Common octopus (*Octopus vulgaris*) Performance When Including Fasting on Feeding Schemes: Preliminary Data Regarding a Formulated Feed

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TRG, JCV, AVS and BGG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author TRG managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

The common octopus (*Octopus vulgaris*) has aroused great interest in recent years as a new species for aquaculture. The current research is focused on developing a formulated feed, although a proper diet management has also promoted rearing success of other commercial cultured species. It is documented that wild animals eat depending on prey availability and most experience fasting in nature. Hence, *O. vulgaris* subadults were subjected to two different feeding schemes, with a similar semi-moist diet, including either 2 (2FDb, control) or 3 (3FDb) non-consecutive days of fasting per week. Growth, feed efficiency (FE), digestibility and condition were assessed after 56 days of rearing. Both feeding schemes promoted similar growth and digestibility (P>0.05), 100% of survival and higher food-intake after fasting. Interestingly, feed efficiency (FE) was enhanced with the 3

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fasting days scheme (58.6% vs. 48.3% for 2FDb scheme; P<0.05). Results might indicate that *O. vulgaris* has the ability to compensate fasting days through an increase in food intake on the subsequent day or a better use of its reserves. Moreover, a reduction on feeding days might promote a decrease in production costs at commercial scale.

1. INTRODUCTION

The common octopus (*Octopus vulgaris*) is considered a potential species for industrial aquaculture [1,2]. Currently, research is focused on developing a formulated feed, with an adequate nutritional composition and palatability [3–6]. Still, it must be considered that, in nature, cephalopods are subject to fasting [7] but still attain higher growth rates. This points to the possibility of establishing a feeding regime including fasting days when these species individuals are kept in captivity. In fact, this type of feeding scheme is already a common practice in other commercially reared species, such as salmonids [8]. A proper feed management has promoted a decrease in production expenses while maintaining culture success of fish species [8,9] through a compensatory growth strategy [10].

Octopus food intake increases after fasting days, when individuals are fed with either formulated [3,5] or natural diets [11]. Contrariwise, these species display an irregular ingestion pattern when fed daily. In this sense, some days they practically reject all food supplied which might indicate, as occurs in the wild, that this species doesn't need a daily feeding regime. Accordingly, Morillo-Velarde et al. [5] and García-Garrido et al. [12] proved the use of energetic reserves stored in the digestive gland during starvation, principally as triglycerides. In addition, amino acids and carbohydrates can also be used during fasting periods [13,14]. This has been reported not only in this species but also in other cephalopods, such as *Sepia officinalis* [15,16]. A suitable feeding regime for *O. vulgaris* could avoid an excess of lipids storage in the digestive gland when fed with formulated diets [17,18], which might have a negative effect on ingestion and in the condition of individuals in the long term. Moreover, a reduction in nourishing days in feeding schemes also denotes a cost reduction and economic benefits for growing or maintenance of commercial production of this species. In this sense, studies testing feeding schemes that include fasting might allow a better comprehension on how it affects the growth performance. Hence, we tested if an increase in fasting days included in feeding schemes affects octopus production performance.

2. MATERIALS AND METHODS

In the present study, two feeding regimes were applied to octopus, reared with the same semi-moist diet (Table 1) but fasting either two (2FDb, fasting on Wednesdays and Saturdays, [19]), as control, or three (3FDb, fasting on Mondays, Wednesdays and Saturdays) non-consecutive days per week. Growth, feed efficiency, digestibility and condition of *O. vulgaris* were assessed after 56 days of rearing.

2.1 Acclimation and Experimental Conditions

A total of 16 *O. vulgaris* juveniles were captured by trawling in the Mediterranean Sea (Murcia, SE, Spain), transported to the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) facilities and kept in group in 1970L circular tanks for captive acclimation for two weeks. A week before the start of the experiment, octopuses were re-allocated individually in 216L circular tanks, of a recirculated seawater system described in [19], and were acclimated to isolation conditions for a week. Until the start of the experiment, individuals were fed to satiety on crab (*Carcinus mediterraneus*). The experiment was performed at 19.9±1.5°C, salinity of 36.8 ‰, dissolved oxygen concentration above 80% saturation and a photoperiod of 12L: 12D.

