Aquaculture 567 (2023) 739259

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Evaluation of *Moringa oleifera* and corn starch as feed for seed production of the pearl oyster *Pteria sterna* (Gould,1851)

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ARTICLE INFO

Keywords:
Mariculture
Microalgae
Corn starch
Nursery culture
Fatty acids

ABSTRACT

In the search for complementary diets to improve performance in bivalve farming, the use of terrestrial plants with nutritional and nutraceutical properties has been proposed as an alternative. The effectiveness of moringa leaf meal *Moringa oleifera* (Mo) was evaluated under controlled laboratory conditions (30 days), as a dietary supplement during the pre-growth stage of *Pteria sterna* seeds (7.2 ± 0.59 mm), as well as its combinations with microalgae and corn starch (Co), on growth and survival in the laboratory and its subsequent initial suspended culture in the sea. Diets were formulated with a mixture of the microalgae *Tetraselmis suecica* and *Chaetoceros gracilis* (M), diet M; M and 5% Mo (M + Mo); diet M and 5% corn starch (M + Co); 100% moringa leaf meal (Mo); 100% corn starch (Co), and diet M with 2.5% Mo and 2,5% Co (M + Mo + Co). The Mo diet did not provide pre-seed sustainability, resulting in 100% mortality at 30 days. From the rest of the diets, M obtained the lowest oyster survival, while M + Mo and M + Mo + Co showed the highest growth rates. At the end of the laboratory bioassay, the seeds were sown in a culture system in the open sea (50 days), where the highest growth occurred in the juveniles previously fed with M + Mo + Co. The results suggest that, in the nursery, *P. sterna* pre-seeds can be maintained with a diet of 100% corn starch, but not with 100% moringa flour, probably due to its poor digestibility. However, moringa used as an additive to the microalgae diet provided a higher yield in the oyster, which is reflected in a higher yield in the initial culture outdoors.

1. Introducction

The pearl oyster or winged oyster, *Pteria sterna* (Gould, 1851), is a tropical bivalve that offers a dual benefit, since it can serve as a matrix for the development of pearls (Monteforte and Garcia-Gasca, 1994), as well as its meat that has an important nutritional value (Vite-García and Saucedo, 2008). In the American subtropical Pacific, particularly in Baja California Sur (Mexico), its aquaculture has been developing with advances in hatchery, maturation, spawning, and initial phases of cultivation in a controlled environment (Hoyos-Chairez et al., 2020; Chavez-Villaba et al., 2022). Additionally, in the tropical Pacific (Ecuador-Peru) various studies have been carried out on the development of technologies for its cultivation (Lodeiros et al., 2018; Treviño and Vélez-Falcones, 2019; Freites et al., 2019; Gregori et al., 2019; Rojas et al., 2021) and pearl production (Freites et al., 2020; Rojas et al., 2021; Jara et al., 2022), whose results demonstrate the feasibility of cultivation that lead to project *Pteria sterna* as an emerging species for aquaculture. The collection of wild *P. sterna* seeds using artificial substrates placed in marine environments could support its cultivation for commercial purposes (Lodeiros et al., 2018). However, in recent years, the availability of seeds from the natural environment has been variable and decreasing, which demands new strategies for seed production under controlled

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https://doi.org/10.1016/j.aquaculture.2023.739259

Received 23 August 2022; Received in revised form 11 January 2023; Accepted 13 January 2023

Available online 18 January 2023

0044-8486/

laboratory conditions.

In the hatchery, 100% microalgal diets fully cover the nutritional needs of bivalve molluscs, from the maturation of broodstock to the culture and larval fixation, and the pre-fattening of seeds prior to their sowing in the sea for the grow-out process until reaching commercial size (Brown et al., 1997). These diets are usually combinations of 2 or more species, commonly flagellates and diatoms, generating a complementary nutritional synergy that allows production to be optimized (Mazón-Suástegui, 2005; Maeda-Martínez et al., 2016). Alternative and complementary diets have been studied for the partial substitution of live microalgae, as an option to maintain nutritional quality and, at the same time, to reduce the high costs of microalgae cultivation in a hatchery. This situation is particularly important when the demand for food grows exponentially, which happens during nursery from post-larvae (spat) to seed (seed, early juvenile), and is when the bivalve acquires a manageable size and greater resistance to the conditions expected during their cultivation (grow-out) in the sea.

Non-living or "artificial" diets include microalgae pastes (Southgate et al., 2016), microencapsulated, yeast, dry microalgae, as well as cereal flours due to their high carbohydrate content (Mazón-Suástegui et al., 2008; Mazón- Suástegui et al., 2019) and lipid emulsions (Espinoza, 2014). The complementary diets have shown advantages and disadvantages as single diets or as ingredients in mixed diets, but none of them has been able to fully replace microalgae (Mazón-Suástegui, 2005; Mazón- Suástegui et al., 2019).

