interval (Table 1), among group differences (P < 0.05) were found for the SH group from

Table 1
Weights of common octopus fed with the three test diets during the experiment

Day	Octopus weight (g)					
	Control		SQ		SH	
1	486.3 ± 104.6	(10)	505.7 ± 114.1	(10)	468.1 ± 100.7	(10)
10	530.9 ± 101.2	(10)	566.4 ± 105.3	(9)	485.7 ± 102.1	(10)
20	666.5 ± 130.0a	(10)	665.3 ± 125.5a	(9)	512.9 ± 107.0b	(10)
30	767.8 ± 151.6a	(9)	708.4 ± 98.8a	(6)	541.2 ± 100.8b	(10)
40	867.6 ± 119.0a	(8)	796.4 ± 143.9a	(6)	582.8 ± 123.4b	(10)

Results represent means ± standard deviation (S.D.). Different letters in the same row means significant differences (P < 0.05). Numbers between brackets indicate the alive animals used to calculate the average (n).

Table 2
Growth rates of common octopus fed the three test diets for the different weighing periods

Period	Octupos growth rates %BWd^{-1}								
	Control			SQ			SH		
1–10	1.0±0.6	ab	(10)	1.8±0.7	a	(10)	0.4±0.5	b	(10)
10–20	2.3±0.8	a	(10)	1.6±0.9	a	(9)	0.5±0.4	b	(10)
20–30	1.7±0.5	a	(10)	1.0±0.9	ab	(9)	0.6±0.5	b	(10)
30–40	1.6±0.5	a	(9)	1.0±0.8	ab	(6)	0.6±0.8	b	(10)
Overall	1.6±0.4	a	(8)	1.4±0.6	a	(6)	0.6±0.3	b	(10)

Results represent means±standard deviation (S.D.). Different letters in the same row means significant differences ($P<0.05$). Numbers between brackets indicate the alive animals used to calculate the average (n) %BWd^{-1} means percentage of the body weight per day.

day 20 until the end of the trial using ANOVA. The control and SQ groups did not differ during the sampling intervals.

Overall growth rate showed the same differences than weights obtained at each sampling (Table 2). However, differences of SH group growth rates in every sampling interval were not always found significant, compared to the other groups, due to the high variability. No mortalities other than escapes from the tanks (2 and 4, for the control and SQ groups, respectively) were observed.

3.2. Feeding

Table 3 shows the results of feeding rates for each weighing interval and for the entire feeding trial. Significantly lower ($P<0.05$) feeding rates were found for the control group, compared to that of the SQ and SH groups. Feeding rate did not differ between the SQ and SH groups, both for the whole trial or for every weighing interval, except for periods 10–20.

Conversion efficiency values for each weighing interval and the whole trial (Table 4) were higher for the SQ group compared to the SH group. Exceptions were observed for periods 20–30 and 30–40 for which values did not differ. Conversion efficiencies were higher ($p<0.05$) for the control group compared to the other two groups, for the whole feeding trial and at each weighting interval; with the only exception being for the first 10 days, for which values did not differ compared to the SQ group.

4. Discussion

Results of the present experiment showed that minced squid was a better dietary ingredient than minced shrimp. When agglutinated with gelatin, it promoted higher growth and conversion efficiency. The poorer values for the shrimp based diet suggest that this SH diet could possibly have a worse nutritional value compared to SQ and control diets. Samples of the three diets, digestive gland and mantle of the octopuses were collected and total protein, amino acids, lipid and lipid classes will possibly be analysed in the future. This information

Table 3
Feeding rates (%BWd^{-1}) (g wet weight diet/octopus wet weight) of common octopus fed with the three test diets for the different weighing periods

Period	Octopus feeding rates %BWd^{-1}								
	Control			SQ			SH		
1–10	5.9±1.9	b	(10)	9.6±1.9	a	(10)	9.6±0.6	a	(10)
10–20	6.6±0.5	c	(10)	7.3±0.9	b	(9)	8.3±0.7	a	(10)
20–30	7.2±0.7	b	(10)	8.1±1.9	a	(9)	9.1±0.7	a	(10)
30–40	5.9±0.2	b	(9)	7.8±1.5	a	(6)	8.2±0.7	a	(10)
Overall	5.8±0.6	b	(8)	8.6±0.8	a	(6)	8.5±0.8	a	(10)

Results represent means±standard deviation (S.D.). Different letters in the same row means significant differences ($P<0.05$). Numbers between brackets indicate the alive animals used to calculate the average (n) %BWd^{-1} means percentage of the body weight per day.