Two experimental groups of eight individual replicates were established depending on the feeding regime, being fed to satiety a semi-moist prepared diet, provided as cube/rectangular shaped pieces, previously tested by Rodríguez-González et al. [19] (see Table 1 for details regarding raw materials and proximate composition).

Table 1. Basal mixture (%) and proximate composition (% dry weight) of freeze-dried bogue (FDb) formulated diet [19]

		FDb diet
Basal mixture (%)	Water	40
	Gelatin [1]	22
	Egg yolk [2]	10
	Bogue [3] (*Boops boops*)	10
	Squid [3] (*Todarodes sagittatus*)	5
	Crab [3,4] (*Carcinus mediterraneus*)	5
	Fish oil	2
	Glucose [5]	3
	Starch [6]	3
Proximate composition (%)	Moisture	43.48±0.13
	Crude protein	66.14±0.01
	Crude lipid	22.17±0.46
	Ash	5.77±0.02
	NFE [7]	5.93±0.51
	AIA [8]	0.0938±0.0247
	Gross energy (kJ/100 g)	2400.00±9.50
	P/E [9] (g/MJ)	26.22±0.10

Data as mean±SD; [1]Granulated Gelatin, Bloom 220, supplied by Productos Sur, S.A. (Pol. Ind. Oeste, San Ginés, Murcia, Spain); [2]Egg yolk powder, supplied by Avícola San Isidro S.L. (Los Belones, Cartagena, Murcia, Spain); [3]Freeze-dried ingredients; [4] Whole animal freeze-dried; [5]Glucose anhydride, supplied by Guinama S.L.U. (Alboraya, Valencia, Spain); [6]Starch from potato soluble, supplied by Panreac Química S.L.U., (Castellar del Vallés, Barcelona, Spain); [7]NFE, Nitrogen-free extract, calculated by difference; [8]AIA, Acid Insoluble Ash; [9]P/E= protein/energy ratio

Every individual was weighted at day 0 (mean weight of 675±65 g), 28 and 56 days of rearing to avoid a handling stress. On the last day, individuals were euthanized by immersion in iced seawater. The experiment was performed under Project INIA-Project RTA2012-00072-00-00, which was approved before the entry into force of Directive 2010/63/EU as national legislation in Spain.

2.2 Preparation, Conservation and Water Stability of the Diets

The formulated diet based on freeze-dried ingredients (diet FDb) was prepared according to Rodríguez-González et al. [19], vacuum-packed and maintained frozen at -20°C until use.

The water stability was defined as feed disintegration percentage (WSI) of dry weight after soaking in seawater for 24 h. WSI was calculated following WSI (%)= ((DWi – DWf)/DWi)x100, where DWi and DWf are the initial and final dry weights, respectively.

2.3 Sample Collection and Determinations

During the experiment, the remaining food (moistened and less cohesive cube/rectangular shaped piece) of each replicate was collected (using a small net and removing the water excess) on the consecutive day of feeding and dried in an oven (105°C for 48 h) to calculate the individual daily feed intake. Similarly, faeces (filamentous, soft and high water-content structures that can appear rolled) were daily collected during the experiment (aspirated by a syphon, collected using a small net and removing the water excess) and preserved frozen (-80°C) to estimate macronutrient digestibility. In addition, formulated feed and faeces samples were freeze-dried and pulverized before macronutrient analyses.

After euthanasia, tissue samples were collected, grinded and frozen (-20°C) until biochemical determination analyses were performed (in triplicate) in eight individuals of each treatment. Four individuals were used for macronutrient composition of the digestive gland and muscle

tissue determinations, and another four for whole animal determinations.

