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EVALUATING THE EFFECTS OF FORMULATED MOIST DIETS ON JUVENILES OF PATAGONIAN OCTOPUS *ENTEROCTOPUS MEGALOCYATHUS* (GOULD 1852)

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ABSTRACT The aim of this study was to evaluate the performance of Patagonian octopus fed with moist diets formulated with several local feed ingredients. All formulated diets were based on crab paste (70%) and the experimental feed ingredient (30%). Experiment 1 assayed salmon meal, prime sardine meal, and wheat gluten, using fresh fish as a control; experiment 2 assayed prime fish meal and macroalgal meal against crab paste alone as a control. The ingestion rate was lower than expected for all diets except those of fresh fish, crab paste alone, and crab paste plus prime sardine meal. No significant differences were found in the observed digestibility of the diets, indicating, in general, low digestibility, even for fresh fish. The highest protease values were observed for crab paste plus prime sardine meal in both experiments. The better growth of *Enteroctopus. megalocyathus* was obtained when these were fed fresh fish, which was associated with the greater consumption observed in this diet, as neither the digestibility nor the enzymatic activities of the hepatopancreas were related to this greater growth.

KEY WORDS: octopus, *Enteroctopus megalocyathus*, digestibility, enzymes, consumption rate, nutrition, moist diets

INTRODUCTION

Enteroctopus megalocyathus (Gould 1852) is 1 of 2 commercially significant species of Chilean octopus exploited on a small scale by local fishermen. Since October 2008, the species has been protected by a 3-y restriction on catches. *E. megalocyathus* can reach weights of approximately 4,000 g, and its natural diet consists mainly of crustaceans of the Cancridae and Porcellanidae families, without changes in dietary composition in relation to octopus sex (Ibañez & Chong 2008). Experimental rearing of this species has led to the need to develop formulated diets to supplement fresh diets and to permit sustainable rearing in the future. Recent studies have attempted to substitute fresh diets with a balanced diet for octopuses of other species, revealing that one of the greatest problems with the formulated diets is low consumption on the part of the octopuses, leading to poor growth and even weight loss (Aguila et al. 2007, Domingues et al. 2007). Nutritional studies of cephalopods have been carried out mainly with squid and cuttlefish (Lee et al. 1991, Lee 1994). In the case of octopodids, nutritional studies of *O. vulgaris* and *O. maya* are more recent (Aguila et al. 2007; Domingues et al. 2007, Rosas et al. 2007, Cerezo-Valverde et al. 2008, Quintana et al. 2008, Rosas et al. 2008). To date, no reports show specific growth rate (SGR) exceeding 0.7%/day with the use of formulated diets. However, Rosas et al. (2008) obtained an SGR of 2.0%/day using a reference diet of moist crab paste. This study was aimed at determining the effect of several local feed ingredients (available in southern Chile) on ingestion rates, digestibility, and the activity of digestive enzymes in *E. megalocyathus* in an attempt to evaluate how the octopuses of this species perform with formulated diets based on ingredients from diverse origins.

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MATERIALS AND METHODS

Animals

Juveniles of *E. megalocyathus* were collected by scuba diving at Hueihue ($n = 30$) (41° 52′S, 73° 51′W) in the coastal zone of Chilean Patagonia, with a mean weight of 152 ± 28 g in the first experiment and 136 ± 13 g in the second experiment. The individuals were transported to the Hatchery of Marine Invertebrates, Universidad Austral de Chile (Aquaculture Institute at Puerto Montt; 41° 42′S, 72° 50′W), where they were placed in individual flow-through 80-L black tanks with aerated seawater kept at 30–31‰ salinity; in a semidark environment with a 12:12 h light/dark photoperiod. They were held there for 5 days prior to beginning the experiment. A PVC tube (50 mm in diameter) was placed in each tank as a refuge. During the first 2 days of acclimation, the octopuses were not fed; afterward, they were fed frozen white fish (*Odontesthes* spp.) until the onset of the experiments. The first experiment was done in July at 11 ± 0.2°C and the second in November at 12.5 ± 0.3°C. The live weight of each octopus was measured weekly to correct the feed ration, and all data were used to calculate the SGR per octopus using the fit to an exponential regression according to Uriarte et al. (2009).

