Aquaculture Reports 35 (2024) 102017

Contents lists available at ScienceDirect

Aquaculture Reports

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

Evaluation of sustainable feeds for "caviar" production in the Mediterranean sea urchin *Paracentrotus lividus* (Lamarck, 1816)

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ARTICLE INFO

Keywords:
Echinoculture
Sustainability
Waste
Discard
Diet

ABSTRACT

To improve the sustainability of aquaculture practices, a step towards the use of alternative nutrient sources (such as food processing discards) may secure the future of aquaculture sector, namely for emergent species, such as sea urchins. In this context, adult females of the commercial sea urchin *Paracentrotus lividus* were reared using four feeds based on lettuce discards (72%) and enriched (8%) with an animal-source ingredient (fish *Sardina pilchardus*, Feed-S; krill *Euphausia superba*, Feed-K; mussel *Mytilus galloprovincialis*, Feed-M; anchovy *Engraulis encrasicolus* discards, Feed-AD). A fifth feed, used as control treatment, was composed of macroalgae (*Laminaria* sp. and *Ulva* sp., Feed-UL). Feed performance was evaluated employing a new productive protocol, the Raking method, which propose testing feed effects on sea urchin caviar (oocytes rather than gonads) production. Thus, ingestion rates and absorption efficiency were measured to evaluate feed palatability. Somatic growth and caviar production, expressed introducing the ovosomatic index (OI) instead of the traditional gonadosomantic index, were measured to assess feed productive performances. Caviar quality was assessed by nutritional content and color. Ingestion rate results showed that all feeds were palatable, while findings on absorption efficiency showed differences between the five proposed feeds, with Feed-M and Feed-AD presenting the worst results. Somatic growth was promoted regardless the provided feeds, while OI resulted higher with Feed-K and Feed-M than the other feeds. All produced caviar resulted suitable for human consumption with high protein and fatty acid content, but caviar produced by Feed-UL showed the poorest nutritional profile. Similarly, Feed-UL led to the production of caviar with the lowest quality color, while Feed-S showed the best orange color. Lettuce-based feeds were therefore effective for feeding *P. lividus* as they stimulated production of high quality caviar. Findings support the exploitation of food discards for the production of eco-friendly feeds for sea urchin aquaculture.

1. Introduction

Sea urchin gonads, commonly referred as roe, are considered one of the most valuable seafood products, especially in Japan which accounts for around 80% of global demand of sea urchin gonads (Stefánsson et al., 2017). Several sea urchin species are harvested for human consumption such as *Loxechinus albus* (Molina, 1782) and *Strongylocentrotus* spp. (*S. franciscanus* (Agassiz, 1863), *S. intermedius* (Agassiz, 1864) and *S. pulcherrimus* (Agassiz, 1864) in particular), which provide to the greatest global landings (FAO, 2020; Stefánsson et al., 2017). In Europe, the most exploited sea urchin is *Paracentrotus lividus* (Lamarck, 1816), widely distributed along Mediterranean and North Atlantic coasts (Boudouresque and Verlaque, 2020) and appreciated due to its bright orange roe characterized by a delicate and slightly saline flavor. A great increase in catches has been observed in recent years (Stefánsson et al., 2017), for instance in Portugal, landings of *P. lividus* raised from 3 tons in 2010–324 tons in 2020, with an overall value of 843 000 € (Instituto Nacional de Estatística, 2021). This increasing fishing pressure is leading to a decline in natural stocks, with a detrimental impact on the whole ecosystem, since *P. lividus* plays also a key role in controlling the macroalgal beds assemblages in benthic ecosystems (Boudouresque and Verlaque, 2020).

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https://doi.org/10.1016/j.aqrep.2024.102017
Received 11 August 2023; Received in revised form 2 December 2023; Accepted 4 March 2024
Available online 8 March 2024

Aquaculture of sea urchins appears to be a valuable tool to contrast stock depletion. Thus, the attention to sea urchins rearing is increasing. Today, the lack of proper hatchery and breeding techniques able to obtain high larval survival rates are the major issues affecting the first stages of sea urchin production (Carboni et al., 2013; Rubilar and Cardozo, 2021). In addition, sea urchins are characterized by a low growth rate. So, several months are required to achieve the commercial size (Boudouresque and Verlaque, 2020). Accordingly, adult sea urchins need 3 or 4 months to produce gonad biomass for aquaculture goals (Walker et al., 2015). Gonad biomass and/or size increase are influenced by nutrient accumulation in specialized cells, called nutritive phagocytes. Number and size of nutritive phagocytes change according to nutrient accumulation and progression of the sea urchins' reproductive cycle, as gonads play a dual function as storage tissue and reproductive organ (Walker et al., 2015). For aquaculture goals, the best gonads are those of sea urchins sexually mature, just prior to gamete emission, as they are characterized by a large size and better nutritional content. On the contrary, when spawning occurs, the gonads are emptied from gametes, resulting in a loss of biomass and nutritional content (Marsh et al., 2013; Walker et al., 2015). In order to promote nutrient accumulation and thus, gonad biomass increase, several diets or feeds were prepared using high quality ingredients (Baião et al., 2019; Pearce et al., 2004; Sartori and Gaion, 2015; Shpigel et al., 2005) and tested for the effects on juvenile and adult gonad production. Positive results in gonad growth and quality were obtained using algal-based diets, diets composed of animal and vegetal ingredients, dry, wet and pelleted diets (Fernandez and Boudouresque, 2000; Baião et al., 2019; Castilla-Gavilán et al., 2019; Grosso et al., 2022). However, the lack of cost effective and sustainable feeds, able to combine high gonad yield and quality is still an important issue. Ingredients, such as wheat or soybean meals, have been added as a partial replacement of macroalgae, aiming to a better nutritional quality, and positive effect on somatic and gonadic growth, as well as on roe organoleptic characteristic (Grosso et al., 2022; Pearce et al., 2004, 2002b, 2002a) were found. The encouraging results obtained in gonad growth and quality, promote the exploitation of terrestrial vegetables instead of macroalgae, which resulted low performant (Raposo et al., 2019; Santos et al., 2019; Sartori and Gaion, 2015). Today, however, there is scarce availability of high-quality ingredients for aquaculture feeds. Fish meals and oils from low-quality pelagic fish were also used to produce feeds for swine and poultry breeding, while cereal and terrestrial vegetables could be destined to direct human consumption (Hua et al., 2019). It has become clear that the success and sustainability of commercial sea urchin aquaculture, its economic viability and market acceptance, strongly depend on the development of suitable cost-effective diets, capable of producing high-quality gonads (Pearce et al., 2002b). Following the principle of the green economy, the reuse of food by-products could be a valuable alternative in aquaculture feed production (de la Caba et al., 2019). Today, food discards and waste are an important issue worldwide, with a great amount of vegetal and animal biomass being thrown away with high disposal costs (Gustavsson et al., 2011; Tedesco et al., 2021). The European Union produces more than 88 million tons of food waste, and it is estimated that this value will continue to grow up to 40% in the future (Stenmarck et al., 2016). In addition, a large amount of waste is made up of edible fruits and vegetables discarded because they do not fit the food quality standards that retailers and consumers demand (Gustavsson et al., 2011). These wastes and discards are characterized by a great content of carbohydrates, micronutrients and bioactive compounds (Tedesco et al., 2021), which may be reused as ingredients for feed production. Use of food discards in animal feeds have been investigated (Wadhwa et al., 2015), namely for fish (Mo et al., 2020) and shrimp aquaculture (Sajitha et al., 2011) with positive results.