The macronutrient composition (moisture, ash, crude protein and lipid content, and nitrogen-free extract, calculated by difference) were determined according to AOAC [20] methodologies as described in Rodríguez-González et al. [19].

2.4 Calculated Parameters and Indices

Growth, ingestion and feed efficiency indices were calculated as follows: (1) Weight gain (Wg; g) = $Wf - Wi$, where Wi = initial weight (g); Wf = final weight (g); (2) Absolute growth rate (AGR; g.day-1) = $(Wf-Wi) / t$, where t = time in days; (3) Specific growth rate (SGR; %BW.day-1) = $(LnWf - LnWi) \times 100 / t$, where BW refers to body weight; (4) Absolute feeding rate (AFR; g.day-1) = IF / t, where IF is the Corrected ingestion (IF; wet weight in g) = (dry feed supplied in g – uneaten dry feed in g x F) + moisture feed supplied in g and F is a Correction factor for ingestion (F) = DWi / DWf, which considers disaggregation rate in water being DWi = initial dry weight (g) and DWf = final dry weight (g); (5) Absolute protein or lipid feeding rate: AyFR (g.day-1) = Iy / t, where y could be either protein or lipid, Iy = ingested protein or lipid (g); (6) Specific feeding rate (SFR; %BW.day-1) = $(AFR \times 100) / Wa$, where Wa = Average weight in g = $(Wi+Wf) / 2$; (7) Feed efficiency (FE; %) = $(Wf - Wi) \times 100 / IF$; (8) Feed conversion ratio: FCR = $IF / (Wf - Wi)$; (9) Productive value for lipids or proteins (LPV and PPV, respectively; %) = 100 x retained (Lipids or Proteins) / Intake (Lipids or Proteins) and (10) Digestive gland index (DGI; %) = $DGW \times 100 / Wf$, where DGW refers to digestive gland weight (g).

The variation of the daily food intake was analysed through the instantaneous feeding rate (IFR), according to [11]. Similarly, the variation of the weekly intake was analysed through the feeding rate per week (WFR), expressed as a percentage of the weekly ingested food, by each, with respect to its body weight estimation, from the obtained SGR for each one, at the 4th day of each week.

2.5 Statistical Analysis

The obtained results were expressed as mean ± standard deviation (SD). Normality and homogeneity of variances were tested using the Shapiro-Wilk test and the Levene's test [21], respectively. An arcsine square root transformation was applied on percentages and data when normality and/or homoscedasticity where not met. A t-test for independent samples [21] was used to compare: growth, ingestion and feed efficiency indices for each experimental group; the collected faeces macronutrient composition; digestibility; and the proximate composition in octopus tissues fractions (digestive gland, muscle and whole animal). A Mann-Whitney U test was used when a normal distribution and/or homogeneity of the variances were not achieved after arcsine square root transformation [21].

3. RESULTS AND DISCUSSION

3.1 Results

The semi-moist formulated diet (FDb, Table 1) was accepted by both groups and promoted growth, faeces production and 100% survival. Ingestion and growth were similar between experimental groups considering the lapses between samplings and the whole experimental period (P>0.05; Table 2). Nonetheless, the 3FDb group presented higher FE and PPV than 2FDb (P<0.05, Table 2) without differences on animal condition.

The macronutrient composition of faeces was similar between both feeding regimes (P>0.05; Table 3). Alike, the apparent digestibility coefficients (dry matter, protein, lipids and nitrogen-free extract) were similar (P>0.05; Table 3) and the whole animal, digestive gland and muscle proximate composition were also similar between groups (P>0.05, Table 4).

When Instantaneous Feeding Rate (IFR) values were compared on a daily basis (Fig. 1), significant differences were identified (P<0.05). Both groups displayed the highest IFR peaks after fasting. Additionally, the 2FDb group ingestion exceeded 4% IFR in some cases.

The Feeding Rate per Week (WFR) displayed dissimilarities between different weeks (Fig. 2) but showed a similar ingestion trend, which was characterized by an intake reduction followed by an increase in the same weeks. In any case, ingestion and growth were similar for both fasting regimes through all the experimental period (P>0.05, Table 2).