Diets

All the diets were based on a paste of fresh crab meat (*Cancer edwarsii*) mixed with gelatin as a binder. Experiment 1 consisted of 300 g/kg protein ingredient (either sardine meal, salmon meal, or wheat gluten) mixed with 520 g/kg crab paste and other minor ingredients (Table 1). In experiment 2, we evaluated 2

TABLE 1.

Ingredients (measured in gram per kilogram dry weight) and proximate composition of experimental diets.

Ingredients	*E. megalocyathus* Experiment 1			*E. megalocyathus* Experiment 2		
	Salmon	Prime	Gluten	Crab Paste	Prime	Macro algae
Salmon meal*	300					
Prime sardine meal*		300			275	
Wheat gluten†			300			
Commercial macroalgal meal‡						275
Crab paste	520	520	520	940	665	665
Fish oil*	8	160	20			
Starch	40	40	40			
Common ingredients§	61	61	61			
Filler	15	7	3			
Gelatin	35	35	35	50	50	50
Celite¶	21	21	21	10	10	10
Approximate composition						
Crude protein (g/kg)	572	616	637	822	772	489
Total lipids (g/kg)	106	94	85	26	77	17
Ash (g/kg)	199	224	155	130	134	181
NFE** (g/kg)	124	67	123	22	17	313
Energy†† (MJ/kg)	19.9	19.5	20.6	20.9	21.6	17.6
Protein-to-energy ratio (g protein/ MJ)	28.7	31.6	30.9	39.3	35.7	27.8

* Kindly provided by Pesquera Pacific Star.

† Kindly provided by BIOMAR.

‡ From Multiexport.

§ Vitamin mixture (2.5%), Stay–C (1.5%), vitamin E (0.02%), and mineral mixture (2.1%), kindly donated by BIOMAR; sodium benzoate (0.02%); choline chloride (0.21%); BHT (0.02%).

¶ Celite acid washed (SIGMA).

* * NFE calculated by differences including fiber and soluble carbohydrates.

† † Theoretical values according to Guillaume et al. (2004): protein, 23.7 KJ/g; lipids, 39.5 KJ/g; carbohydrates, 17.2 KJ/g.

ingredients (275 g/kg) mixed with crab paste (665 g/kg): one that was highly digestible (the ingredient from experiment 1 that gave the best results) and one that had a supposed low digestibility (macroalgal meal). For experiment 1, we used frozen fish as a control and for experiment 2 we used crab paste alone (Table 1). Each experimental tank held 1 octopus, which was fed for 15 days as a conditioning period for the different diets; afterward, consumption rates were determined over 6 consecutive days, and feces were collected for other 15 days. Four tanks were distributed randomly per diet for experiment 1 and, in experiment 2, 3 randomly distributed tanks were used. At the end of fifth week of the experiment, 3 individuals per diet were kept without food for 12 h prior to being anesthetized with ice-cold seawater and then sacrificed to obtain the hepatopancreas.

Experimental diets were prepared by thoroughly mixing ingredients with cold water until achieving a semimoist diet (Table 1; 52% final moist). Celite (Sigma) was used as a marker; it was included at 20 g/kg in experiment 1 and at 10 g/kg in experiment 2. The mixture was molded in known amounts within mussel shells to allow for the appropriate manipulation and sucking on the part of the octopus. The artificial diet was prepared on a weekly basis and stored at –20°C. The proximal composition of the fresh fish was 728 ± 14 g/kg raw protein, 49 ± 2 g/kg lipids, 200 ± 45 g/kg ash, and 23 g/kg nonnitrogenated extract.

Octopuses were fed once a day (0900 h) at 10% body weight for all regimens (Farías et al. 2009). Unconsumed feed was siphoned every day (1700 h) and the tanks were cleaned by siphoning. Overnight feces were removed from the tanks before the octopuses were given the initial feeding.