In the search for new inputs that can improve yield in bivalve farming, the use of terrestrial plants with nutritional and immunomodulatory properties has been proposed as a novel and interesting alternative (Elumalai et al., 2021), as in the case of *Moringa oleifera* Lam. 1783. Moringa is a Moringaceae plant species, widely distributed in tropical and subtropical regions of Asia, Africa, and Latin America (Olson, and y Fahey, J., 2011; FAO, 2020), to which antibacterial, antiviral, antiparasitic, and antidiuretic properties are attributed, among other beneficial characteristics for human health (Fahey, 2005). Its leaves are well known to be rich in protein, vitamins A, B, and C, and minerals, and are recommended as a fortifying food supplement for pregnant women, infants, and young children (FAO, 2020).

The inclusion of moringa leaf meal in different proportions has been used particularly in in fish aquaculture (Al-Dubakel and Taher, 2020; Abdel-Latif et al., 2022) and in shrimp (Kaleo et al., 2019; Akbary et al., 2021; Abidin et al., 2022) considering a promising alternative to immunostimulants in Aquaculture diets, not only because their antimicrobial properties are cost-effective and eco-friendly, with negligible side effects, but also because they improve the growth performance (Elumalai et al., 2021).

Despite the nutritional benefits that are reported for humans, and the studies on its inclusion in fish diets that improve their growth and survival rates, the implications and benefits of its use as a diet for bivalve molluscs are unknown. For this reason, this research evaluates the effectiveness of moringa leaf meal (*Moringa oleifera*) as a usable dietary supplement during the pre-seed cultivation phase of *P. sterna* and its possible synergistic effect associated with mixed diets formulated with microalgae and corn starch, an additive of proven utility in the production of bivalve mollusc seeds (Mazón-Suástegui et al., 2008; Mazón-Suástegui et al., 2019), on growth and survival both under controlled laboratory conditions and subsequent initial cultivation in the sea.

2. Materials and methods

2.1. Origin of the pre-seeds and experimental design

The study comprised two phases, the first consisted of the cultivation of pre-seeds of *P. sterna* with a supply of different diets under controlled laboratory conditions (nursery) for 30 days, and the second consisted of evaluating subsequent effects of these diets, once the seeds were transferred for their suspended culture (Grow-out) in the open sea (50 days). The experimental design in the first phase involved six (6) dietary treatments, with 3 replicates each. In the second phase, the performance of the pre-seeds was evaluated, following up on the treatments and replications of the previous experimental design applied in the nursery phase. The experimental procedure was carried out following the recommendations of MEWE and Kilkenny et al. (2010) for bioethical research and responsibility in the use of animals.

The pre-seeds of *P. sterna* (6–8 mm maximum antero-posterior length; mass 0.01 ± 0.002 g) were supplied by the National Center for Aquaculture and Marine Research of the Escuela Superior Politécnica del Litoral, Ecuador (CENAIM- ESPOL) obtained from the wild on artificial collectors located in the Bay of Ayangue, Santa Elena, Ecuador. The pre-seeds were transferred to the Aquaculture Laboratory of "Luis Arboleda Martínez" Higher Technological Institute (ISTLAM), Ecuador, where they were kept for acclimatization in tanks with circulating seawater (temperature 24.1 ± 0.12 °C, dissolved oxygen 7.0 ± 0.15 ppm, Salinity 34.9 ± 0.17 ‰ and pH 8.1 ± 0.14) for 2 days. At the beginning of the experiment, the pre-seeds were previously selected, establishing a homogeneous average population size (7.2 ± 0.59 mm-SD in antero-posterior length). The pre-seeds were arranged in plastic baskets 25 cm long x 20 cm wide and 10 cm high with 5 mm openings throughout their surface at a density of 42 individuals/basket. These baskets were placed in 22 L rectangular tanks, with a continuous flow of seawater (0.2 L/min) and constant aeration; similar conditions to those referred to above (Fig. 1).