Table 2. Growth, ingestion and feed efficiency for two (2FDb) or three (3FDb) days of fasting

Index/Diet		2FDb	3FDb
Growth	W(g)		
	Day 0	661±62	689±67
	Day 28	872±102	939±126
	Day 56	1029±144	1127±123
	Wg_{0-28} (g)	211±63	249±70
	AGR_{0-28} (g/day)	7.55±2.27	8.91±2.50
	SGR_{0-28} (%BW/day)	0.98±0.25	1.09±0.22
	Wg_{28-56} (g)	157±71	188±49
	AGR_{28-56} (g/day)	5.59±2.52	6.71±1.76
	SGR_{28-56} (%BW/day)	0.58±0.22	0.66±0.19
	Wg_{0-56} (g)	368±112	437±80
	AGR_{0-56} (g/day)	6.57±2.01	7.81±1.42
	SGR_{0-56} (%BW/day)	0.78±0.19	0.88±0.12
Ingestion	AFR_{0-56} (g/day)	13.41±2.07	13.37±2.46
	$APFR_{0-56}$ (g/day)	5.01±0.77	5.00±0.92
	$ALFR_{0-56}$ (g/day)	1.68±0.26	1.68±0.31
	SFR_{0-56} (%BW/day)	1.59±0.14	1.47±0.16
Feed efficiency	FE_{0-56} (%)	48.31±9.70	58.65±6.47*
	FCR_{0-56}	2.15±0.47	1.72±0.18
	$FCRdm_{0-56}$	1.22±0.27	0.97±0.10
	PPV_{0-56} (%)	16.80±4.85	23.90±2.61*
	LPV_{0-56} (%)	10.12±4.44	9.74±0.85
Condition	DGI_{0-56} (%)	4.34±1.23	5.55±1.09

*Data as mean±SD. * $P<0.05$. Weight (W), weight gain (Wg), absolute growth rate (AGR), specific growth rate (SGR), absolute feeding rate (AFR), absolute protein feeding rate (APFR), absolute lipid feeding rate (ALFR), specific feeding rate (SFR), feed efficiency (FE), feed conversion ratio (FCR), dry matter (dm), protein productive value (PPV), lipid productive value (LPV) and digestive gland index (DGI)*
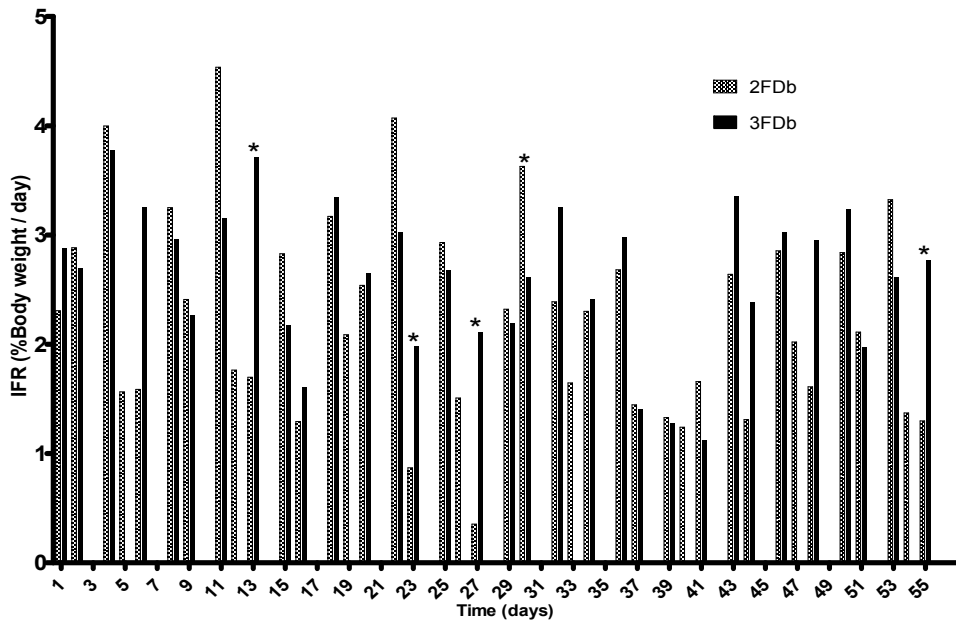

