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Effects of dry pelleted diets on growth and survival of the edible sea urchin *Arbacia stellata* (Blainville, 1825) for an echinoculture feasibility

Efectos de las dietas de granulado seco sobre el crecimiento y la supervivencia del erizo de mar comestible *Arbacia stellata* (Blainville, 1825) para una factibilidad del equinocultivo

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ABSTRACT | The sea urchin, *Arbacia stellata* is part of a new biofood landscape for the global marine non-food organisms to be used as nutraceuticals. A 98-day feeding trial (a simple completely randomized experimental design) was performed using specimens of *A. stellata* (n= 88; 29.3 ± 0.2 mm and 14.5 ± 0.3 g mean initial test diameter and weight, respectively) to evaluate the efficacy of three isoproteic diets containing crustacean and vegetable protein sources in different combinations, for developing a low-cost dry formulated diet for promoting their growth (in test diameter and weight), gonadal yield and survival. Diet 1 (shrimp, *Penaeus vannamei*), Diet 2 (Sacha Inchi, *Plukenetia volubilis*) and Diet 3 (mixed diet) were prepared. Six individuals per treatment were used. They were fed 1% in relation to the average body weight per container (0.86-0.97 g), every 48 hours under *ad libitum* condition. Diets 2 and 3 produced a significantly better performance for growth in test diameter 31.58 ± 0.21 mm, SGR= 0.0066 ± 0.0001 mm mo^{-1} and 31.18 ± 0.35 mm, SGR= 0.0064 ± 0.0001 mm mo^{-1}, respectively. Similar results of growth in weight were obtained with diets 2 and 3, 19.13 ± 0.52 g, SGR= 0.0413 ± 0.0019 g mo^{-1} and 19.08 ± 0.48 g, SGR= 0.0377 ± 0.0017 g mo^{-1}, respectively. Feed conversion ratio and gonadal index were better with diets 2 and 3. In general, the lowest performance was obtained with diet 1. Survival was greater than 54% and the starved group died on day 42. This study indicates that plant-based diets and the combination of various protein sources in the diets produced remarkable biological responses to *A. stellata* growth.

Palabras clave
Alimentación alternativa
Nutrición
Tasas de crecimiento
Supervivencia
Arbaciidae
Ecuador

RESUMEN | El erizo de mar, *Arbacia stellata*, es parte de nuevos escenarios bioalimentarios para el empleo como nutracéuticos de organismos marinos del mundo sin uso actual como alimento. Se realizó una prueba de alimentación durante 98 días (en un diseño experimental completamente aleatorizado simple) usando especímenes de *A. stellata* (n= 88; 29,3 ± 0,2 mm y 14,5 ± 0,3 g de diámetro de la testa y peso inicial promedios, respectivamente) para evaluar la eficacia de tres dietas isoproteicas que contenían fuentes de proteínas de crustáceos y vegetales en diferentes combinaciones, con el propósito de desarrollar una dieta seca formulada de bajo costo que promoviera su crecimiento (en diámetro de la testa y peso), rendimiento gonadal y supervivencia. Se elaboraron la Dieta 1 (camarones, *Penaeus vannamei*), Dieta 2 (Sacha Inchi, *Plukenetia volubilis*) y Dieta 3 (dieta mixta). Se utilizaron seis individuos por tratamiento. Se alimentaron al 1% con relación al peso corporal promedio por envase (0,86-0,97 g), cada 48 h en condiciones *ad libitum*. Las dietas 2 y 3 produjeron un rendimiento significativamente mejor para el crecimiento en el diámetro de la testa 31,58 ± 0,21 mm, SGR= 0,0066 ± 0,0001 mm mo^{-1} y 31,18 ± 0,35 mm, SGR= 0,0064 ± 0,0001 mm mo^{-1}, respectivamente. Similares resultados de crecimiento en peso se obtuvieron con la dieta 2 y 3, 19,13 ± 0,52 g, SGR= 0,0413 ± 0,0019 g mo^{-1} y 19,08 ± 0,48 g, SGR= 0,0377 ± 0,0017 g mo^{-1}, respectivamente. La tasa de conversión alimenticia y el índice gonadal fueron mejores con las dietas 2 y 3. En general, el rendimiento más bajo se obtuvo con la dieta 1. La supervivencia fue superior al 54% y el grupo mantenido en inanición murió el día 42. Este estudio indica que las dietas basadas en plantas y la combinación de varias fuentes de proteína en las dietas produjo respuestas biológicas notables en el crecimiento de *A. stellata*.

INTRODUCTION

The interest in aquaculture of sea urchins has increased worldwide (McBride 2005; Hagen and Siikavuopio 2010; Mendes *et al.* 2019). Nowadays, this production activity represents more than the edible part of the prized gastronomic fresh seafood industry as a delicacy or seed production for stock restoration (Fernandez and Boudouresque 2000; Andrew *et al.* 2002; Guidetti *et al.* 2004; Pais *et al.* 2007; Micael *et al.* 2009; Martínez-Pita *et al.* 2010; Lawrence 2013; Sonnenholzner-Varas *et al.* 2018). Some sea urchin species, such as *Arbacia* spp. are being considered as part of the newest *Blue Industry* focused to manage high-qualified functional food and therapeutic diets. In this sense, sea urchin aquaculture has gained relevance in the nutraceutical and pharmaceutical industry at the service of human and animal health from sustainable marine environments (Gates 2010; Pangestuti *et al.* 2022); since some pigments known as spinochromes (Polyhydroxylated 1,4-naphthoquinones) found in their shells, spines, gonads, coelomic fluid, and eggs are a unique source of bioactive compounds with biomedical applications (Brasseur *et al.* 2017; Vasileva *et al.* 2017; Shikov *et al.* 2018; Rubilar *et al.* 2021).