In this context, the aim of this study was to test four feeds produced using common lettuce discards (*Lactuca sativa*, Linnaeus, 1753) as main ingredient (72%) to feed adult *P. lividus*, together with a low amount (8%) of animal meal. Namely, a meal of sardine fillets (*Sardina pilchardus,* Walbaum, 1792, Feed-S), krill meal (*Euphausia superba,* Dana, 1850, Feed-K), a mussel (*Mytilus galloprovincialis*, Lamarck, 1819) meal, with shells included (Feed-M), and a meal of anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) processing discards (viscera, heads, bones, skin, Feed-AD). The effectiveness of the four feeds was evaluated in terms of production, employing the Raking method, a new generation method that introduces an innovative productive approach in echinoculture (Rakaj et al., under review). Unlike the traditional methodology that envisages the entire gonad as the final consumer product, thus imposing the sacrifice of adult sea urchins at the end of the breeding cycle for gonad extraction, the Raking method provides oocytes as a final product in form of "sea urchin caviar". With this method, caviar is obtained by breeding female-only batches, which are induced to spawn with cyclical cadence through a combined wet-dry and thermal shock, until the spawn is ended. Thus, to measure production, the ovosomatic index was used in spite of the traditional gonadosomatic index, as these indices can be considered comparable since gametes and gonads differ mainly in cellular composition (different ratio of nutritive:reproductive cells) rather than in biomass (Byrne, 1990; Marsh et al., 2013; Walker et al., 2015). This approach allows to overcome the main traditional constraints, due to the long breeding time to reach the market size. With this method, in fact, it is not necessary to sacrifice sea urchins at the end of the rearing cycle, but the same stock can undergo multiple reproductive cycles (Rakaj et al., under review). To test the effect of the four lettuce-based feeds, ingestion rate, absorption efficiency, somatic growth and caviar production were evaluated. Finally, a first evaluation on caviar quality was conducted by assessing nutritional content (proteins, lipids and fatty acids) and color, which is considered an important quality parameter as it can be assessed at first sight (McBride et al., 2004). Findings were compared with those obtained from a control group of sea urchin fed with an algal-based feed composed of *Laminaria* sp. and *Ulva* sp. These macroalgal species were chosen as both: i) can be present in environments populated by *P. lividus*; ii) are often used as control treatments in feeding experiments; iii) have been included as an ingredient in several sea urchin diets (Candeias-Mendes et al., 2020; Carboni et al., 2013; Cook and Kelly, 2007; Fabbrocini et al., 2012; Shpigel et al., 2005; Spirlet et al., 2001; Zupo et al., 2019).

2. Materials and methods

2.1. Feed

Five feeds were tested: four were obtained using discarded leaves of the lettuce *Lactuca sativa* as the main ingredient (72%), and a small amount of animal meal (8%) (Table 1). Namely, sardine (*Sardina pilchardus,* Walbaum, 1792) fillet meal for Feed-S; krill (*Euphausia superba,*

Table 1
Ingredients (%) of the five experimental feeds, algal feed with *Laminaria* and *Ulva* (Feed-UL), feed based on common lettuce discards with *Sardina pilchardus* fillet meal (Feed-S), krill meal (Feed-K), mussel meal (Feed-M), and a meal obtained from *Engraulis encrasicolus* processing discards (Feed-AD).

Ingredients (%)	Feed-UL	Feed-S	Feed-K	Feed-M	Feed-AD
Laminaria sp.	40	-	-	-	-
Ulva sp.	40	-	-	-	-
Vegetal discards (*Lactuca sativa*)	-	72	72	72	72
Fish meal (*Sardina pilchardus*)	-	8	-	-	-
Krill meal (*Euphausia superba*)	-	-	8	-	-
Mussel meal (*Mytilus galloprovincialis*)	-	-	-	8	-
Fish discards (*Engraulis encrasicolus*)	-	-	-	-	8
Lithothamnium calcareum	16.5	16.5	16.5	16.5	16.5
Binder	3.5	3.5	3.5	3.5	3.5

Dana, 1850) meal for Feed-K; a meal made from mussels (*Mytilus galloprovincialis*, Lamarck, 1819), including shells, for Feed-M and a meal made from anchovy (*Engraulis encrasicolus,* Linnaeus, 1758) processing discards (viscera, heads, bones, skin) for Feed-AD, were used. The fifth feed, composed by equal proportions of *Laminaria* sp. and *Ulva* sp., was used as control feed (Feed-UL). *Lithothamnium calcareum* (Areschoug, 1852) was integrated in each feed (16%) to provide an appropriate content of inorganic carbon. In all experimental formulations, agar (3%) and arabic gum (0.5%) were used as binders. Each formulation was prepared with boiling water and mixed to obtain a homogeneous mixture. Pellets (8 mm diameter) were produced using an industrial extruder and left to air dry for 24 hours. Finally, the pellets were steamed for 15 minutes in a steamer and stored at −20°C. Aliquots from each feed were analysed for proximate composition.