Fig. 1. Instantaneous Feeding Rate (IFR) for two (2FDb) or three (3FDb) fasting days throughout the experiment
* P<0.05

Table 3. Faeces proximate composition (% dry weight) and apparent digestibility coefficients (ADC) obtained with two (2FDb) or three (3FDb) days of fasting

Index/Diet		2FDb	3FDb
Proximate composition	Crude protein	17.69±2.42	16.14±1.43
	Crude lipid	24.46±1.03	26.28±5.22
	Ash	29.70±1.50	28.34±1.98
	NFE	28.15±3.33	29.25±5.03
	AIA	0.7092±0.1811	0.8654±0.1894
ADC	ADCDM	86.22±3.25	88.78±2.57
	ADCPROT	96.30±1.02	97.23±0.86
	ADCL	84.78±3.67	86.46±4.83
	ADCNFE	35.21±13.68	46.06±3.29

No differences found in any comparison (P>0.05). NFE- Nitrogen-free extract, calculated by difference; AIA- Acid Insoluble Ash; ADCDM- apparent digestibility coefficients of the dry matter; ADCPROT- apparent digestibility coefficients of the protein; ADCL- apparent digestibility coefficients of the lipids; ADCNFE- apparent digestibility coefficient of the nitrogen-free extract

Table 4. Macronutrient composition (% dry weight) of the different fractions of common octopus fed applying two different feeding schemes: two (2FDb) or three (3FDb) days of fasting

		2FDb	3FDb
Digestive gland	Moisture	62.12±6.57	56.38±0.93
	Crude protein	43.57±11.16	39.40±3.69
	Crude lipid	44.28±13.77	53.14±6.91
	Ash	3.77±1.41	3.12±0.89
	NFE	8.39±2.83	4.34±4.76
Muscle	Moisture	81.09±0.81	80.86±1.26
	Crude protein	81.76±3.02	76.72±3.55
	Crude lipid	2.44±0.18	2.72±1.80
	Ash	11.56±0.73	11.43±1.36
	NFE	4.24±3.14	9.13±4.52
Whole animal	Moisture	79.66±1.27	79.04±1.04
	Crude protein	73.55±3.02	74.24±3.17
	Crude lipid	5.89±1.84	4.95±0.20
	Ash	10.74±1.23	10.10±0.75
	NFE	9.82±4.12	10.71±3.74

No differences found in any comparison (P>0.05). NFE = Nitrogen free extract
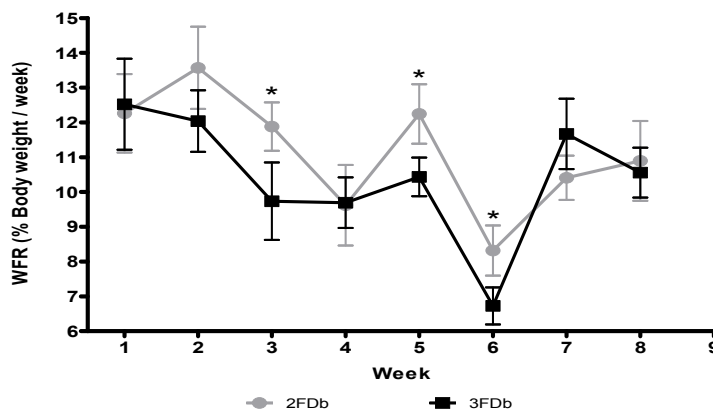

Fig. 2. Feeding Rate per Week (WFR) for both fasting protocols, 2FDb and 3FDb
*P<0.05

3.2 Discussion

It seems that *O. vulgaris* subadults display a similar ingestion behaviour (see Fig. 1) as observed in fish species after fasting. In fact, Skalski et al. [22] reported that fish usually show higher food intake after fasting. Moreover, Ali et al. [10] recognized hyperphagia as a mechanism of growth compensation, which is characterized by a higher food intake after fasting by fish that are continuously fed ad libitum. The duration of hyperphagia depends on the frequency and duration of fasting, and food consumption might be equal or higher than in common conditions [10].