Digestibility measurements

Feces were collected by siphoning and retained in a fast paper filter. Fecal samples were immediately rinsed with distilled water and stored at –20°C. Fecal samples collected from the same tank per treatment were pooled. The ash in feed and feces was obtained by burning samples at 500°C for 6 h. The insoluble ash in acid was obtained according to Montaño-Vargas et al. (2002), following the modifications of Rosas et al. (2008), in which the ashes were placed into boiling 4N HCl for 20 min and filtered in a low-fiber filter (Whatman 42), after which they were incinerated in a muffle at 500°C for 6 h.

We calculated the coefficient of apparent digestibility (Montaño-Vargas et al. 2002, Tibbets et al. 2006) for the dry material (ADC_{DM}) as

$$ADC_{DM}(\%) = 100 - [100 \times (\text{marker in the diet}/\text{marker in the feces})]$$

Ingestion Rate

The ingestion was calculated as the difference between the delivered food and that remaining after 8 h, based on the dry

weight, and corrected for the percentage of loss not as a result of feeding, in accord with Farías et al. (2003). Briefly, the percentage of leached nutrients was measured using tanks identical to the experimental tanks but without any octopuses. These tanks (controls) received the same amount of the corresponding diet. The food remaining after 8 h was siphoned and dried at 100°C for 24 h for both experimental and control tanks. The percentage of humidity of the pelletized diets was measured by drying samples of 1 g at 100°C for 24 h. The percentage of loss not as a result of feeding was calculated as the ratio of dry matter retained after leaching in the control tanks and dry matter from the original samples.

Enzymatic Activity

Enzymatic activity was measured individually in octopuses fed with each experimental diet. All the animals were starved for 12 h before sampling. The digestive glands were dissected and stored at –20°C until analysis. Frozen samples were homogenized at 4°C in 500 µL ice-cold pyrogen-free water. Homogenates were centrifuged at 13,200*g* for 20 min at 4°C. The supernatant was diluted in 10 volumes of ice-cold pyrogen-free water. The homogenates were immediately used for enzyme analysis. The soluble protein content was measured according to Bradford (1976) using bovine serum albumin (2.0 mg/mL) as the standard. The spectrophotometer used for measuring absorbance in all cases (enzymes and protein content) was a Multiskan microplate reader.

Assays were duplicated for each sample. The total proteolytic activity was assayed with azocoll 2.4% (Sigma Aldrich A4341) as a substrate in a phosphate buffer, following the method described by Van Wormhoudt et al. (1980). Briefly, a 5:1 mixture of the substrate and sample was incubated at 37°C for 1 h. The reaction was stopped by adding TCA (110 mmol/L). Reaction tubes were transferred to ice for a few minutes and then centrifuged for 10 min at 10,000*g* and 4°C. The absorption of the supernatant was measured in a spectrophotometer at 442 nm. The activity was expressed as units per milligram protein in the extract, where 1 unit corresponds to the variation (by 0.001) of optic density per minute.

One unit is defined as the amount of enzyme that catalyzes the release of azo dye causing a $\Delta A/\Delta t = 0.001$/min. Assays were duplicated for each sample. Trypsin and chymotrypsin activities were measured in diluted (1:100) homogenates using 0.1 mol/L Na-benzoyl-L-Arg-p-nitroanilide (BAPNA) and 0.1 mol/L[1] succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAAPNA) as substrates, respectively, in a buffer with a pH value of 7.5 at 4°C (0.1 M Tris; 0.05 M NaCl; pH, 7.5). Absorbance was read at 405 nm. Acid phosphatase activity was determined according to Perrin et al. (2004) using p-nitrophenyl-phosphate (2%) as a substrate. After 30 min of incubation at 25°C, the reaction was stopped by adding 1 mL NaOH (1 mol/L). Absorbance was measured at 405 nm. Total acid phosphatase activity was expressed as specific activity (units per microgram protein), with 1 enzymatic unit corresponding to 1 µmol/L p-nitrophenol/min.