Six (6) diets were established: a base diet composed of a mixture of the microalgae *Tetraselmis suecica* and *Chaetoceros gracilis* at a ratio of 1:1 (diet M), two mono-diets (Mo and Co) with 100% meal of leaves of moringa *M. oleifera* and 100% corn starch (all the percentages were in relation to the dry mass of the microalgal diet), two diets with the mixture of microalgae M added with 5% of leaf meal of moringa *M. oleifera* (M + Mo) and with 5% corn starch (M + C), finally, a mixed diet (M + Mo + Co) was included with the mixture of microalgae M, 2.5% moringa and 2.5% corn starch (Table 1). All diets were established with 3 replicates. Maicena® was used for the corn starch diet, by virtue of comparison, as this is a product used successfully as an additive to microalgal diets for the development of bivalve pre-seeds (Mazón-Suástegui et al., 2008; Mazón- Suástegui et al., 2019). The mono-diets with moringa and 100% corn starch were supplied as emulsions. The moringa emulsion was mixed with carboxymethylcellulose as a gelling agent (without a nutritional value), at a percentage of 0.5%. The corn starch emulsion was prepared in an aqueous medium, and at a temperature of ~70 °C, on a thermal plate until a consistent emulsion was obtained (Mazón-Suástegui et al., 2008). Both emulsions were cooled and mixed in the proper proportion to constitute the different mixed diets. The control microalgal diet was 50% *T. suecica* and 50% *Ch. gracilis* (1:1) in relation to their dry masses, according to Mazón-Suástegui et al. (2008). The daily food ration was established at 8% of the total dry mass of the bivalves for each experimental unit, during the first 15 days, and the ration was twice the initial mass of the diets for the following 15 days. After 30 days of the bioassay, the organisms from each replicate were arranged in culture baskets (5 lantern nets), maintaining their replicate order. The individuals were placed on a floor of the first three levels of the lantern net, distributing the replicates heterogeneously in the lanterns, and were taken to the sea for suspension culture for 50 days (initial culture phase). Although our design involved pseudo-replication (Hurlbert, 1984), apart from the heterogeneous redistribution of the replicates of each diet (floors of lantern nets), concurrent studies using the same type of basket show that the contribution to variability due to differences between basket floors within treatments were insignificant compared to variability between treatments (Lodeiros and Himmelman, 1996). The initial culture in the sea was evaluated during the equatorial summer season, where the environmental parameters remained in ranges very similar to those used in the preliminary laboratory tests with an average temperature of 25.8 ± 0.36 °C.

The evaluation of the experimental nursery diets and their

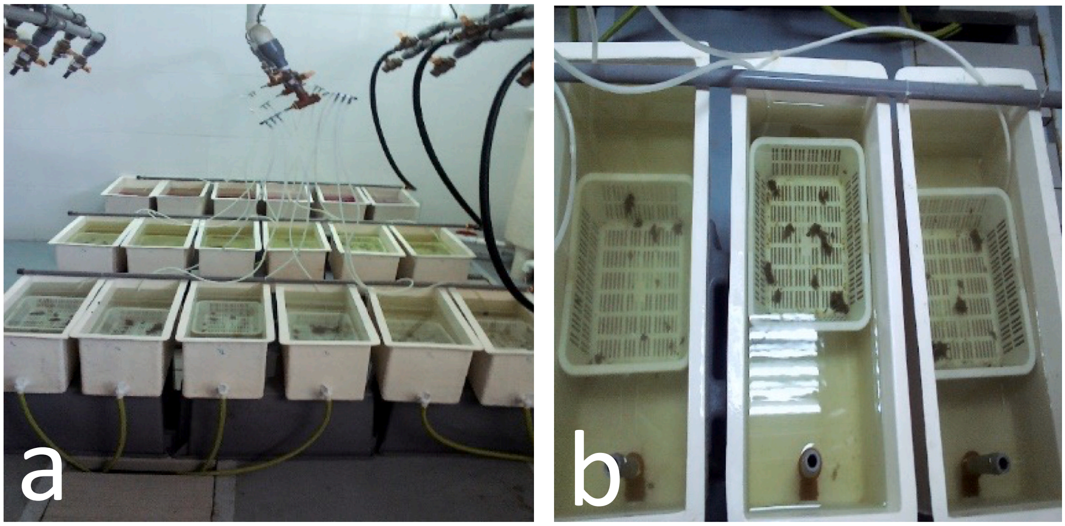

Fig. 1. System showing the experimental replicates, containers with 22 L of water with continuous flow and constant aeration (a) with 42 individuals in each container (b). The photo belongs to the end of the experiment organisms in seed size.

Table 1
Diets tested showing their components and proportions.

Diets	Components	Proportions
M	*Tetraselmis suecica* and *Chaetoceros gracilis*	50% *T. suecica* and 50% *Ch. gracilis*
Mo	Meal of leaves of *moringa Moringa oleifera*	100% Mo
Co	Corn starch flour	100% Co
M + Mo	Microalgal diet and Mo as an additive	100% M + 5% Mo
M + Co	Microalgal diet and Co as an additive	100% M + 5% Co
M + Mo + Co	Microalgal diet and Mo and Co as additives	100% M + 2.5% Mo + 2.5% Co

All the percentages were in relation to the dry mass of the microalgal diet and the daily feed ration was estimated as 8% of the dry mass of the total bivalves in each experimental unit. M = microalgae, Mo = Moringa and Co = corn starch.

subsequent effect during the initially suspended culture in the sea was carried out by monitoring the survival and growth of the organisms in length and weight. For this, the organisms were evaluated at 15 and 30 days in the nursery phase and at the end of the initial grow-out study in the field. In these periods, the living organisms were counted, discarding those that did not close their shells after a mechanical stimulus or whose valves were empty. The growth in antero-posterior length was calculated by means of a photometry system applying a public domain program Image J (National Institutes of Health, USA). The mass of the shell and soft tissues was estimated on an electronic scale with a precision of 0.0001 g and for this, five organisms from each replica of the treatments at times 15 and 30 days of the laboratory phase, and five organisms at the end of the initial culture were dissected and dehydrated in an oven at 65 °C for 24 h.