Ali et al. [10] did not observe growth depression when fish were starved for short periods with enough food being supplied between fasting episodes, but higher food intake rate was verified. Hence, it seems that growth depression was avoided with similar ingestion by octopus in the present experiment.

The obtained results with *O. vulgaris* were according to those reported by Teskeredžic et al. [23], who had already verified no differences in growth or body composition of rainbow trout fed daily or three times per week.

In the present study, no differences were found between 2 or 3 days of non-consecutive fasting, which indicates that octopus fed on this prepared diet were able to store enough reserves to be used to maintain the homeostasis during fasting. *O. vulgaris* is known for having the ability to regulate food intake when a previous meal was ingested in short-term [24]. Therefore, individuals experience fasting in nature and have the ability to adapt its metabolism to these specific conditions, as described in *S. officinalis* [15,16].

After fasting, both groups displayed higher food-intake as compensation. On the other hand, both groups displayed a similar trend of food intake when data were normalized as weekly food-intake. It is interesting that FE was better with 3 days of fasting, while no statistical differences were identified regarding FCR probably due to individual data variability.

Interestingly, both treatments displayed a similar trend on feed intake, growth and conversion to those obtained by García García and Cerezo Valverde [11] in octopus fasted and fed on a crab-based diet (Fig. 3). These authors obtained similar Specific Feeding (SFR), Specific Growth (SGR) and Food Conversion Rates (FCR) on animals fed with crab when comparing daily food supply with 1 fasting day per week. However, 2 fasting days per week caused a significant reduction in SFR and FCR, while SGR was similar. Analogously, despite statistical differences were not verified for any index, a similar trend was verified on mean values supplying the FDb formulated diet when increasing fasting from 2 to 3 days per week: SGR displayed a slight increase while FCR was slightly reduced (Fig. 3).

At the moment, octopus ongrowing development at industrial scale is limited by scarce specific production technology, i.e. the absence of massive production of paralarvae and juveniles in hatcheries, which implies the capture of wild juveniles, and the lack of a commercial feed that promotes high growth and survival. Specific economic studies performed on the production of octopus fed on natural diets, i.e. by-catch species with low commercial value, reared in sea cages related high-risk and low-profit to this activity. In these, juveniles capture and feeding were referred as the main production costs: 40% and 12% in the NE of Spain [25] or 17-23% and 38-40% in the Mediterranean coast [1], respectively. Wild subadults and food price are dependent on availability and market demand fluctuations. When using formulated diets, the costs of feed must consider the cost of raw materials but also those related to the ingredients processing and diet preparation. Independently of feed, this study was conducted with isolated individuals fed a formulated diet and further research must be performed to quantify the real relevance on profitability of the obtained results at industrial scale. In this sense, it is important to consider mortality on octopus reared in group. In the latter, octopus mortality is frequently observed after high-stress conditions or results from cannibalism and is significantly dependent on the food supplied (either fish or crab), stocking density, temperature, weight and size dispersion [26].