Table 4
Conversion efficiency (wet weight gain/wet weight diet) of common octopus fed with the three test diets for the different weighing periods

Period	Conversion efficiency (%)								
	Control			SQ			SH		
1–10	17±11	a	(10)	19±8	a	(10)	6±6	b	(10)
10–20	41±10	a	(10)	25±11	b	(9)	7±5	c	(10)
20–30	25±11	a	(10)	11±12	b	(9)	9±10	b	(10)
30–40	28±16	a	(9)	14±9	b	(6)	8±9	b	(10)
Overall	28±5	a	(8)	16±8	b	(6)	7±4	c	(10)

Results represent means±standard deviation (S.D.). Different letters in the same row means significant differences ($P<0.05$). Numbers between brackets indicate the alive animals used to calculate the average (n).

might help to explain the poorer performance of the SH diet. On the other hand, the same artificial diets (SQ and SH) have been supplied to cuttlefish (*S. officinalis*) and they have not promoted significant growth (unpublished data). These results suggest that the dietary requirements of *O. vulgaris* may be different than those of the cuttlefish.

In the current study, adequate weight gain, growth rates and conversion efficiency were obtained for *O. vulgaris* sub-adults fed with a wet artificial diet based on squid (SQ), compared to values reported for feeding natural prey (Cagnetta and Sublimi, 1999; Iglesias et al., 2000; García García and Aguado Giménez, 2002; García García and Cerezo Valverde, 2006). Growth obtained by feeding the SQ diet (57% of initial weight in 39 days) was higher compared to that reported by Cerezo Valverde et al. (2008) (6–20% in 28 days), using wet artificial diets at similar culture temperatures. Although the animals used in the current study were smaller, this difference in weight cannot explain the higher growth obtained here. Furthermore, unlike Cerezo Valverde et al.'s (2008) study, growth of the octopuses fed with the SQ diet was not lower than that obtained by feeding the control diet (frozen squid). Feeding rates cannot be compared between the two experiments because of the lixiviation rates in the current experimental diets, which could not be accurately measured. However, in spite of these high lixiviation rates, conversion efficiencies were similar in both studies (16 vs 16–22, for the current and Cerezo Valverde et al. (2008) studies, respectively). It can be concluded that these rearing parameters indicate that gelatin, used as agglutinant, did not have any negative effect on the quality of the diet.

As in the current study, the artificial diets used by Cerezo Valverde et al. (2008) were in essence a natural food agglutinated with a binding material, although these authors used two different agglutinant materials (alginate and gelatin). When comparing results obtained with the gelatin agglutinated diet used by Cerezo Valverde et al. (2008) with the one we used, the difference between the two studies could be associated with a possible lower nutritional value of the raw material (mainly fish) compared to the squid we used. However, the methodology used for preparing the two diets was also different and the heating process applied by those authors to the gelatin may have influenced the nutritional value or palatability of the diet. Also, weight gain, growth rate and conversion efficiency values obtained with the SQ diet represent a significant improvement, compared to the dry artificial diets tested in octopuses (*O. maya*) by Domingues et al. (2007a) which did not promote any growth.

The lower growth and conversion efficiency obtained with the SH diet could be related to a lower feed intake (data not showed), or lower palatability of this diet; especially if higher lixiviation in this diet occurred compared to the SQ diet. The better consistency of the SQ diet could be associated with its better cohesion due to a higher percentage of collagen in the squid used in the SQ diet (Ando et al., 2001).

Average feeding rate of the SQ diet indicates that the gelatin was well accepted as an agglutinant, and the resulting artificial diet had an acceptable palatability. Average feeding rates with the artificial diets were higher than with the control. However, if we take into consideration that both artificial diets had higher lixiviation; this could have increased the estimated values for feed intake values for those diets, and also decrease the conversion efficiency values obtained for these diets. This would result in an over- and under-estimate of these values, respectively.

Feeding rates, when compared for the different weighing periods, showed similar trends to those obtained for the entire feeding trial (SQ=SH>control). On the other hand, our results indicate that gelatin did not reduce the palatability of the SQ diet, compared to the control diet. Octopuses were also able to manipulate the SQ diet without losing a significant amount of the diet, which differs from observations reported for other artificial diets (García García and Cerezo Valverde, 2006).

The better results obtained with SQ diet support the hypothesis that this type of wet diet (a natural diet plus an agglutinant) could form the basis for other prepared diets. As indicated before, diets agglutinated with gelatin also resulted in good results for early juveniles of *O. maya* (Quintana and Rosas, 2007; Rosas et al., 2008). In this type of diet, different proportions of raw materials might be substituted by dried, hydrolyzed or freeze-dried raw materials, in different phases and proportions, which could help develop to a semi-moist or dry artificial diet. In this sense, a nutritional profile of the raw materials similar to the squid used in the present study, would probably provide better growth rates and conversion efficiency values.

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