2.2. Nutritional analyses of the diets

The inputs used in the diets were previously analyzed. Meal from dehydrated leaves of *M. oleifera* from the National Ecotype (Guayaquil, Ecuador) was used and its quality was evaluated in terms of lipid and amino acid profile analyses. These studies were carried out in the food security and sustainable development service of the Scientific-Technological Research Support Center (CACTI) of the University of Vigo, Spain, reflecting their analysis and methodology in a previously published article (Estay-Moyano et al., 2021). The microalgae used (*T. suecica* and *Ch. gracilis*) belong to the CENAIM-ESPOL/Ecuador strain collection; they were cultivated following their protocols and using f/2 medium (Guillard, 1975) enriched with 1% sodium metasilicate for diatom cultivation. The nutritional quality of this mixture of microalgae was described by Rodríguez-Pesantes et al. (2020). The composition of the corn starch (commercial Corn starch®) was mainly carbohydrates according to Mazón- Suástegui et al. (2019).

2.3. Statistical analyses

The data was verified for normality using the Shapiro-Wilk test and homogeneity of variances using the Bartlett test. A one-way analysis of variance (ANOVA) and Fisher's *a posteriori* test were applied to test the effect of the diet treatments at each of the sampling times on the survival and growth of pre-seeds and seeds during the grow-out phase (Sokal and Rohlf, 2012). The analyses were performed with the statistical package STATGRAPHICS CENTURION v. XIX (http://Statgraphics.net) and a significance level of $P < 0.05$.

3. Results

3.1. Survival

During acclimatization, the mortality of organisms was very low (<0.5%). At 15 days from the start of the bioassay in the laboratory (nursery), significant differences could already be observed among the applied experimental treatments (supplied diets). In the pre-seeds fed with the Mo diet, a notable decrease in survival was recorded to 60.3 ± 15.5%, with total mortality occurring at 30 days (Fig. 2). However, with the remaining diets, at 15 days no significant difference was recorded in survival among the treatments, but at 30 days the M + Co diet significantly provided lower survival (92.06 ± 1.56%). The pre-seeds fed with the other diets were not significantly different and showed survival rates >95%.

In the subsequent initial culture in the sea (Grow-out), the same nursery trend was observed, being the organisms previously fed with the M + Co diet, the ones that presented significantly lower survival.

At the conclusion of the study, the cumulative survival of the organisms previously fed in the nursery with the different experimental diets showed significant differences, with the lowest percentage for the M + Co diet (80.7 ± 3.58%), compared to the other diets. Diets whose survivals were not significantly different and >90% (Fig.3).

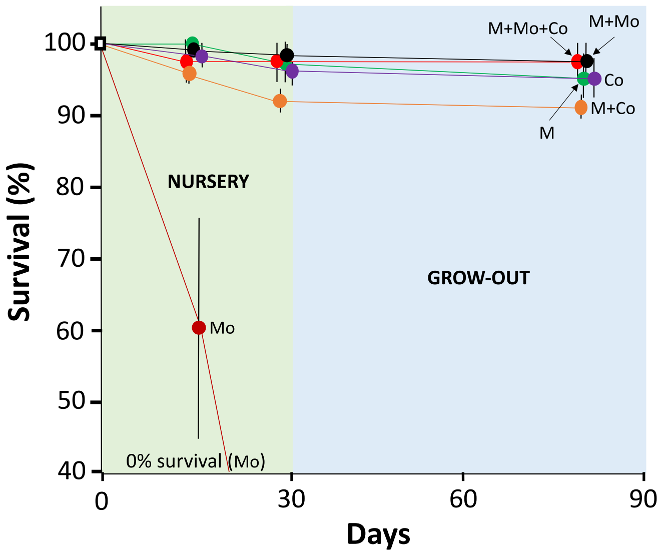

Fig. 2. Average survival of pre-seeds (nursery) and seeds (growout) of *Pteria sterna* fed with the established diets. M: Microalgae diet, Mo: Moringa diet, Co: cornstarch diet, M + Mo: microalgae diet with 5% Moringa flour, M + Co: microalgae diet with 5% cornstarch, M + Mo + Co: from microalgae with 2.5% Moringa and 2.5% corn starch. Vertical lines indicate the confidence interval at 95%.