Statistical Analysis

One-way ANOVA was applied to the growth rate, ingestion rate, ADC_{DM}, and enzyme activity (Sokal & Rohlf 1995). The Kruskal-Wallis test was used when violations of homogeneity variance occurred in the raw data (Sokal & Rohlf 1995).

RESULTS

All diets were well accepted by *E. megalocyathus*. For experiment 1, only octopuses fed fresh fish showed a positive growth rate (1.47 ± 0.04%/day), whereas animals fed the 3 formulated diets showed negative growth rates ($P < 0.05$; Table 2). In experiment 2, the octopuses fed crab paste alone showed a positive growth rate (0.27 ± 0.09%/day), although this was only one fifth of the SGR observed in animals fed fresh fish in experiment 1 (Table 2). In the other 2 diets of experiment 2, the growth rate was negative; nonetheless, the octopuses fed a crab-based diet plus prime fish meal showed an SGR near 0 (–0.06 ± 0.62%/day).

Ingestion Rate

In experiment 1, the ingestion rate of octopuses fed fresh fish was higher (1.39 ± 0.35 g/d; 2.9% BW/day in wet diet) than for the other semipurified diets (mean value of 0.47 ± 0.19 g/d; 0.8% BW/day, in wet diet; $P < 0.05$; Table 2). By converting all the diets to dry weight, the diet with wheat gluten was found to be the least consumed (constituting only half the fish consumption). In experiment 2, animals fed a diet of prime fish meal had an ingestion rate of 0.59 ± 0.35 g/day (1.4% BW/day, in wet diet); this did not differ significantly from the octopuses fed only

TABLE 2.

Biological and nutritional indices obtained for juvenile Patagonian octopus (*Enteroctopus megalocyathus*) fed experimental diets.

Experiment	Ingredients	SGR (%/day)	Consumption in Wet Diet (% bwt/day)	Consumption in Dried Diet (% bwt/day)	ADC (%)
1	Salmon meal	–0.9 ± 0.1 [a]	0.8 ± 0.2 [a]	0.4 ± 0.1 [ab]	49.3 ± 4.8
	Prime sardine meal	–1.1 ± 0.2 [a]	1.0 ± 0.5 [a]	0.5 ± 0.2 [ab]	61.8 ± 3.1
	Wheat gluten	–1.2 ± 0.1 [a]	0.7 ± 0.3 [a]	0.3 ± 0.1 [a]	52.6 ± 7.9
	Fresh fish	1.5 ± 0.1 [b]	3.0 ± 0.5 [b]	0.7 ± 0.1 [b]	47.1 ± 4.0
2	Commercial macroalgal meal	–0.3 ± 0.3	1.0 ± 0.6	0.4 ± 0.2	55.1 ± 10.4
	Prime sardine meal	–0.1 ± 0.6	1.4 ± 0.3	0.7 ± 0.2	61.7 ± 4.5
	Crab paste	0.3 ± 0.2	2.0 ± 0.4	0.5 ± 0.1	59.5 ± 3.4

Four replicate tanks per diet for experiment 1 and 3 replicate tanks per diet in 3xperiment 2. Values are mean ± SE. Values in the same column with different superscripts are statistically different at $P < 0.05$.

crab paste or a diet of macroalgal meal (mean value of 0.73 ± 0.16 g/day; 1.6% BW/day, in wet diet; Table 2). By converting all the diets to dry weight, we saw that the diet had some effect on consumption, and the consumption values in experiment 2 were similar to consumptions of experiment 1 (Table 2).

ADC_{DM}

ADC_{DM} values for *E. megalocyathus* during experiment 1 were between 47% and 62%. No differences were found between octopuses fed fresh fish and a formulated diet. The average ADC_{DM} of this experiment was 52.8 ± 4.9% (Table 2).

No statistical differences were observed in ADC_{DM} values (55–62%) during experiment 2. A mean value of 58.8 ± 6.1% ADC_{DM} was calculated for all the foodstuffs used in this experiment (Table 2).