Regular echinoids have a wide range of feeding habits which makes it difficult to classify them in a particular trophic niche. Under vegetable sources availability, sea urchins are often regarded as herbivores, but they can be considered opportunistic since they are able to obtain nutrients from different food sources (Lawrence 2007; Lawrence *et al.* 2007). For this reason, seaweeds have been traditionally used for feeding edible sea urchins in captivity systems, but not all sea urchins feed solely on algae, since some act as omnivores (McBride 2005), and other groups, such as the species of the *Arbacia* genus, present a strong tendency to carnivory (Penchaszadeh and Lawrence 1999; Silva *et al.* 2004; Gianguzza and Bonaviri 2013; Gianguzza 2020). Additionally, seaweeds have several drawbacks for utilizing them for sea urchin aquaculture: large amounts are required, low-quality and variable seasonal protein content, high-water content, pathogens presence, high costs, and other issues that affect growth, production of gonads and perivisceral fluid volume (McBride 2005).

Several studies have demonstrated that, in most cases, formulated feeds out-perform macroalgae as a diet for sea urchins (Frantzis and Grémare 1992; Klinger *et al.* 1998; Meidel and Scheibling 1999; Fernandez and Boudouresque 2000; Lawrence *et al.* 2003; Eddy *et al.* 2012; Sartori and Gaion 2015; Muñoz-Entrena 2019). In this sense, fishmeal has been the fundamental protein source incorporated in artificial diets due to its high protein content, ω3 polyunsaturated fatty acids, and is very rich in amino acids (lysine, methionine, and cysteine) which enhances digestibility, and is also rich in vitamins (Complex B and D) and minerals (such as calcium and phosphorus) (Zhoug *et al.* 2004; Gutiérrez-Espinosa *et al.* 2011). However, fishmeal is a highly expensive and limited resource, thus, alternative dietary high-quality protein sources constitute a priority objective for its total or partial substitution to support healthy animals, and with reduced costs (Hardy 2010; Kamarudin *et al.* 2011; Miranda-Gelvez and Guerrero-Alvarado 2015; Araújo-Dairiki *et al.* 2018). Therefore, it is crucial to gain a better understanding of diets preferably formulated from locally available feed ingredients, to make the formulation easier and keep costs low, but with high nutritional quality feeds. This forces the discovery of more food alternatives, especially for various protein sources (animal and vegetable), since suitable diets for appropriate feeding regimes with high feed efficiencies will be essential for the successful commercialization of sea urchin farming (Lawrence and Lawrence 2004; Rubilar *et al.* 2016).

Recent studies have experimented with ingredients of unconventional animal origin, such as by-products of the shrimp industry. Shrimp cephalothorax has been used in various studies for the preparation of diets, because it contains long-chain saturated fatty acids and high protein values (Carrillo 1994; Carranco *et al.* 2003; Senphan and Benjakul 2015). New sources of plant origin have also been tested, such as phytonutrients from seeds of Sacha Inchi, *Plukenetia volubilis*, containing 24.5 to 29.6% crude protein, nearly 41.4% oil, and high levels of vitamins A and E (Fanali *et al.* 2011; Ruiz *et al.* 2013), while the Sacha Inchi cake (by-product obtained from the extraction of oil from the seed), reaches high protein levels, which can vary between 54.9 to 65.5%, according to several studies carried out (Ruiz *et al.* 2013; Ortiz-Chura *et al.* 2018; Zambrano-Andrade *et al.* 2021). These food sources have been suitable for the formulation of diets according to the dietary response and digestive physiology of aquatic species of herbivorous and omnivorous fish (Rani 2014; Li *et al.* 2018) and shrimp (Sudaryono *et al.* 1995) in order to reduce and optimize costs related to food. However, there are currently no data available on the feeding of subadult and adult individuals of *Arbacia stellata* (Blainville, 1825), except for juvenile organisms, a study in which it was shown that a microencapsulated formulated feed has better results than the seaweed alone (Muñoz-Entrena 2019).

To date, sea urchin fisheries in the temperate eastern Pacific have been focused on three slow-growing species: *Loxechinus albus* in Chile (Olave *et al.* 2001) and *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus* in México (Solís-Marín *et al.* 2013), where their wild stocks have been massively depleted, and aquaculture for restocking their populations is still incipient. In the tropical eastern Pacific, *A. stellata* is not fished yet but has become appraised in aquaculture for its rapid growth, as a ruderal species (Lessios *et al.* 2012; Gianguzza and Bonaviri 2013; Gianguzza 2020) and the high quality of its gonads and fluids (favorable organoleptic characteristics: taste, firmness, and color) (Palma-Chávez *et al.* 2021). Nonetheless, due to these characteristics, *A. stellata* is facing a rapidly expanding exploitation for different biomedical applications in the pharmaceuticals and cosmetics industries within the entire Latin American region (Gates 2010; Rubilar *et al.* 2016; Sonnenholzner-Varas 2021).