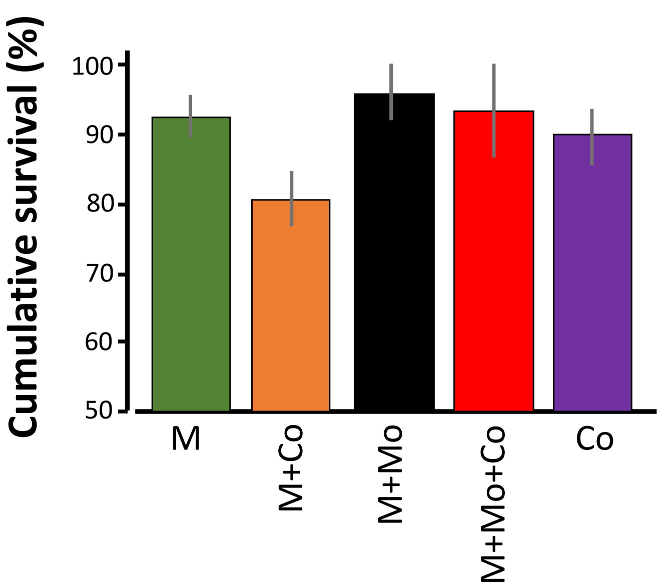

Fig. 3. Average cumulative survival at the end of the study of *Pteria sterna* fed with the established diets. M: Microalgae diet, Co: cornstarch diet, M + Mo: microalgae diet with 5% Moringa flour, M + Co: microalgae diet with 5% cornstarch, M + Mo + Co: microalgae with 2.5% Moringa and 2.5% corn starch. Vertical lines indicate the confidence interval at 95%.

3.2. Growth

3.2.1. Antero-posterior lengths

In the nursery phase, the size values reached at 15 days showed slight but consistent variations with respect to the values reached at 30 days (Fig.4). The lowest growth rate was recorded in the experimental groups fed with the Co diet; however, the differences were not significant.

At the end of the initial grow-out phase in the sea, the organisms fed the different diets established in the nursery reached significantly different sizes, with growth rates of 133 ± 14 μm/day for M + Mo and 129 ± 23 μm/day for M + Mo + Co, which were higher than those of Co (109 ± 24 μm/day) and M + Co (99 ± 14 μm/day), and all of these higher than those recorded in organisms previously fed in the nursery with the diet M formulated 100% with microalgae (74 ± 11 μm/day).

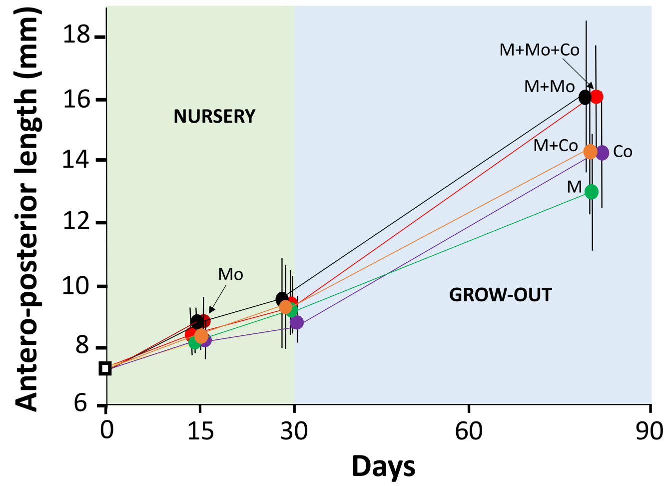

Fig. 4. Growth in average antero-posterior length of pre-seeds (nursery) and seeds (growout) of *Pteria sterna* fed with the established diets. M: Microalgae diet, Mo: Moringa diet, Co: cornstarch diet, M + Mo: microalgae diet with 5% Moringa flour, M + Co: microalgae diet with 5% cornstarch, M + Mo + Co: from microalgae with 2.5% Moringa and 2.5% corn starch. Vertical lines indicate confidence interval at 95%.

3.2.2. Biomass

In general, the growth in biomass of both shell and tissues was similar in the laboratory phase (nursery) for the seeds fed with the different experimental diets. However, at 30 days the organisms fed with Co and M diets showed the lowest growth rates of shell biomass (4.9 ± 1.91 and 3.9 ± 0.62 mg/day, respectively), while the growth rates in the remaining diets were significantly higher (5.8–6.5 mg/day; Fig. 5a). Regarding the soft tissues biomass, the 100% microalgal diet (M) presented the least growth rate (0.2 ± 0.16). For the remaining diets, growth rates >0.4 mg/day were obtained, with the M + Mo + Co diet providing a significantly higher growth rate (0.6 ± 0.20, Fig. 5b).

The results observed in growth during the period of the initial culture (Grow-out) in the sea, were largely coincident in terms of the responses to the diets provided in the nursery. Shell and soft tissue growth rates were significantly lower for individuals previously fed diet Co, followed by those fed only with Diet M and diet M supplemented with corn starch (M + Co) or with moringa leaf flour (M + Mo), which formed a statistically similar group. The mixed M + Mo + Co diet was the one that significantly increased growth, as individuals reached masses of ~1400 mg of shell and ~ 460 mg of tissues. Individuals receiving the other diets reached <1000 mg of shell and < 300 mg of tissues (Fig. 5).