Activity of Digestive Enzymes

General protease activity fluctuated between 3,383 U/mg protein and 10,775 U/mg protein. Values were low for octopuses fed a crab paste diet, wheat gluten, and macroalgal meal, and were high for those fed prime fish meal. Intermediate values were recorded for animals fed salmon meal and fresh fish (Fig. 1A).

Acid phosphatase activity was high in octopuses fed salmon fish meal, followed by animals fed prime fish meal, wheat gluten, a crab-based diet, and macroalgal meal. Octopuses fed fresh fish showed lower acid phosphatase activity than the rest of the treatments ($P < 0.05$; Fig. 1B).

Trypsin activity ranged from 3.29–6.52 U/mg in experiment 1. No activity of trypsin was recorded in the hepatopancreas of octopuses fed a crab-based diet, macroalgal meal, or prime fish meal during experiment 2 (Fig. 1C). Chymotrypsin activity ranged from 0.06–0.15 U/mg protein for all treatments in both experiments (Fig. 1C).

DISCUSSION

SGRs of 1.46%/day and 0.27%/day were recorded in octopuses fed fresh fish and crab paste, respectively. It is interesting to note that the greater weight losses occurred in experiment 1 when the temperature was 11°C, and seemed to be reduced in experiment 2 when the temperature was 12.5°C.

Values of 0.49–1.96%/day have been observed in *E. megalocyathus* fed fresh crab for different rearing periods; specimens fed a diet based on fresh mytilids had negative values of –0.32%/day (Pérez et al. 2006). This indicates that the range of growth and weight loss in experiments 1 and 2 falls within that observed for *E. megalocyathus* fed fresh diets and is not necessarily a typical characteristic of formulated diets. Even the variation that the previous authors found for a single diet (fresh crabs) is within the same range as the variation in SGR found in this study for the 2 best diets: fresh fish and crab paste.

In other octopuses such as *Octopus vulgaris*, an SGR of 0.22%/day was observed when these animals were fed formulated diets based on fish (400 g/kg) plus shrimp (100 g/kg) and bound with gelatin. An SGR of 0.71%/day was found when fed diets with fish (500 g/kg) plus shrimp (100 g/kg) bound with alginate (Cerezo-Valverde et al. 2008).

Semipurified diets were accepted by the animals; however, the ingestion rate was lower than expected. Only the octopuses fed fresh fish, crab paste, and crab paste plus prime fish meal showed ingestion rates higher than 1% BW/day (wet weight diet). Cerezo-Valverde et al. (2008) found ingestion rates between 1.31% BW/day and 3.1% BW/day (wet weight diet) when *O. vulgaris* were fed diets bound with gelatin and alginate, respectively, and suggested that the lower growth rate observed in experimental animals fed both diets could be related to the low acceptability of mixed diets. This low acceptability could be similar to that found for *E. megalocyathus* for all the diets that included a mixture with crab paste. It was even possible to observe a tendency for lower consumption and, therefore, lower growth when other minor ingredients were added along with the main ingredient.

On the other hand, Quintana et al. (2008) showed ingestion rates of 5.8, 8.6, and 8.5% BW/day (wet weight diet) for *O. vulgaris* subadults fed fresh squid, fresh squid paste bound with gelatin, and shrimp paste bound with gelatin, respectively, suggesting that single-item diets of squid and shrimp could be more attractive for *O. vulgaris* than the fish–shrimp mix used by Cerezo-Valverde et al. (2008).