2.2. Proximate composition

Samples were freeze-dried and grinded. Lipids were measured following Bligh and Dyer (1959) after modification: a solution of MilliQ distilled water, methanol (CARLO ERBA Reagents, Chaussée du Vexin, France) and chloroform (Panreac Quimica Sau, Barcelona, Spain) ratio 1:2:1 (v:v:v) with 0.01% of butylated hydroxytoluene (Sigma-Aldrich®, St. Louis, United States of America) as an antioxidant was added to samples. Samples were then sonicated to improve lipid extraction and centrifuged twice to separate the lipid and aqueous phases. The lipid extracts were evaporated to dryness under a gentle nitrogen stream and weighed. Protein content was estimated by analysing the total nitrogen content in an Elemental Analyzer (Thermo Fisher Scientific EA 1112), which was subsequently converted to protein content by applying the conversion factor of 6.25 (Horowitz and Latimer, 2006). Ash content was determined by combustion in a muffle furnace at 550°C for 4 hr according to Nielsen (2010), while carbohydrate content was also estimated, according to Baião et al. (2019) as follows:

carbohydrates = (100 – (ash + protein + lipid))

2.3. Feed stability

Before starting the feeding experiment, a preliminary evaluation of stability in seawater was conducted in a recirculating aquaculture systems (RAS) with stable environmental condition: seawater temperature, 20.0 ± 1.0 °C, salinity, 35.8 ± 0.3 PSU, pH 8.20 ± 0.20 and natural photoperiod. As a first step, three replicates of each feed were weighed (WW), oven dried at 60°C until constant weight (≈ 48 h), and then weighed again (DW), in order to assess the standard dry weight (DW_s% = DW/WWx100) of each feed. Next, three replicates per feed were weighed and left in water for 24 h, after which they were oven dried until constant weight and then weighed again to assess the final dry weight. Feed loss was calculated as follow:

DW_{loss} (%) = $[(DW_i\text{-}DW_f)/DW_i]$ x 100

where DWi is the dry weight of each feed pellet, calculated on the standard dry weight as follow:

DW_i (g) = (WW_i x DW_s%)/100)

2.4. Feeding experiment

In March 2020, 220 adult specimens (Test Diameter, TD: 47.41 ± 4.04 mm) of *Paracentrotus lividus* were collected by snorkeling (1–5 m depth) at Santa Marinella, Italy (42°3′0″Nord, 11°49′9″Est). Sea urchins were transported to the Laboratory of Experimental Ecology and Aquaculture (L.E.S.A) of the University of Rome "Tor Vergata" inside a 150-L tank equipped with aerators and dry ice. Organisms were maintained in a 600 L indoor tank in a closed circulation aquarium, and fed with macroalgae collected in the same site of the sea urchins for one week, in order to adjust specimens to laboratory conditions. Environmental conditions were maintained stable: seawater temperature, 20.0 ± 1.0 °C, salinity, 35.8 ± 0.3 PSU, pH 8.20 ± 0.20 and natural photoperiod. After one week spawning was induced in all specimens, employing the Raking protocol (Rakaj et al., under review). Sea urchins were removed from the 600 L tank and left to dry for three hours. Subsequently, each sea urchin was assigned to a separate basket, in order to isolate the gametes produced by each specimen. Sea urchins were then induced to spawn by thermal stimulation, filling each basket with heated seawater (24°C), and left to release gametes for few hours. When the spawning ended, sea urchins were removed from the basket and 90 females were randomly selected for the experiment. Fifteen females were randomly selected and wet weighed to evaluate the initial (Wild) ovosomatic index (OI). Released oocytes were collected and transferred to 50 ml tubes and then centrifuged at 2000 rpm for 10 minutes, to assess the total volume. Finally, the residual of water was removed and the oocytes weighed.

The others 75 specimens were tagged trough a Passive Integrated Transponder (PIT) tag, measured (TD: 47.58 ± 4.26 mm), wet weighed (total wet weight: 48.89 ± 15.07 g) and maintained in the RAS (Fig. 1), in floating boxes for 4 weeks before the start of the feeding experiment. During the two first weeks, sea urchins were fed with macroalgae collected in the sampling site, while in the lasts two weeks sea urchins were kept fasting to standardize initial appetite levels and to synchronize their reproductive stage (Castell et al., 2004; Ciriminna et al., 2021; Raposo et al., 2019). Then, sea urchins were divided in 15 tanks each containing 5 sea urchins. Three tanks were randomly assigned to each feed with a total of 15 sea urchins for treatment. The feed was provided *ad libitum* six days a week for four months. The experiment was conducted in a RAS maintaining the same environmental conditions during the whole experiment. Water exchange of 50% of the whole volume was undertaken at least twice a week, using 5 μm filtered and UV-sterilized seawater. Ammonia, nitrate and nitrite concentration were checked every two days by means of spectrophotometer (HI83303). Temperature, salinity and pH were daily monitored through a multiparameter probe (the sensor EUTECH PCD 650) directly immerged in the aquaria.

2.5. Metabolic rates

The ingestion rate (IR) and the absorption efficiency (AE) were assessed daily for three weeks, after one month of the controlled feeding provision, to avoid the influence of the initial fasting period. Provided feed was wet weighed before each distribution and respective dry weight was calculated from the standard dry weight (DW_s%), like-wise the previous stability trial. Before each feed provision, faeces and food leftovers were carefully siphoned off, filtered with a sieve (500 μm), oven-dried (60°C for 48 h) and then weighed. The IR was calculated as the difference between the total provided biomass (dry weight) and the total uneaten biomass on number of specimens (n) as follows:

IR (g day^{-1} $individual^{-1}$) = (total provided biomass – total uneaten biomass)/tn

Absorption efficiency was calculated as follows:

AE (%) = [(total biomass ingested – total faeces biomass)/total biomass ingested] x 100

Where total biomass ingested is measured as follows: total provided biomass – uneaten biomass -biomass loss in seawater.