We tested the hypothesis that a high-quality mixed (plant and animal protein) formulated diet would result in increased growth (in diameter and weight), survival, and performance of gonad formation in the sea urchin *A. stellata*, in contrast to diets containing a single protein source. To identify an effective feed with good storage qualities and suitable for the culture of *A. stellata*, our aim was to demonstrate that growth performance, feed conversion ratios, survival, and gonad production can be maintained when the inclusion of vegetables (*Plukenetia volubilis*) and shrimp (*Penaeus vannamei*) with high protein levels, are included in the diets for omnivorous echinoids. The concept was to conduct a small-scale experimental trial of limited duration testing the introduction of new low-cost diets for the sea urchin industry.

MATERIALS AND METHODS

All experimental procedures used in this study were strictly performed following the relevant national guidelines and regulations of Ecuador and in accordance with the Care and Use of Laboratory Animals guidelines of Universidad Técnica de Manabí. In addition, the guidelines for ethical and responsible research with *in vivo* animals and experiments with echinoderms were followed (Rubilar and Crespi-Abril 2017).

Sea urchins supply

Specimens of *A. stellata* (test diameter: 29.3 ± 0.3 mm and weight: 14.6 ± 0.4 g; *n*= 104) were harvested from an intensive oyster *Magallana gigas* hanging culture system with lantern nets between 3 and 5 m depth of a fishery cooperative facility along the coast off Monteverde, Santa Elena, Ecuador (2°02′53.005” S 80°44′24.799” W). Sea urchins were then transported in a portable styrofoam container with seawater at 25.0 ± 1.0 °C to the Laboratory of Experimental Biology and Aquaculture of Echinoderms of the Aquaculture and Fishery Department of the Universidad Técnica de Manabí (UTM), Bahía de Caráquez, Manabí, Ecuador, on 28th July 2020, and they were kept in a tank (180 cm length x 100 cm width x 80 cm depth). During the experiment, seawater quality was enhanced with a biofiltration system and continuously aerated with a constant flow of 35 L min^{-1}. One-third of the seawater (filtering it through a sieve of 1 and 5 μm) was renewed three times a week. Water temperature was 24.6 ± 0.9 °C, pH 8.2 ± 0.1, salinity 33 g L^{-1} and dissolved oxygen 5.85 ± 0.27 mg L^{-1}. Test diameter, body weight, and gonad weight (for gonadal index, *GI %*) were measured in ten sea urchins randomly chosen before the experiment to evaluate their initial conditions (time 0) and to know the GI. Test diameter was measured using a vernier caliper (precision: 0.01 mm), and the body weight was measured using a digital balance (precision: 0.01 g). All measurements were made at intervals of 14 days.

Experimental design

Diets used for the experiment were based on the gut content analysis of adult *A. stellata* collected in shrimp aquaculture ponds and channels at Santa Elena, Ecuador. The relative abundance of materials was 75% shrimp, 21% fish, and 4% sediment-inert material (J. Sonnenholzner-Varas, unpublished data). The present study was performed with three treatments (diets): Diet 1 -animal protein source- flour of hepatopancreas of the commercial Pacific white shrimp: *Penaeus vannamei*; Diet 2 -vegetal protein source- flour of a perennial Euphorbiaceae plant, Sacha Inchi: *Plukenetia volubilis*, and Diet 3 a mixed diet (equal proportions in wet weights of diets 1 and 2). Diets were formulated and elaborated at the Nutrition Laboratory facility of the Aquaculture and Fishery Department of the Universidad Técnica de Manabí, Bahía de Caráquez, Manabí, Ecuador. A total of 88 sea urchins were selected for the feeding trial (test diameter: 29.3 ± 0.2 mm and weight: 14.5 ± 0.3 g). Sea urchins were deprived of food for 40 days prior to the experiment, with the aim of emptying their stomachs (Prato *et al.* 2018). They were fed 1% in relation to the average body weight per container (0.86-0.97 g), every 48 hours under *ad libitum* condition. Sea urchins were randomly distributed into fifteen 20 L plastic meshed floating containers (45 cm x 32 cm x 13 cm) in a rectangular tank of 1000 L capacity (220 cm x 150 cm x 43 cm) to undergo the experimental treatments and replicates (*n*= 4) following a completely randomized experimental design. Six urchins were placed per replicate, and the control was used for starved animals with the aim to determine that diets did not cause mortality. Uneaten remains were removed from the tanks with each feed change, while the tanks were thoroughly cleaned every week. The trial was conducted using natural photoperiod (12 h light:12 h dark). The experiment lasted 14 weeks (September 6th - December 14th, 2020).

Diet procedures

Three diets were elaborated (all with approximately 26% protein content). Three types of meals were used: *(i)* Shrimp Cephalothorax Flour (SCF) from the by-product of the white shrimp *Penaeus vannamei*; *(ii)* Sacha Inchi Flour (SIF) from the by-product resulting from the extraction of oil from the *Plukenetia volubilis* plant, and *(iii)* Yellow Corn Flour from a commercial source. All flours were sieved at 300 µm. Other commercial ingredients were used for the preparation and formulation of the diets, such as shrimp cephalothorax concentrate, vitamin and mineral premix, fish oil, and Carboxymethylcellulose (as agglutinant). To prepare the granules of the three diets, an electric meat grinder was used, obtaining pellets of 5 mm in diameter. Table 1 shows components used for balancing diets.