3.3. Nutritional analyses of the diets

3.3.1. Microalgae and moringa flour

The analyses of the fatty acid composition of *T. suecica* indicated high abundances of long-chain fatty acids such as linolenic acid 18:3(n-3), followed by palmitic acid (16:0) and hexadecadienoic acid (16:2(n-4)). In comparison, *Ch. gracilis* presented a higher proportion of Myristic acid (14:0), palmitic acid, and palmitoleic acid (16:1) (Table 2). The specific analysis of the lipid profile of the flour of *M. oleifera* ecotype showed the presence of a greater proportion of α-linolenic acid 13.9 mg/g (33.6 ± 4.79%), palmitic acid with 9.33 mg /g (22.55 ± 1.35%); also, to a lesser degree, linoleic acid (18:2(n-6)) with 3.13 mg/g (7.57 ± 0.69%) and stearic acid (18:0) with 3 mg/g (7.25%) (Table 2). The protein percentage of the *M. oleifera* leaf meal was 29.51 + 0.21%, with a profile composed of 17 amino acids, 9 of which were essential, among

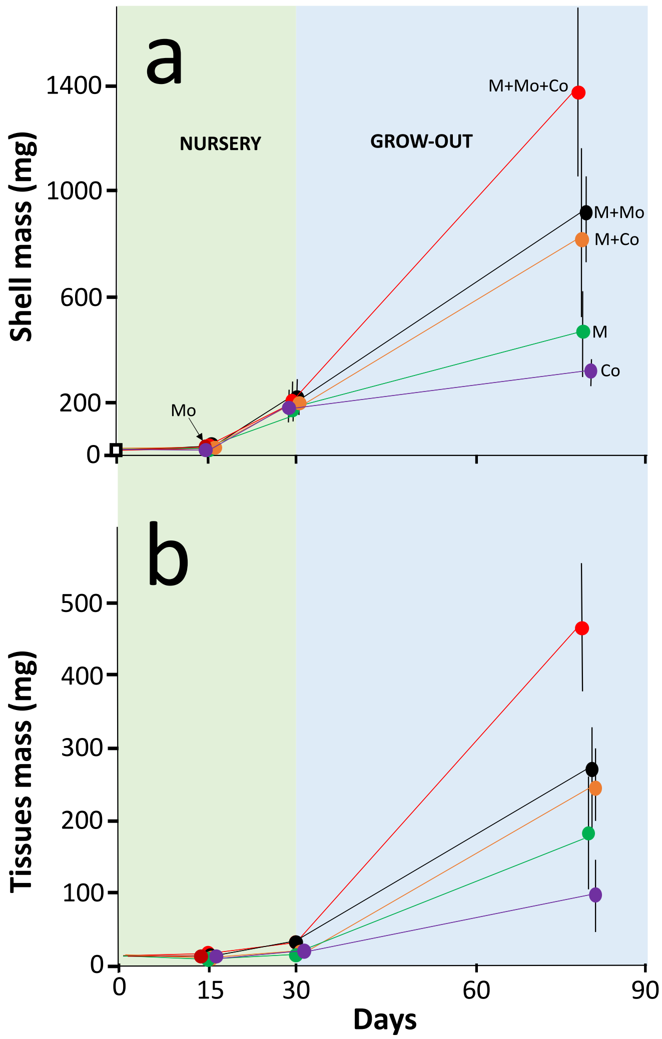

Fig. 5. Growth in biomass of the shell (a) and of the soft tissue (b) averages of pre-seeds (nursery) and seeds (growout) of *Pteria sterna* fed with the established diets. M: Microalgae diet, Mo: Moringa diet, Co: cornstarch diet, M + Mo: microalgae diet with 5% Moringa flour, M + Co: microalgae diet with 5% cornstarch, M + Mo + Co: from microalgae with 2.5% Moringa and 2.5% corn starch. Vertical lines indicate confidence interval at 95%.

them, leucine presented the highest percentage (7.9 ± 0.2%) and threonine, arginine, lysine, phenylalanine, valine with values in the range 3–5% and the rest was tyrosine, histidine, and methionine with values < 3% (Table 3).

3.3.2. *Corn starch (Maicena®)*

Corn starch is an industrialized product for human consumption, derived from part of the corn grain, presented in the form of powdered flour. Corn starch (Maicena®) according to the nutritional table for every 100 g of flour contains carbohydrates (82.8 g), protein (5.59 g), fat (1.39 g), fiber (1.9 g), K (90 mg), P (60 mg) and Mg (18 mg), Vitamin A (214 IU), Vitamin B-9 (48 mg) and Vitamin B-3 (2.7 mg).

4. Discussion

The results obtained showed that there was a direct and statistically differentiated influence among the supplied diets, on the growth and survival of *Pteria sterna* pre-seeds, under controlled nursery conditions, which was concomitant with the performance of the seeds during the second season phase of the study (Grow-out). The best development was promoted by the mixed diet formulated with microalgae (M) and enriched with additives of moringa and corn starch (M + Mo + Co), indicating that the additives used promoted a better general physiological condition in the organisms, with respect to the traditional diet based 100% on cultivated microalgae.

Table 2
Fatty acid composition of *Tetraselmis suecica* and *Chaetoceros gracilis* in exponential phase (Reference composition: Rodríguez-Pesantes et al., 2020); Moringa olifera leaf meal (Reference composition: Estay-Moyano et al., 2021). The fatty acid analyses were realized in relation to total lipids. Abbreviations: ND, not detected.