The somatic growth of sea urchins was evaluated measuring sea urchin wet weight in three times: before the start of the feeding trial (before acclimatization and starvation, T0), after 10 weeks (T1), and at the end of the experiment (T2). Sea urchins were always weighed after 48 h from the last feed provision, in order to empty sea urchin digestive system before being weighed with a precision balance (ORMA BC180, ± 0.1 mg accuracy). In addition, before weight measurement the specimens were kept for 1 minute outside the aquaria to drip external water. Specific Growth Rate (SGR %) was calculate as follows:

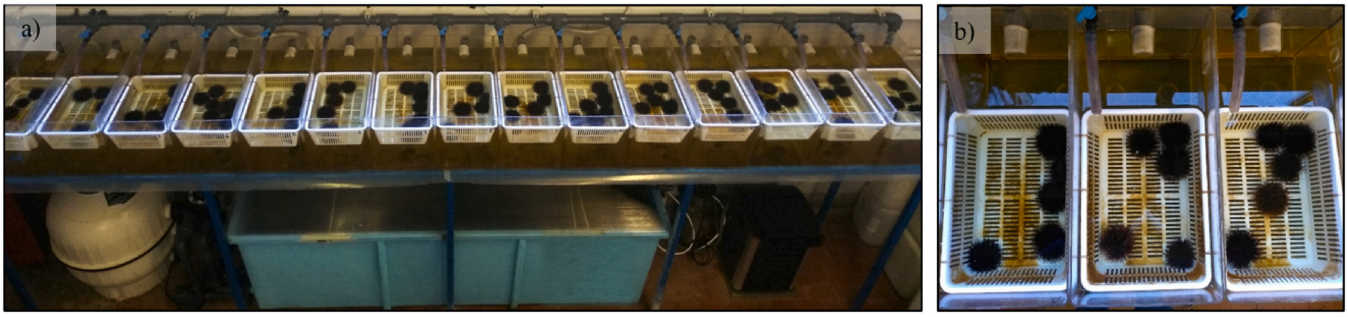

Fig. 1. Recirculating aquaculture system (RAS) used for the feeding experiment (a). Detail of the tanks and sea urchins randomly assigned to each experimental feed (b).

SGR: $((\ln TWW_f - \ln TWW_i) / t) * 100$

where "TWW_f" and "TWW_i" are the final and initial wet weight (g) respectively of each sea urchin specimen, "t" represents time in days of the experiment.

Caviar production was measured through the ovosomatic index. Oocyte release was induced through the Raking method (Rakaj et al., under review), as described before, after four months of controlled feeding.

Ovosomatic index (OI %) was calculated from each sea urchin in each condition:

OI: OWWg/ TWWg x 100

Where OWWg is the wet weight (g) of the oocytes and TWWg is the total wet weight (g) of the sea urchins.

After OWW measurement, oocytes were freeze-dried and stored at −80°C for further analyses.

2.6. Sea urchin caviar quality: nutritional content and color

To assess nutritional quality of *P. lividus* caviar for human consumption, protein, lipid and fatty acid content were measured. Protein and lipid contents were measured following the same procedures as for feed proximate composition, while fatty acids were extracted following a modified version of the Bligh and Dyer (1959) method: after being weighed, lipid extracts were suspended in n-hexane and subjected to acid-catalyzed transesterification using methanolic hydrogen chloride to obtain fatty acid methyl esters (FAME). FAME were then analysed by a gas chromatograph (GC-2010, Shimadzu) equipped with a BPX-70 capillary column (30 m length; 0.25 mm ID; 0.25 μm film thickness, SGE Analytical Science) and detected by a flame ionization detector (FID). Peaks were identified by retention times from mixed commercial standards (37 FAME from Supelco; QUALFISH and BACTERIAL MIX from Larodan). Tridecanoic and tricosanoic acids (C13:0 and C23:0) were used as surrogate standards, while pentacosanoic and dodecanoic acid methyl ester (ME C25:0, ME C12:1) were used as internal standards for quantification. To evaluate caviar quality, fatty acids considered biomarkers of nutritional quality for human consumption were selected: α-Linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), essential fatty acids (EFA), ω3-polyunsaturated fatty acids (ω3-PUFA), ω3-highly unsaturated fatty acids (ω3-HUFA), PUFA/SFA ratio and ω3/ ω6 ratio (Kaur et al., 2014). Finally, atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic/hypercholesterolemic ratios (HH) were calculated according to Prato et al. (2018):

AI= (C12:0 + 4 x C14:0 + C16:0) / (ΣMUFA + ΣPUFA)

TI= [(C14:0 + C16:0 + C18:0) / (0.5 x ΣMUFA + 0.5 x Σω6-PUFA + 3 x Σω3-PUFA + (ω3/ω6))]

HH= (C18:1*cis*9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3) / (C14:0 + C16:0)

To assess the color, sea urchin caviar was placed in clean Petri dishes and compared with Pantone® color standard chart (Color Formula Guide 1000, 1991) under standard artificial daylight (Reer, 4000 K) by an observer with extensive experience in evaluating sea urchin gonad color. Each sample was assigned to a single color category among those defined by Pearce et al. (2002a) and listed below according to a decreasing quality level:

1 = bright yellow or orange
2 = paler yellow or orange, mustard
3 = yellow-brown, orange-brown, red-brown, cream
4 = any other color (e.g., dark brown, gray)

2.7. Statistical analysis

Permutational analysis of variance (PERMANOVA; Anderson et al., 2008) was conducted before the start of the feeding trial to exclude possible differences in size (total weight and test diameter) between the organisms assigned to each experimental formulation. Then, PERMANOVA was carried out to test differences in feed stability, feed and caviar nutritional composition, ingestion rate and absorption efficiency (factor "Feed" fixed with five levels: Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD). PERMANOVA was also conducted to test differences in sea urchin somatic growth among feeds across times (factor "Feed" fixed with five levels: Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD; factor "Time" fixed with three levels T0, T1, T2), and caviar production obtained from reared sea urchins (factor Feed with 6 levels: Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD plus Wild sea urchins at T0). Analyses were conducted on untransformed data resembled with Euclidean distance.

3. Results

Feed-UL was different from all the lettuce-based feeds, which were similar to each other except for Feed-K that was different from Feed-M and Feed-AD. The macroalgae-based feed was characterized by a high carbohydrate content (about 55%) and a low protein and lipid content (about 8 and 1%, respectively), whereas lettuce-based feeds had more balanced nutritional compositions with a carbohydrate content of about 30–35%, a protein content of 25–26% and a lipid content of 4.5–6.3% (Table 2).