Table 1. Formulation and proportion of the experimental diets for *Arbacia stellata* (g kg^{-1} dry matter).

Ingredients	Diet 1	Diet 2	Diet 3
Shrimp cephalothorax flour[a]	44	0	22
Sacha Inchi flour[b]	0	45	22
Corn flour[c]	45	44	45
Shrimp cephalothorax concentrate[d]	2	2	2
Fish oil	1	1	1
Vitamin premix[e]	1	1	1
Mineral premix[f]	1	1	1
Agglutinant (CMC)	6	6	6

[a]*Penaeus vannamei* shrimp cephalothorax flour and concentrate were provided by Procesadora y Exportadora del Pacífico S.A. (PROEXPACSA), Pedernales, Manabí, Ecuador.

[b]Sacha Inchi, *Plukenetia volubilis* vegetable flour was provided by the Sacha Inchi Agricultural Production Association (ASOSACINC), San Vicente, Manabí, Ecuador.

[c]Corn flour from DOÑAREPA commercial source.

[d.]Fish oil FEEDPAC OIL: Fish oil 99.93; Antioxidant 0.05; Vitamin E 0.02.

[e]ADIMIX for shrimp (g kg-1): Laitgrass (refatted whey) 43.32; Vitamin C= 10.00; Inositol= 7.58; Vitamin E= 3.30; Vitamin K_3= 1.74; Vitamin B_{12}= 1.50; Calcium pantothenate= 1.28; Biotin= 1.25; Vitamin A= 1.25; Niacin= 1.01; Copper sulfate= 0.80; Organic selenium= 0.75; Vitamin B_2= 0.63; Vitamin D_3= 0.50; Vitamin B_1= 0.50; organic iron= 0.50; cobalt sulfate= 0.36; Vitamin B_6= 0.25; Organic manganese= 0.25; organic zinc= 0.25; Potassium iodide= 0.17; Folic acid= 0.15; Potassium citrate= 0.08; BHT= 0.08.

[f]MINERFEED mineral premix: Monocalcium phosphate; Antioxidant (citric acid, monosodium phosphate); red iron oxide; Zinc sulfate; Sodium selenite; Cobalt sulfate; Copper sulphate; Magnesium carbonate; Zeolite.

Microbiological analysis was done in all the flours and shrimp cephalothorax concentrate to determine the presence of fungi/yeasts (Potato Dextrose Agar) and *Salmonella* spp. (Salmonella Shigella Agar). For this, peptone water was used as an enrichment medium. The stability of the food in water was evaluated, as follows: (*i*) diets that remained completely intact in periods of 12, 24, 36, and 48 h were scored 3, (*ii*) diets that were partially intact (broken into intact pieces and powder) were scored 2, and (*iii*) diets that were disintegrated were scored 1 (Eddy *et al.* 2012). For the main ingredients and the three elaborated diets, moisture was determined by means of the vacuum oven method, ashes by means of the muffle incineration method, while for proteins and lipids the Kjeldahl and Soxhlet methods were applied, respectively (Aurand *et al.* 1987; Association of Official Analytical Chemists [AOAC] 2016; Nielsen 2017).

Data analysis

Growth performance

Specific Growth Rate, SGR (in diameter and weight) was calculated as follows: SGR (% day^{-1})= [(ln TD_2, W_2 - ln TD_1, W_1)/Δt] x 100; where ln is the natural logarithm, TD_1 or TW_1 and TD_2 or TW_2 are the respective means of whole test diameter (in millimeters) or weight (in grams) of specimens in a bucket at the time of sampling, and Δt is the growth interval in days (t_2 - t_1). SGR is expressed as a percentage of sea urchin size or weight increase per day. Feed Conversion Ratio, FCR was expressed as the ratio of feed intake on gain, as follows: FCR (g/g)= C/(W_2 - W_1); where C is the weight of feed consumed, W_2 and W_1 are the mean whole weight (g) of animals in a bucket at the time of sampling, and the mean whole weight (g) of animals at the beginning of the experiment, respectively (Sudaryono *et al.* 1995; Sonnenholzner-Varas *et al.* 2019).

Gonadal index (GI %)

The GI was calculated by the percentage ratio of the weight of the gonad in relation to the total weight of the sea urchin, following this equation: GI= (Wg/Wt) x 100; where Wg represents the value of the gonad weight and Wt represents the total weight of the sea urchin (Martínez-Pita *et al.* 2010).

Survival

The survival of the organisms was measured in each experiment by counting the number of dead animals. The survival of organisms was calculated based on the initial number of organisms and total number of organisms at the end of the experimental period, as follows: S(%)= (N_2/N_1) × 100; where N_2 represents final number of organisms divided by N_1 initial number of organisms (Sudaryono *et al.* 1995).

Statistical analysis

Data were tested for homogeneity of variance and normal distribution before following statistical analysis. Performance data were analyzed by one-way ANOVA to test the main effects on the response variables: growth performance, gonadal index, and survival of *A. stellata*. Tukey´s *post hoc* comparison was performed if significant differences were found. All somatic and gonadal growth data are reported as mean ± standard error. Software STATISTICA 10.0 (StatSoft, Inc., USA) was used. A significance level of 5% is reported.