Fatty acids	Tetraselmis suecica	*Chaetoceros gracilis*	Moringa olifera
6:0	ND	ND	0.5
12:0	ND	ND	0.7
13:0	ND	ND	1.0
14:0	ND	20.3	1.5
14:1	ND	7.4	0.3
16:0	21.1	21.7	9.4
16:1(n-9)	7.2	22.4	0.8
16:2(n-4)	11.5	6.5	ND
17:0	ND	ND	0.5
18:0	3.0	4.4	3.0
18:1(n-9)	6.6	4.9	1.5
18:2(n-6)	8.7	6.2	3.2
18:3(n-3)	33.0	8.4	14.0
18:4(n-3)	ND	ND	ND
20:0	3.9	ND	0.8
20:3 (n-6)	ND	ND	0.7
20:4(n-6)	ND	ND	0.4
20:5(n-3)	ND	5.3	ND
22:0	ND	ND	1.0
23:0	ND	ND	0.5
24:0	ND	ND	1.6

Table 3
Aminoacidic profile (essential and non-essential) of dehydrated *Moringa oleifera* leaf flour, expressed as a percentage (%) of the molecular weight.

Essential	μg g-1	Non-essential	μg g-1
Leucine	7.90	Alanine	13.10
Threonine	4.93	Glycine	11.00
Lysine	4.43	Glutamic acid	10.17
Phenylalanine	4.33	Aspartic Acid	9.33
Valine	3.90	Serine	6.43
Isoleucine	2.90	Cysteine	5.93
Tyrosine	2.83	Proline	5.20
Histidine	1.63	Arginine	4.80
Methionine	1.17		

The pre-seeds fed with the mono-diet consisting of 100% moringa leaf meal did not survive the nursery bioassay (30 days), while those fed the mono-diet of 100% corn starch showed similar survival to that registered with the other diets evaluated. However, the development and production of somatic tissue of the pre-seeds and seeds that only received corn starch were lower than the other diets, which indicates a low efficiency of the use of corn starch as a sole diet, despite its great applicability as a complementary diet or as an aid in situations of contingencies of low or null microalgal production in *P. sterna* nurseries. If the nutritional quality of moringa *versus* corn starch is compared, the former has a greater diversity of macro and micronutrients with a high nutritional load of essential components (fatty acids and amino acids). For this reason, this plant is considered a highly nutritional and even immunostimulant raw material, with high projections for use in aquaculture (Elumalai et al., 2021). Based on the results obtained, it is possible to assume that the mono-diet formulated with 100% moringa flour was not fully available for ingestion for *P. sterna* pre-seeds and that its subsequent digestion was low, at least for this species. Unlike moringa, corn starch mostly provides carbohydrates such as starch, highly digestible and easily accumulated in the form of glycogen in the different organs of *P. sterna* (Hernández-López, 2012). Starch is a glucose polymer that can supply the body's energy needs and can even be transformed into other more complex substrates (proteins and lipids), providing essential compounds for its development. In this sense, corn starch has been used as a supplement in diets for the reproductive conditioning of *P. sterna* and other bivalves (Mazón-Suástegui, 1988) and for the nursery of pre-seeds and juveniles of other species (Gabbott, 1975; Mazón-Suástegui and Avilés, 1988; Mazón-Suástegui et al., 2008; Mazón- Suástegui et al., 2019).

The results obtained with microalgae and additives (moringa flour, corn starch) produced the highest yield, based on a larger percentage of growth (shell, tissues) and survival. These findings lead us to infer that the inclusion of moringa flour in small amounts (2.5% of the total diet), could be a key factor to stimulate growth, even more so if it is applied in synergy with the inclusion of corn starch in the said diet. In perspective, it is possible to assume that some pre-digestion treatments of moringa flour could facilitate the availability of its nutrients, regardless of the size of particles whose importance is high (20–25 μm, data not shown). Although the moringa leaf meal was supplied as a nutritional emulsion to the pre-seeds (an effective process for corn starch), this procedure was possibly not effective enough. The method of enzymatic digestion or hydrolyzation under acid media could be tested to increase the absorption of nutritional compounds from the leaves of *M. oleifera*, since its leaves are fermentable, which could lead to the decrease in pH as well as to the bacterial growth associated with its digestion (Dou et al., 2019).

Regardless of the perspective and the nutritional potential of the non-traditional ingredients evaluated during the present study, the results show that moringa flour and corn starch, combined or not at a rate of 5% with microalgal diets, can be used to improve the condition of the seeds of *P. sterna*, and probably of other bivalves. Comparing the growth rates obtained in the nursery phase, the mixed diets provided greater growth than the traditional mono diet of microalgae, in agreement with other studies. The growth rates obtained in the present study ranged between 70 and 77 μm/day and were like those reported by Hoyos-Chairez et al. (2020), using a trialgal diet (71 μm/day). However, survival (>92%) obtained during the present study was significantly higher than that estimated (80%) by Hoyos-Chairez et al. (2020). This comparison may be inadequate because our pre-seeds were not produced in the laboratory but were collected from the natural environment.