Results of the stability trial showed a 24 h-feed loss of 21.70 ± 1.29%, 28.39 ± 1.60%, 27.99 ± 2.94%, 29.26 ± 0.65% and 30.53 ± 0.44% for Feed-UL, Feed-S, Feed-K, Feed-M and Feed-AD respectively, with significant differences among Feed-UL and the other four lettuce based feeds (Table 3).

No mortality or disease were observed across the entire trial (1 month of maintenance and starvation period, 4 months of feeding experiment), and all sea urchins maintained the pit tag without

Table 2
Proximate composition (mg/g, dry weight) of the five experimental feeds. Algal feed with *Laminaria* and *Ulva* (Feed-UL), feed based on common lettuce discards with *Sardina pilchardus* meal (Feed-S), krill meal (Feed-K), mussel meal (Feed-M), and a meal obtained from *Engraulis encrasicolus* processing discards (Feed-AD). Different letters highlighted significant differences.

Proximate composition	Feed-UL[a]	Feed-S[b,c]	Feed-K[c]	Feed-M[b]	Feed-AD[b]
Proteins	8.66	25.20	26.41	25.54	26.04
Lipids	1.92	4.95	6.20	6.29	6.05
Ashes	34.06	34.50	32.99	37.23	37.18
Carbohydrates	55.35	35.32	34.38	30.94	30.73

Table 3
Univariate permutational analysis of variance (a, main test and; b, pair-wise tests) of feed stability of the five experimental feeds. Significant *p* values are highlighted in bold.

MAIN TEST					
Source of variation		**df**	**MS**	**Pseudo-F**	**P(perm)**
Feed		4	32.88	21.74	**0.006**
Residual		10	1.51		
PAIR-WISE TESTS					
Between feeds		**t**	**P(perm)**	**Unique perms**	**P(MC)**
Feed-UL *vs*	**Feed-S**	5.65	0.097	10	**0.013**
	Feed-K	6.11	0.106	10	**0.005**
	Feed-M	9.08	0.093	10	**0.003**
	Feed-AD	9.62	0.106	10	**0.003**
Feed-S *vs*	**Feed-K**	0.45	0.597	10	0.684
	Feed-M	0.86	0.476	10	0.473
	Feed-AD	1.16	0.319	10	0.267
Feed-K *vs*	**Feed-M**	0.26	0.786	10	0.807
	Feed-AD	0.54	0.614	10	0.633
Feed-M *vs*	**Feed-AD**	0.54	0.503	10	0.586

collateral effects. PERMANOVA analysis conducted on sea urchin sizes prior to the start of the feeding trial indicated no significant differences (MS= 15.2, Pseudo-$F_{(4,\ 67)}$= 0.06, P= 0.998) between the sea urchin pools assigned to each experimental feed.

Analysis of sea urchin ingestion rate (IR) and absorption efficiency (AE) showed significant differences among feeds (Table 4). Feed-S showed a higher IR, than Feed-UL, Feed-AD and Feed-K, which did not differ each other, while Feed-M showed the lowest value. AE peaked for the Feed-UL, followed by Feed-K and Feed-S, while Feed-M and Feed-AD showed similar and lower values (Fig. 2).

Specific growth rate showed similar values for all the experimental feeds (MS= 0.009, Pseudo-$F_{(4,79)}$= 0.338, P= 0.858), with averaged values of 0.16±0.08% for Feed-UL and 0.24±0.11% for Feed-K. Similarly, total wet weight did not show significant differences among feeds but only between sampling periods (Table 5a), with higher values at T2 than at both T1 and T0, which did not differ each other (Fig. 3a). Ovosomatic index (OI), on the contrary, showed a significant increase with time and differed among feeds (Table 5b). Feed-UL led to lower OI (12.82±8.87%) than Feed-M and Feed-K (21.09±10.59%, 20.69 ±10.34%, respectively), but similar to Feed-S (16.56±11.15%) and Feed-AD (19.73±11.04%) (Fig. 3b), which in turn did not differ with Feed-K and Feed-M.

Analysis on nutritional content of sea urchin caviar showed that Caviar-UL, obtained from sea urchins fed with Feed-UL, was different from all the other caviars (Table 6). In particular, Caviar-UL showed a lower content of proteins and fatty acids, with the exception of total essential fatty acids (EFA) and ω6 polyunsaturated fatty acids (ω6-PUFA). Differently, sea urchins fed with lettuce-based feeds produced caviars with similar nutritional content, except Caviar-AD that was similar only to Caviar-M.

Results of color assessment showed that sea urchins fed with Feed-UL produced 10% dark orange (DO), 30% cream orange (CO) and 60% paler orange (PO) caviar (Fig. 4). Sea urchins fed with Feed-S showed the best performance presenting 100% bright orange (BO) caviar, followed by Feed-M (90% BO and 10% PO). Feed-K and Feed-AD gave the same result with 50, 40 and 10% of caviar characterized by PO, BO and YO color respectively.

4. Discussion

Effectiveness of five experimental feeds for the Mediterranean purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) was assessed through the recently developed Raking method (Rakaj et al., under review), a highly sustainable productive approach for testing feeds without sacrificing sea urchin breeding batches.

Food stability in seawater, i.e. the ability of pellets to remain stable enough time to allow animal feeding, is a crucial issue in the evaluation of experimental feeds, especially for species such as sea urchins that take even 2–3 days to eat the provided feed (Pearce et al., 2002b). The results of stability in seawater highlighted a better performance for Feed-UL, with about 21% of feed loss in 24 h, rather than the lettuce based feeds, which presented about 29% of feed loss. These results were similar with a previous study in which agar was used as binding agent for a feed based on food discards (Ciriminna et al., 2020) and were also in accordance with the good performance of agar used with terrestrial vegetables (Raposo et al., 2019; Zupo et al., 2018). Although Feed-UL showed the best stability in water, for all the experimental

Table 4
Univariate permutational analysis of variance (Main test and Pair-wise tests) of ingestion rate IR (a) and absorption efficiency AE (b) in *Paracentrotus lividus* fed with the five experimental feeds. Significant *p* values are highlighted in bold.