RESULTS

Microorganisms analysis

The raw materials showed the presence of fungi and yeasts in the shrimp cephalothorax flour (2.00 CFU g^{-1}), Sacha Inchi flour (1.00 CFU g^{-1}), and corn flour (1.00 CFU g^{-1}), but in the shrimp cephalothorax concentrate were absent. *Salmonella* spp. was absent in all different flours and in the shrimp's cephalothorax concentrate (25 g of sample).

Nutritional analysis and feed stability

Statistical comparisons among nutritional parameters, such as crude protein, moisture, ash, lipids, and nitrogen-free extracts are presented in Table 2. Diet 2 showed better consistency and resistance in water for 48 h, while diets 1 and 3 showed partial stability during the first 12 and 24 h, respectively, and both disintegrated after 36 h (Table 3).

Table 2. Approximate nutritional composition of ingredients and the experimental diets. SCF: Shrimp cephalothorax flour (*Penaeus vannamei*: animal source of protein); SIF: Sacha Inchi flour (*Plukenetia volubilis*: vegetable source of protein); CF: Corn flour; SCC: Shrimp cephalothorax concentrate. Data are shown as media ± standard deviation.

	Concentration (%) per ingredients				
Components	**Moisture**	**Ash**	**Crude protein**	**Crude lipid**	**NFE**
SCF	9.54 ± 0.09^a	18.42 ± 0.17^a	60.43 ± 3.87^a	10.21 ± 0.29^a	1.41 ± 3.93^a
SIF	9.35 ± 0.04^a	5.48 ± 0.04^b	59.74 ± 5.53^a	6.36 ± 0.36^b	19.07 ± 5.84^b
CF	11.82 ± 0.07^b	1.0 ± 0.01^c	8.90 ± 0.33^b	2.85 ± 0.14^c	75.44 ± 0.25^c
SCC	30.04 ± 0.12^c	8.55 ± 0.03^d	22.00 ± 1.50^c	8.00 ± 0.52^d	31.42 ± 1.37^d
Diets	**Moisture**	**Ash**	**Crude protein**	**Crude lipid**	**NFE**
Diet 1	8.38 ± 0.08^a	10.46 ± 0.04^a	24.75 ± 0.20^a	5.11 ± 0.28^a	51.31 ± 0.43^a
Diet 2	8.36 ± 0.15^a	4.92 ± 0.08^b	27.00 ± 0.17^b	3.69 ± 0.13^b	56.04 ± 0.21^b
Diet 3	7.27 ± 0.17^b	7.53 ± 0.12^c	26.86 ± 0.15^b	5.17 ± 0.48^a	53.16 ± 0.48^c

Ingredient and diet values in the same column that have different letters (superscript) are significantly different ($P<0.05$). NFE: Nitrogen-Free Extracts.

Table 3. Stability of formulated diets in water measured by time (12 - 48 h).

	Time (h)			
Treatments	**12**	**24**	**36**	**48**
Diet 1	Partially intact	Disintegrated	Disintegrated	Disintegrated
Diet 2	Intact	Intact	Intact	Intact
Diet 3	Partially intact	Partially intact	Disintegrated	Disintegrated

Growth performance in diameter and weight

No significant differences were found among the treatments in the initial test diameter ($F_{(2,9)}$= 0.002, p>0.05; Figure 1a) or weight ($F_{(2,9)}$= 0.074, p>0.05; Figure 1b). Diets 2 and 3, both showed the highest performance in terms of growth in test diameter (TD) of *A. stellata*, with a final TD of 31.58 ± 0.21 mm and 31.18 ± 0.35 mm, respectively; while diet 1 showed the lowest performance for growth in TD of 29.80 ± 0.62 mm ($F_{(2,9)}$= 4.780, p<0.05; Figure 1a). Regarding weight, diets 2 and 3 presented the best performance on the growth of *A. stellata*, with a final weight of 19.13 ± 0.52 g and 19.08 ± 0.48 g, respectively; while diet 1 showed the lowest performance for growth, with a final weight of 17.35 ± 0.42 g ($F_{(2,9)}$= 4.562, p<0.05; Figure 1b).

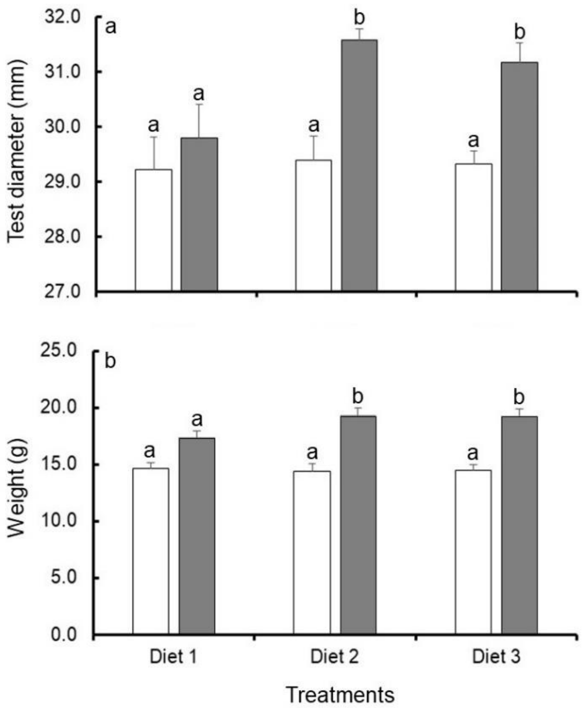

Figure 1. Growth performance of the sea urchin *Arbacia stellata* fed with three formulated diets between time initial (t_0; white bars) and at the end of experiment ($t_{98;}$ gray bars), in test diameter (a) and weight (b).