Rosas et al. (2007) used artificial diets enriched with squid paste and obtained negative growth associated with low assimilation efficiency. These authors concluded that the lipid level could be the main factor limiting the ADC. The natural diet of *E. megalocyathus* consists predominantly of crustaceans, molluscs, and teleost fish (Ibañez & Chong 2008) and, although its main prey include fish and crab, it presents an important consumption of fish, crab, and octopus eggs (25%), suggesting that *E. megalocyathus* is not only well adapted to digesting foods with high protein levels (crustaceans), but also to digesting those with high lipids (e.g., fish, crustacean, and octopus eggs). This adaptation could be interesting, but dietary lipids have already been recognized as a nutritional factor limiting the growth rate of *O. vulgaris* (García-García & Aguado-Giménez 2002, Giménez & Garcia 2002, Petza et al. 2006) and *O. maya* (Domingues et al. 2007).

Digestibility in *E. megalocyathus* was low for all the diets and did not differ among the ingredients, despite the use of nearly 300 g/kg macroalgal meal in one of the diets, which was expected to act as an example of low digestibility. ADC values of 800 g/kg dry matter have been reported for *O. maya* using a diet that was highly similar to the basic diet of experiment 2 (ashes resistant to acid were used as a marker (Rosas et al. 2008)). For *E. megalocyathus*, an absorption value of 72% was reported for fresh fish (*Odontesthes* sp.) using the Conover method (Farías et al. 2009); an assimilation efficiency value of 99.2% and an absorption efficiency of 0.99 were reported by Pérez et al. (2006) for a mix of crabs (*Petrolisthes* sp., *Acanthocyclus hassleri*, *Hemigrapsus crenulatus*) using total ashes and feces collections, respectively. Given such different methods, it is difficult to establish comparisons for this study. The only exception was *O. maya*, for which the same method and a diet based on crab paste were used. In this context, the values of digestibility (78.6%) and SGR (2%/day) for *O. maya* were much higher than those recorded with a similar diet for *E. megalocyathus*. The largest difference between the 2 species may be their adaptation to warm and cold water, respectively, which could have a strong effect on the different strategies applied to the use of digestive enzymes and the protein metabolism, as well as the efficiency of the use of energy (Farías et al. 2009).

For the hepatopancreatic enzyme activity analyzed in this study, we found that total proteases and phosphatase acids were