MAIN TEST		**a) IR**			**b) AE**		
Source of variation	**df**	**MS**	**Pseudo-F**	**P(perm)**	**MS**	**Pseudo-F**	**P(perm)**
Feed	4	0.017	9.00	**0.001**	946.010	71.58	**0.001**
Residual	40	0.002			13.215		
PAIR-WISE TESTS		**t**	***P*(perm)**	**Unique perms**	**t**	***P*(perm)**	**Unique perms**
Feed-UL *vs* Feed-S		2.15	**0.046**	969	7.33	**0.001**	975
Feed-UL *vs* Feed-K		0.83	0.402	980	4.33	**0.003**	977
Feed-UL *vs* Feed-M		3.36	**0.005**	974	12.47	**0.001**	978
Feed-UL *vs* Feed-AD		0.43	0.667	982	12.81	**0.001**	975
Feed-S *vs* Feed-K		3.17	**0.005**	981	2.71	**0.013**	976
Feed-S *vs* Feed-M		6.11	**0.001**	968	7.26	**0.001**	982
Feed-S *vs* Feed-AD		3.07	**0.009**	976	7.90	**0.001**	977
Feed-K *vs* Feed-M		2.65	**0.016**	984	8.33	**0.001**	980
Feed-K *vs* Feed-AD		0.54	0.576	985	8.93	**0.001**	980
Feed-M *vs* Feed-AD		3.70	**0.002**	974	1.92	0.079	977

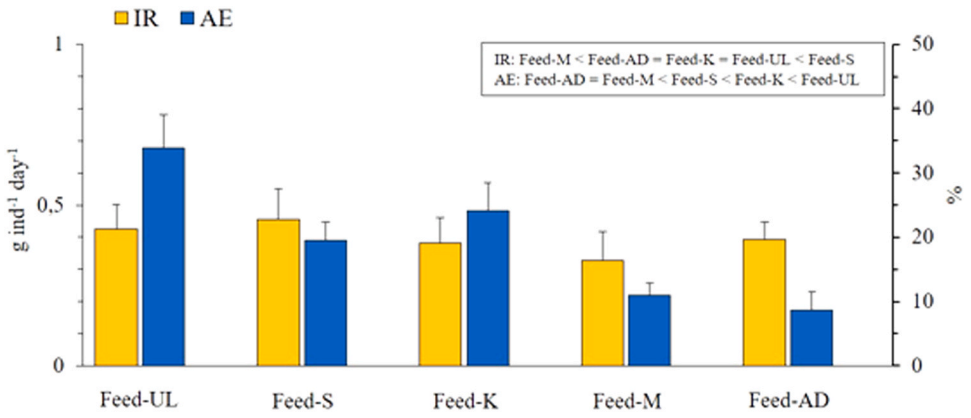

Fig. 2. Ingestion rate (IR, mean ± standard deviation) and absorption efficiency (AE, mean ± standard deviation) of *Paracentrotus lividus* fed with the five experimental feeds. In the box, significant differences between feeds are indicated.

Table 5
Results of permutational analysis of variance (Main test and Pair-wise tests). a) sea urchin total weight was tested for the factors Feed (5 levels: Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD), Time (T0, T1, T2) and their interaction; b) ovosomatic index obtained at the end of the experiment (T2) was tested for the factor Feed (6 levels: Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD, plus wild sea urchins at T0). Significant *p* values are highlighted in bold.

a) Total weight				
MAIN TEST				
Source of variation	**df**	**MS**	**Pseudo-F**	**P(perm)**
Feed x Time	4	217.31	0.72	0.564
Time	2	6720.8	22.43	**0.001**
Feed x Time	8	61.91	0.21	0.992
Residual	201	299.52		
PAIR-WISE TESTS		**t**	**P(perm)**	**Unique perms**
T0 vs T1		0.96	0.366	998
T0 vs T2		5.94	**0.001**	998
T1 vs T2		5.10	**0.001**	998
b) Ovosomatic Index				
MAIN TEST				
Source of variation	**df**	**MS**	**Pseudo-F**	**P(perm)**
Feed	5	691.08	7.71	**0.001**
Residual	82	89.61		
PAIR-WISE TESTS		**t**	**P(perm)**	**Unique perms**
Wild *vs* Feed-UL		4.00	**0.001**	996
Wild *vs* Feed-S		4.53	**0.001**	997
Wild *vs* Feed-K		6.44	**0.001**	997
Wild *vs* Feed-M		6.44	**0.001**	997
Wild *vs* Feed-AD		5.71	**0.001**	996
Feed-UL *vs* Feed-S		1.01	0.345	997
Feed-UL *vs* Feed-K		2.25	**0.026**	995
Feed-UL *vs* Feed-M		2.23	**0.028**	997
Feed-UL *vs* Feed-AD		1.89	0.072	998
Feed-S *vs* Feed-K		1.08	0.271	997
Feed-S *vs* Feed-M		1.03	0.303	998
Feed-S *vs* Feed-AD		0.76	0.456	998
Feed-K *vs* Feed-M		0.10	0.925	995
Feed-K *vs* Feed-AD		0.33	0.736	995
Feed-M *vs* Feed-AD		0.24	0.808	995

formulations food loss between consequent daily feed provisions were always lower than 30%, confirming pellet efficacy for feeding sea urchins in controlled conditions. Sea urchins consumed food with very low rates and thus pellets are expected to remain for long times in the water with consequent nutrient loss, water soaking and low efficiency (Fabbrocini et al., 2015; Pearce et al., 2002b).

4.1. Metabolic rates

Sea urchin growth performance is the resultant of physiological processes (ingestion, digestion, absorption, assimilation and egestion), which in turn are strongly influenced by the adequacy and quality of the feeds administered (Lawrence, 2013). Therefore, metabolic rates, offspring quality and biomass production are excellent measures of the relative suitability of a given food source.