The highest amounts of growth in test diameter (in SGR) during 98 days of experiment were recorded for diet 2 and 3 (Figure 2 a). The Specific Growth Rate (SGR) in diameter was 0.0066 ± 0.0001 mm mo^{-1} for diet 2 and 0.0064 ± 0.0001 mm mo^{-1} for diet 3, being the best performance for the development of *A. stellata*. In contrast, diet 1 had the lowest growth performance for SGR, which was 0.0037 ± 0.0001 mm mo^{-1}, showing significant differences compared to diet 2 and 3 ($F_{(2,9)}$= 309.21, p<0.05; Figure 2 b).

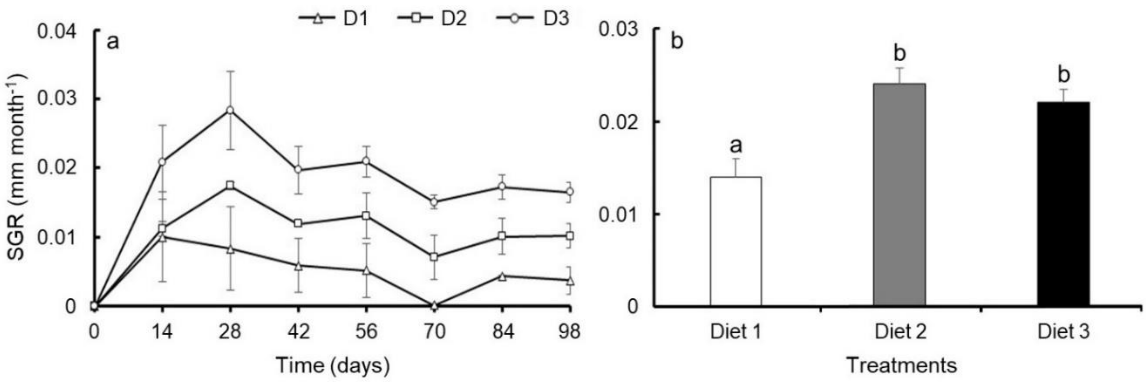

Figure 2. Effect of three formulated diets on the Specific Growth Rate (SGR, in test diameter) of the sea urchin *Arbacia stellata*. SGR variation during the 98 days of the experiment (a) and at the end of the experiment (b) by type of diet formulated.

The highest amounts of growth in weight (in SGR) during 98 days of the experiment were registered for diet 2 and 3 (Figure 3 a). The Specific Growth Rate (SGR) in weight was 0.0413 ± 0.0019 g mo^{-1} for diet 2 and 0.0377 ± 0.0017 g mo^{-1} for diet 3, presenting these two diets the best performance for the development of *A. stellata*. Diet 1 had the lowest growth performance for SGR, which was 0.0235 ± 0.0012 g mo^{-1}, showing significant differences compared to diets 2 and 3 ($F_{(2,9)}$= 32.446, p<0.05; Figure 3 b).

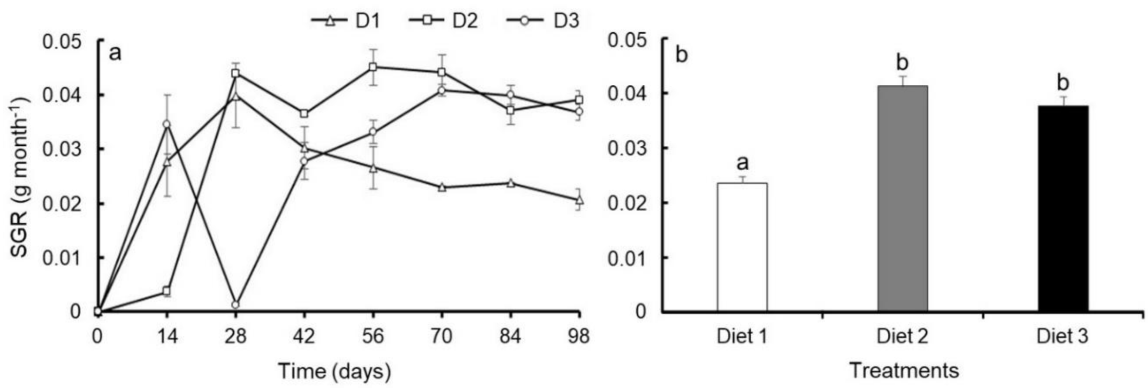

Figure 3. Effect of three formulated diets on the Specific Growth Rate (SGR, by weight) of the sea urchin *Arbacia stellata*. SGR variation during the 98 days of the experiment (a) and at the end of the experiment (b) by type of diet formulated.

Feed conversion ratio

Diets 2 and 3 showed the best performances with proportional relations of 0.54 and 0.80, respectively. Those FCR values contrasted with diet 1 which was 1.42.