The nutritional profile is a critical factor in the search for alternative diets for the pre-seed culture of aquatic organisms, particularly those that supply highly energetic fatty acids and essential amino acids. In relation to the quality of the diets used, Estay-Moyano et al. (2021) showed the presence of α-linolenic acid (C18:3) and linoleic acid (C18:2) in the meal of dehydrated leaves of *M. oleifera*, two fatty acids characteristic of nutrients of vegetable origin. α-Linolenic acid is a long-chain fatty acid (omega-3 type), which through the action of desaturases and elongases can be transformed into polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid EPA (C20:5) and subsequently in docosahexaenoic acid DHA (C22:6) (Calder, 2006; Wanten and Calder, 2007). These fatty acids are essential for important actions in cellular energy metabolism (Serhan and Chiang, 2008) and can be incorporated into cell membranes in bivalve molluscs (Farías, 2008; Zhukova, 2019). The proportion of palmitic acid (16:0) was high in *M. oleifera* meal, an interesting fact, even though this fatty acid can be synthesized *de novo* in bivalves (Dernekbasi et al., 2015). Regarding the analysis of the microalgae strains *C. gracilis* and *T. suecica* used in this study, Rodríguez-Pesantes et al. (2020), determined that *T. suecica* contains α-linolenic acid (C18:3). The species of microalgae that present saturated fatty acids in their composition are more nutritious for bivalves, due to the greater facility for the release of energy compared to polyunsaturated fatty acids (Thompson et al., 1993).

Based on the previous statement and compared to what was reported by Rodríguez-Pesantes et al. (2020) for the microalgae used, in the present study the saturated fatty acids with the highest value in *M. oleifera* were palmitic acid (22.55%), whose proportion turned out to be similar to that observed in *Ch. gracilis* (21.73%) and *Tetraselmis suecica* (21.08%). Myristic acid (3.7%) in *M. oleifera* was lower in proportion to that observed in *Ch. gracilis* (20.26%) and was not found in *T. suecica*. In addition, it was determined that the proportion of stearic acid (7.25%) in *M. oleifera* was higher than that found in *Ch. gracilis* (4.35%) and in *T. suecica* (2.96%). Also, the presence of arachidic acid (1.76%) was detected in *M. oleifera* and in *T. suecica* (3.68%). When comparing the fatty acid profiles of *M. oleifera* in this study and that of *T. suecica*, a similar and relevant concentration of linolenic acid (~33%) was observed. In relation to *Ch. gracilis*, similar concentrations of palmitic acid (~22%) and linoleic acid (~7.5) were also found, which was not evident in the study carried out by Rodríguez-Pesantes et al. (2020). Additionally, *Ch. gracilis* presented relevant concentrations of the fatty acids eicosapentaenoic (5%) and linolenic (8.44%). In the protein analysis, it was determined that the meal of dehydrated leaves of *M. oleifera* used during this study showed a high proportion of proteins (~30%) with a content of 17 amino acids, 9 of which are essential (Estay-Moyano et al., 2021). This provides a relevant quality since amino acids are important in various physiological functions in living beings, including structural, enzymatic, and immunological functions (Abidin et al., 2022).

Finally, obtaining chemical compounds (fatty acids, proteins, and carbohydrates) that guarantee the energy requirements and biochemical substrates essential for development, growth, and tissue formation in *P. sterna* seeds grown in controlled environments, can be ensured by including artificial dietary supplements in the natural food par excellence which are cultivated microalgae (Flagellate + Diatom). Apparently, the supplementation of a 100% microalgae diet with moringa flour and corn starch is effective in promoting the growth of *Pteria* seeds in the nursery stage, with potential applicability for the massive production of seeds of this and possibly other species in the hatchery, which allows sustaining dual-purpose commercial crops: food and pearl farming.

As a matter for further in-depth studies, persists the potential for total substitution of microalgae with moringa, corn starch, and other non-conventional dietary ingredients that can be used in the pre-seed nursery stage.

CRediT authorship contribution statement

César Estay-Moyano: Writing – original draft, Conceptualization, Methodology, Writing – review & editing. **José M. Mazón-Suastegui:** Writing – original draft, Conceptualization, Methodology, Writing – review & editing, Formal analysis, Methodology, Writing – review & editing. **Edgar Zapata-Vívenes:** Writing – original draft, Conceptualization, Methodology, Writing – review & editing. **César Lodeiros:** Writing – original draft, Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

The authors appreciate the cooperation amongst groups supported by the Aqua-Cibus network funded by the CYTED cooperation program. Funding for the open access charges was covered by Universidade de Vigo/CISUG. This research is a product of the project 'PYTBEC408-2018-FCV0012 "Feasibility of culture of the Pacific oyster Magallana gigas and the winged pearl oyster Pteria sterna in the Chone River estuary, Manabi province, Ecuador" from the Universidad Técnica de Manabí, Ecuador. J. Alió collaborated in the English translation making some input into the manuscript.

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