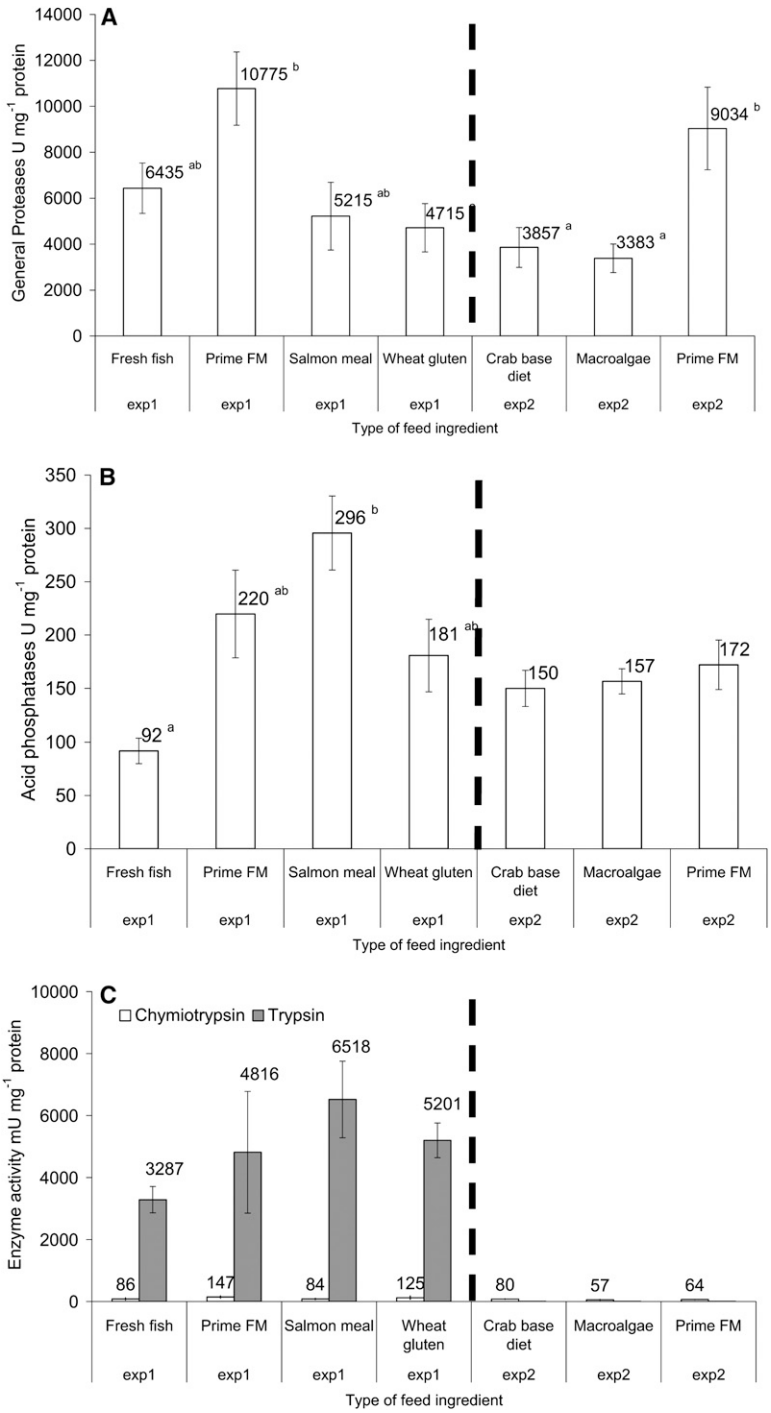

Figure 1. ***E. megalocyathus*****. (A–C) Activity of digestive gland enzymes in octopuses fed different ingredients: general proteases (A), acid phosphatase (B), and chymotrypsin and trypsin (C) . Mean ± SE of 4–6 replicate samples. Different letters mean differences among treatments in an experiment at a level of $P < 0.05$. The dotted vertical line separates both experiments. *Trypsin values < 10 mU/mg protein.**

on the same order of magnitude in both experiments, although some differences arose when comparing diets from experiment 1 and 2. The order of magnitude of the enzymatic activity only showed differences between the 2 experiments for the chymotrypsin and trypsin enzymes, which were found to be greatly reduced in experiment 2.

General protease and trypsin activities reported in the hepatopancreas of *O. maya* (Aguila et al. 2007) were 2 and 7 times higher than those observed for *E. megalocyathus,* respectively, suggesting that the tropical species should have a greater proteolytic capacity than the coldwater species.

Digestive enzyme activity can be induced as a response to growth or in an attempt to drive more nutrients from a deficient meal during digestion (Van Wormhoudt et al. 1980). Prime fish meal induced general proteases in both experiments, whereas the activity of phosphatase acid in *E. megalocyathus* was only

induced in experiment 1 by the salmon meal. The results for *O. maya* (Aguila et al. 2007) suggest that the enzyme capacity of octopuses and, consequently, food digestibility could be improved using nutrients that stimulate enzyme secretion. In this sense, it was found (Best &Wells 1983, Best &Wells 1984) that enzyme secretion can be regulated by visual, chemical, and endocrinological stimuli. Nonetheless, the stimulation of the protease and acid–phosphatase activities observed in *E. megalocyathus* was not related to better digestibility.

In summary, the better growth of *E. megalocyathus* was obtained when they were fed fresh fish, which was associated with the greater consumption observed in this diet, because neither the digestibility nor the enzymatic activities of the hepatopancreas was related to this greater growth. The crab paste alone allowed only one-fifth of the growth observed in octopuses fed fresh fish. Nonetheless, this growth was located within the range observed for the Patagonian octopus fed fresh crab. Therefore, crab paste could be considered to be a reference diet for this species. On the other hand, of all the protein ingredients tested, prime sardine meal offers the best possibilities as an ingredient in future diets, because it was the only ingredient mixed with crab paste that was consumed by the octopuses at a rate of ≥1% BW/day and that did not result in weight loss, maintaining an SGR near 0.

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