Gonadal index

The average GI was <0.01% at t_0. Compared with initial conditions, gonad weight and gonadal index of *A. stellata* increased significantly in diets 3 and 2, with values of 1.69 ± 0.10% and 1.49 ± 0.16%, respectively ($F_{(3,12)}$= 6.6788, p<0.05; Figure 4). The control (average= 0.87%) and diet 1 (0.82 ± 0.07%) were not significantly different (Figure 4).

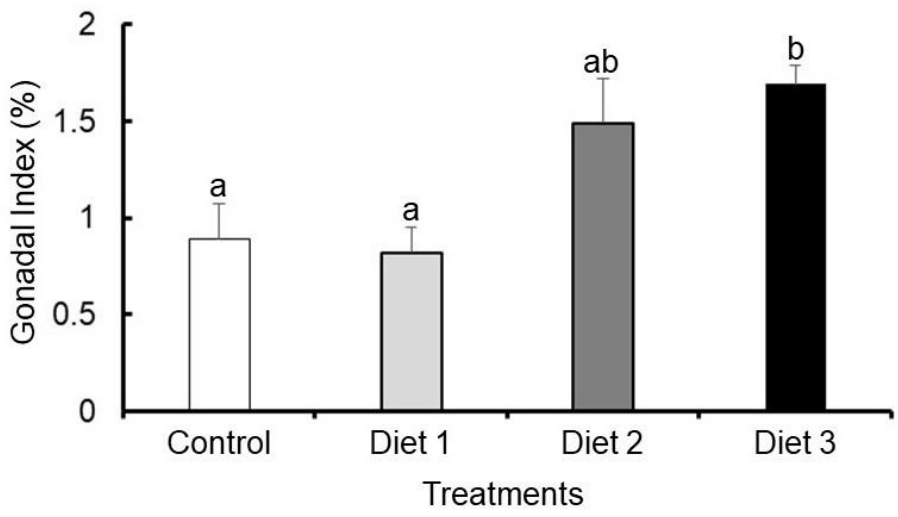

Figure 4. Gonadal yield of the sea urchin *Arbacia stellata* fed with three different formulated diets contrasting to control (initial time).

Survival

Results of the one-way ANOVA showed there was a non-significant effect of diets on survival ($F_{(2,9)}$= 0.5539, p>0.05). The mean survival for the treatments was 62.5 ± 2.1% (diet 1= 62.5%; diet 2= 54.2%; diet 3= 70.8%). All the sea urchins kept under starvation conditions (control treatment) died massively until day 42. Cannibalism was observed in the control, where bites were evident in the shell of the sea urchins, leaving this area stripped of spines and epidermis.

DISCUSSION

The three feeds that were prepared for the sea urchin *Arbacia stellata* showed variability in terms of stability in water, with diet 3, based on a vegetable source, showing the best stability during 48 h of testing. Our results coincide with the study carried out by Eddy *et al.* (2012), in which they determined that there is a variability of stability in water for various diets evaluated, with similar results to those found in the present study. However, they associated these differences with the variable levels of protein in the diets. In contrast, our study determines that these variations are linked to the use of vegetable source ingredients, which presented a robust consistency since the protein levels in the three diets evaluated were similar. The stability and quality of food play a key role for the optimal development of sea urchins (Frantzis and Grémare 1992; Fernandez and Boudouresque 2000; Lawrence *et al.* 2003), therefore, the low stability of the diet 1 (animal source: shrimp cephalothorax) can be considered to have influenced somatic and gonadal growth performance in sea urchins, since it did not maintain a good consistency during the hours evaluated.

The digestive system of regular echinoids is relatively simple (Hyman 1955); however, it produces mucus specialized in synthesizing digestive enzymes (mainly carbohydrases, lipases and proteases, actioned in the stomach) and nutrient absorption (mainly in the intestine), which favors the digestion and assimilation of feed (Lawrence *et al.* 2007; Sonnenholzner-Varas 2011). The echinoid *A. stellata* is a considerable trophic plasticity species, ranging from omnivory with a marked carnivorous habit (Gianguzza and Bonaviri 2013; Gianguzza 2020), like other species of *Arbacia*: *A. lixula*, *A. dufresnii* and *A. spatuligera* (Bay-Schmith 1981; Vásquez *et al.* 1984; Silva *et al.* 2004; Agnetta *et al.* 2013, 2015). In this study, it is demonstrated that *A. stellata* readily accepted the experimental diets from the beginning of the experiment and maintained normal behavior throughout the experimental period. Their growth response (in test diameter and weight) and gonadal production were significantly higher when fed diets 2 and 3, a single and mixed supplementation by the dietary components.

The sea urchin *A. stellata*, effectively uses a great variety of food items, especially of animal origin, but seaweeds are not a major food item in this species (Trenzado *et al.* 2012). In this sense, this study showed an increased growth rate and efficiency in feed utilization (based on FCR), which may have stemmed from the increased efficiency of absorption and assimilation of the digested food (and nutrient utilization from lipids and protein) in the intestine of *A. stellata* (Jangoux and Lawrence 1982). Although in this study the enzymatic activity of *A. stellata* was not measured, it is important that an optimal feed for the species be designed considering its complex enzymatic machinery. Here, we presume that four enzymes are present: amylase (by their omnivorous feeding habit), protease and lipase (when consuming animal protein source), and hydrolases (when consuming vegetable protein source) (Klinger 1984; Sweijd 1990; Cabral de Oliveira 1991; Gómez-Pinchetti and García-Reina 1993; Zhang and Kim 2010; Trenzado *et al.* 2012). This issue is important to consider for developing an optimal feed designed for *A. stellata* aquaculture.