Studies regarding feeding were performed supplying natural food and reported lower mortality rates when crustaceans were used compared to fish [26]. In this sense, a commercially formulated feed will need to be nutritionally balanced and possess organoleptic characteristics to promote ingestion and similar growth performance to those obtained with crustaceans while enhancing efficiency. Rodríguez et al. [27] established 10 kg m-3 as
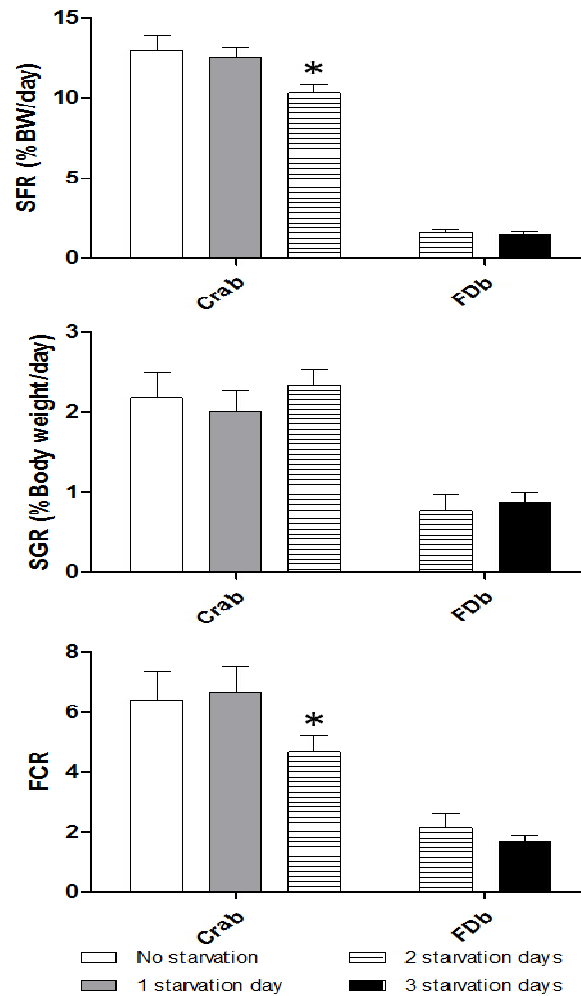
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Fig. 3. Specific feeding rate (SFR, % body weight/day), specific growth rate (SGR, % body weight/day), and food conversion rate (FCR) obtained from the present feeding regimes (2FDb and 3FDb) and compared with those fed with crab [11]
* (P<0.05) Statistical differences assessed independently for each diet

the optimal initial stock density but García García et al. [26] initially established 6.6-35.2 Kg m-3 reaching 46 kg m-3 without observing an impact in growth or survival. A temperature negative effect might be avoided maintaining temperature within the optimal temperature range for common octopus [27,28] of 15-21°C. Since octopus was individually stocked in tanks, the present study didn't test cannibalism while this might be a relevant factor affecting mortality when animals are grown in groups. Since octopuses living in the wild are subjected to fasting/starvation, food supply was estimated applying wide individual margins (minimum 5%BW/day) to ensure an *ad libitum* feeding scheme. Following this reasoning, when octopus is to be reared in group, the food

supply should be estimated according to the total biomass (minimum 5%biomass/day). In contrast, Chapela et al. [29] observed that stressed or malnourished smaller dominated animals, due to the establishment of hierarchies in octopus groups, were more likely to die. García et al. [26] also found an inverse relation between survival and size dispersion, highlighting the relevance of homogeneous sizes in grouped reared octopus.

4. CONCLUSION

Growth, survival, proximate composition of octopus and digestibility were not influenced by a feeding regime including either fasting for 2 or 3

non-consecutive days. *O. vulgaris* subadults seem to have the ability to compensate fasting through an increase in food intake on a subsequent day.

The application of feeding schemes that include fasting days is in accordance with the natural feeding pattern described for this species but is also an interesting option for industrial application, since it promotes a reduction on operational costs achieving similar growth and FE when supplying a formulated diet. In fact, our group current research is focused on octopus grouped rearing applying fasting protocols. Despite the interesting results obtained in the present preliminary study, octopus production at commercial scale is still hampered by the high costs related to food and the purchase of live juveniles. Still, the present results indicate that fasting may be a way to reduce one of the main production costs, the amount of feed. In this sense, further research is needed to achieve a sustainable feed that will allow a massive production of juveniles.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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