Although sea urchins are primarily herbivorous, they are able to consume a great variety of food (Noè et al., 2018), and production is strongly influenced by both ingestion and absorption of consumed food (Lawrence, 2013). Ingestion rate (IR) was rather low for all the experimental feeds (0.30–0.45 g $ind^{-1}day^{-1}$), although with some differences among formulations, suggesting that all the five tested feeds were nutritionally adequate for *P. lividus*. In accordance with the compensatory model, food ingestion is strictly related to food availability and quality, and sea urchins usually show higher ingestion rates for food with low nutrient content, than for high-energy foods (Boudouresque and Verlaque, 2020; Fernandez and Boudouresque, 2000; Hammer et al., 2004). Findings were similar with those presented by Grosso et al., (2021) testing diets composed of fishmeal mixed with maize and carrots, highlighting the palatability of vegetable discards for *P. lividus*. Also, Ruocco et al. (2018) found higher values of IR for *P. lividus* fed on macroalgae (*Ulva rigida*) or seagrass (*Posidonia oceanica*) than on artificial pellets, and Fernandez and Boudouresque (2000) measured lower values for *P. lividus* fed on a mixed diet composed of vegetal ad animal ingredients. Accordingly, Raposo et al. (2019) observed a lower IR for sea urchins fed with a diet composed of terrestrial vegetables but characterized by a high lipid content, than a diet composed only of macroalgae. However, in the present experiment the differences highlighted among feeds suggest that the different origin of the main ingredients did not have a key role in feed palatability. In fact, *P.lividus* fed on the algal feed showed similar IR values as two feeds composed mainly by *Lactuca sativa* outermost leaves.

Absorption efficiency (AE) is very sensitive to the digestibility of the main feed ingredient (Cuesta-Gomez and Sánchez-Saavedra, 2018), giving insights on the nutritional quality of the administrated feed. However, it may be affected by several factors, including sea urchin age and environmental conditions (Lawrence et al., 2020). Differently to IR, AE showed a marked difference between the feeds. The algal feed resulted more efficient than the lettuce-based feeds in accordance with other studies (Cyrus et al., 2015; Shpigel et al., 2005). Sea urchins are commonly considered herbivorous, able to transform communities dominated by macroalgae into barren areas due to their intense grazing activity. Thus algae-based feeds are usually considered appropriate as similar to wild food sources (Grosso et al., 2022; Lawrence, 2013). In contrast, the four lettuce-based feeds were less effective than macroalgae, despite the appropriate nutritional quality. Proximate composition of the experimental feeds showed that the lettuce-based feeds were characterized by a higher protein (25–27%) and lipid (4–6%) content than algae feed (about 8% and 2% respectively), which instead was

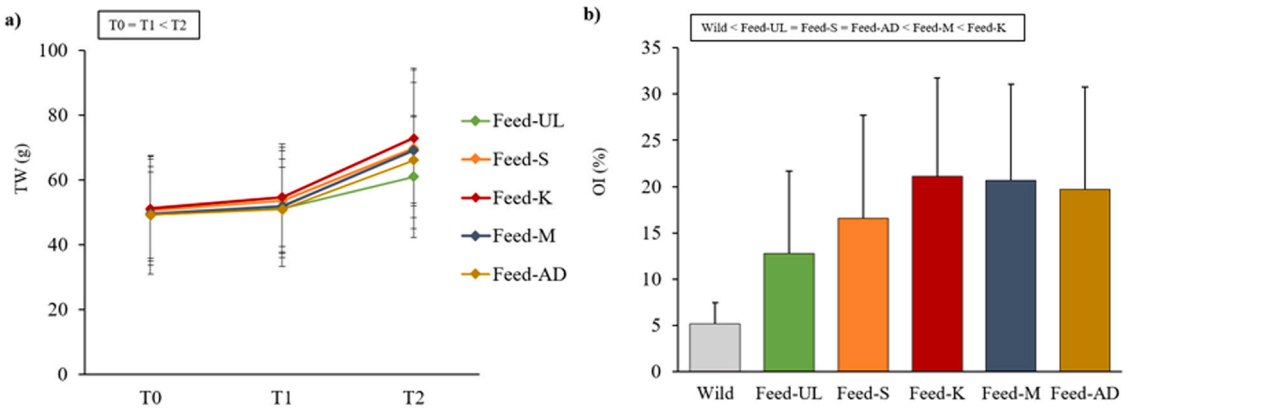

Fig. 3. a) Total wet weight (TW, mean ± standard deviation) of *Paracentrotus lividus* fed with the five experimental feeds across experimental times (T0, T1 and T2). In the box, significant differences between times are indicated. b) Ovosomatic index (OI, mean ± standard deviation) of sea urchin at the start of the experiment (Wild) and after the feeding experiment with the five experimental feeds (Feed-UL, Feed-S, Feed-K, Feed-M and Feed-AD). In the box, significant differences between feeds are indicated.

Table 6
Nutritional content (proteins and lipids, %) of caviar (Caviar-UL, Caviar-S, Caviar-K, Caviar-M, Caviar-AD) obtained from sea urchins fed with the five experimental feeds (Feed-UL, Feed-S, Feed-K, Feed-M, Feed-AD respectively). Fatty acids (mg/g) considered biomarkers of nutritional quality for human consumption were selected: α-Linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), essential fatty acids (EFA), ω3- and ω6-polyunsaturated fatty acids (ω3-PUFA, ω6-PUFA), ω3-highly unsaturated fatty acids (ω3-HUFA). Atherogenicity index (AI), thrombogenicity index (TI,) and hypocholesterolemic/hypercholesterolemic ratios (HH). Different letters indicate significant differences.

	Caviar-UL[a]	Caviar-S[b]	Caviar-K[b]	Caviar-M[b,c]	Caviar-AD[c]
Proteins	45.44 ± 3.16	50.91 ± 2.13	52.14 ± 6.63	48.64 ± 3.21	51.59 ± 3.68
Lipids	25.29 ± 4.27	20.29 ± 3.81	18.68 ± 3.87	19.02 ± 3.77	19.43 ± 4.33
FA					
18:3n3 (ALA)	7.39 ± 0.83	20.14 ± 6.47	19.40 ± 2.04	20.66 ± 5.84	22.15 ± 3.36
20:5n3 (EPA)	12.61 ± 2.31	13.69 ± 2.54	12.93 ± 2.41	11.24 ± 2.19	11.05 ± 2.11
22:6n3 (DHA)	0.19 ± 0.29	3.63 ± 0.96	3.86 ± 1.11	1.35 ± 0.34	1.91 ± 0.28
Σ EFA	33.64 ± 6.57	25.45 ± 4.18	22.61 ± 4.93	20.24 ± 4.17	23.04 ± 5.80
Σ ω3-HUFA	18.62 ± 2.93	19.71 ± 3.90	18.40 ± 3.52	14.07 ± 2.68	14.83 ± 2.65
Σ ω3-PUFA	29.64 ± 4.01	47.27 ± 11.13	45.65 ± 6.48	41.42 ± 9.58	43.50 ± 6.12
Σ ω6-PUFA	36.80 ± 7.06	25.06 ± 3.96	23.54 ± 5.18	25.45 ± 5.65	30.74 ± 6.31
ω3/ω6	0.81 ± 0.08	1.87 ± 0.14	1.97 ± 0.19	1.63 ± 0.18	1.43 ± 0.10
AI	2.08 ± 0.08	2.66 ± 0.42	2.77 ± 0.18	3.00 ± 0.41	2.65 ± 0.14
TI	0.57 ± 0.02	0.48 ± 0.12	0.45 ± 0.03	0.43 ± 0.08	0.04 ± 0.03
HH	0.20 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.02	0.13 ± 0.10