Diet 3 was the best formulated and balanced mix of food based on flours of hepatopancreas of shrimp and Sacha Inchi for *A. stellata* (see Tables 1-3). In relation to growth performance, our data contrast to the results of Rubilar *et al.* (2016) who did not observe an increase in echinoid diameter and growth productivity in *A. dufresnii* that was fed a formulated feed. They suggested that weight gain may be a better indicator of growth than test diameter. Here, we determined that growth performance measured in test diameter and weight are critical for evaluating diets in tropical fast growth sea urchins, like *A. stellata*. There are currently no data available on the feeding of *A. stellata* in subadult and adult organisms, except for juvenile organisms, a study in which it was shown that a microencapsulated formulated feed has better results than the seaweed (Muñoz-Entrena 2019), like the results obtained in the present study. High survival and fast growth rates (in test diameter and weight) are necessary to be able to determine optimum ingredient contents and nutrient requirements in sea urchin diets. It is important to highlight that the growth in size and weight of sea urchins must be carefully analyzed, since, as evidenced in this study, the organisms are not clearly round, and they tend to lose spines due to rubbing against each other. In this respect, Ellers and Johnson (2009) indicate that diameter measurement can be inaccurate because urchins have spines, are not always exactly circular, and diameter measurements do not account for potential height variation (some urchins may be more flattened than others).

The results of the present study showed that diets 2 and 3 were excellent sources of lipids, with optimum inclusion levels of ingredients for a high growth and survival performance and stress resistance for culturing *A. stellata*. Based on our results, elaboration of a formulated artificial diet with different ingredients (the use of waste by-products with high nutritional value of animal and vegetable origins) for *A. stellata* is important to optimize its growth performance (Eddy *et al.* 2012; Sartori and Gaion 2015; Rubilar *et al.* 2016; Sonnenholzner-Varas *et al.* 2018). Shrimp cephalothorax flour is a high protein raw material for feeding aquatic animals (Carranco *et al.* 2003; Espinosa-Chaurand *et al.* 2015; Salas-Durán *et al.* 2015). Similarly, the by-product obtained from the extraction of oil from the plant *P. volubilis* (Sacha Inchi) is a source of protein in diets formulated for aquatic organisms. It has been used to feed fish (tilapia: Miranda-Gelvez and Guerrero-Alvarado 2015 and trout: Ortiz-Chura *et al.* 2018). The flour of shrimp cephalothorax (60.4 ± 3.9%) and the one from Sacha Inchi plant (59.7 ± 5.5%) have a high percentage of protein, contrasting to corn flour and shrimp cephalothorax concentrate, which had lower levels of protein. The results show that these ingredients are of high quality and viable for the development and growth of *A. stellata*, therefore, they are important alternative sources of protein. The search for these alternative food sources constitutes a priority objective for the total or partial substitution of fishmeal in dry feeds, to maintain healthy animals with reduced costs (Hardy 2010; Kamarudin *et al.* 2011; Miranda-Gelvez and Guerrero-Alvarado 2015; Araújo-Dairiki *et al.* 2018).

Gonadal growth requires a specific functional digestive organization that ensures adequate transfer of nutrients to the gonads when the animal stops growing in test diameter (Sonnenholzner-Varas 2011). In this sense, gonadal performance in our experimental study could be affected by water temperature or other variables related to water quality, according to Hill and Lawrence (2006) who evaluated the effect of two types of stress (high temperature and starvation) on gonad production and growth margin in *A. punctulata*. They hypothesized that *A. punctulata* has a stress-tolerant life strategy and would be more stress-tolerant than other echinoids. Furthermore, our results may be related to the presence of bacteria in the ingested food that can promote nutrient availability in *A. stellata* by increasing digestive capacity associated with an exogenous source of digestive enzymes (Lawrence *et al.* 2007). However, further studies are required to determine the optimal content of other specific requirements, since these are species specific (Shearer 1995). In the present study, the final high average survival (>54%) indicated that components and ingredients in diets with vegetable and animal protein sources were the best obtained.

It is important remarking that the results presented in this study are obviously pioneering for the edible tropical sea urchin *A. stellata* and must be considered in the appropriate magnitude. Additional studies building upon our results presented here are needed to allow continuous monitoring of other different protein and lipid matrices of animal and plant sources to give a classification that allows generating more reliable selection criteria. For its part, commercial feeds available on the market offer a wide range of physical-chemical and nutritional characteristics, but when selecting the appropriate feeds, suppliers must adhere to Good Management Practices, to promote the use of food best adapted to the sea urchin farming conditions, with the best cost-benefit ratio. In summary, the results show that *A. stellata* is a species capable of optimally accepting formulated feeds, therefore, it is important to continue with studies that will determine the optimal nutritional values required by this species, to identify the most adequate diet for the maintenance, growth and gonadal development of the species.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ETHICAL APPROVAL

In the current study, sea urchins were collected from an intensive oyster *Magallana gigas* hanging culture system, and they stayed in the Laboratory of Experimental Biology and Aquaculture of Echinoderms of the Aquaculture Department of the Universidad Técnica de Manabí (Sucre, Manabí, Ecuador). All experimental procedures used in this study were strictly performed following the relevant national guidelines and regulations of Ecuador.

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