characterized by a higher content of carbohydrates (> 50%). Feed nutritional composition is usually considered the main driver of food consumption for sea urchins, and in particular, protein intake plays a key role (Heflin et al., 2016; Grosso et al., 2022). However, food efficacy is related not only to food consumption, but also to assimilation, which in turn depends on its digestibility (Boudouresque and Verlaque, 2020; Cuesta-Gomez and Sánchez-Saavedra, 2018), thus the differences highlighted in AE among feeds may be related to the different origin and

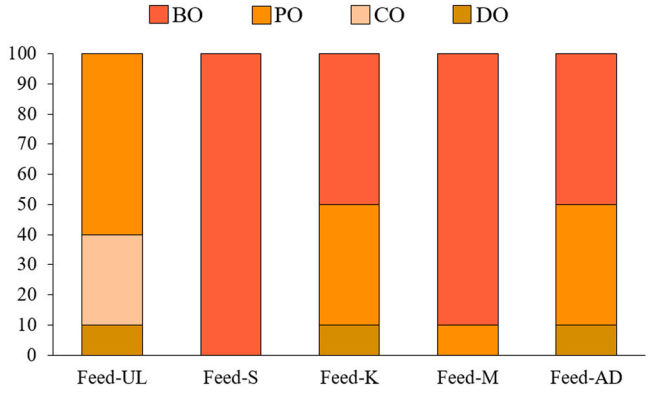

Fig. 4. Relative frequency of the oocyte color categories (BO, bright orange, PO, pale orange, CO cream orange, DO, dark orange) produced by female of *Paracentrotus lividus* fed with the five experimental feeds.

composition of the main ingredients. Terrestrial vegetables, and in particular *Lactuca sativa* leaves, are characterized by a high content of fibers and insoluble carbohydrates (Kim et al., 2016), which may not be easily digested by sea urchins (Marsh et al., 2013). In addition, the inclusion of *Lithothamnium calcareum* as a source of inorganic carbon and other minerals (Aslam et al., 2010) may have contributed to the low efficiency of the sustainable feeds, in particular, for feeds containing mussel shells and anchovy bones, which increase the amount of not digestible matter. As also confirmed by the higher faeces production by sea urchin fed with these two experimental feeds.

4.2. Somatic growth and caviar production

Despite the differences in IR and AE, results of somatic growth showed a similar performance for all the experimental feeds. All feeds were able to promote significantly higher final total weights (TW) values than initial ones, confirming the suitability of administrated feeds for *P. lividus*. Specific growth rates ranged between 0.16% and 0.24%$^{-day}$, in accordance with results obtained in other studies with adult sea urchins (Grosso et al., 2021). Finally, in adult sea urchins, the best measure of sea urchin's well-being is the offspring production (Vadas, 1977). In this study, feed performance was assessed through caviar production expressed as ovosomatic index (OI) instead of the traditional gonadsomatic index (GI). These indices can be considered comparable since oocytes and gonads differ mainly in composition (different ratio of nutritive:reproductive cells) rather than in biomass (Byrne, 1990; Marsh

et al., 2013; Walker et al., 2015). After four months of controlled feeding, all experimental sea urchins showed a marked increase in caviar production, in line with the positive results of TW increase but highlighting differences among the five experimental feeds. Starting from the initial value of 5%, the lowest values were recorded for algal feed (12%) while the four lettuce-based feeds underwent a higher increase (between 16% and 21%), confirming the efficacy of vegetable discards on *P. lividus* gonad growth already reported by Vizzini et al. (2019), (2014). Of the four lettuce-based feeds, Feed-K (krill addition) had the highest OI value (approximately 21%), followed by Feed-M (mussel addition) (about 20%), Feed-AD (anchovy discard addition) (about 19%) and finally Feed-S (fish addition) (about 16%). In particular, results of common anchovy discards confirm findings obtained in a previous study on *P. lividus* gonad enhancement (Ciriminna et al., 2021). Sartori and Gaion (2015) and Santos et al. (2019) presented encouraging but lower increase in *P. lividus* gonad production using maize and spinach (GI of about 13% and 9% respectively). Similarly, Luo et al. (2014) tested banana peel in *S. intermedius* with a final GI of about 8%, while Vizzini et al. (2014), (2018) found gonad growth in both juvenile and adult *P. lividus* fed with only outermost leaves of *L. sativa*, but not when fed with cauliflower (*Brassica oleracea*) or beet (*Beta vulgaris*) discards. Among the four lettuce-based feeds, Feed-K presented the higher value of OI (about 21%), in accordance with results obtained from IR and AE measurements, i.e. a low amount of ingested food coupled with the best absorption efficiency between the lettuce-based feeds. Similarly, despite the low AE, both Feed-M and Feed-AD presented a marked increase in TW e OI, suggesting their feasibility for gonad growth. Finally, Feed-S, which presented the highest IR coupled with about 20% of AE gave positive results but was the worst between the four sustainable feeds. Findings showed that the four lettuce-based feeds were effective for promoting caviar production, leading to higher OI value than the algae-based feed. Better results of mixed diets compared to fully vegetal ones were already observed (Fernandez and Boudouresque, 2000; Grosso et al., 2021; 2022; Shpigel et al., 2005), probably thanks to the high nutritional quality of animal meal. Although no specific analyses were carried out in this case, there is evidence from the literature that lipids from seafood meal are characterized by a large amount of essential fatty acids (EFA), which sea urchins usually synthesize through their metabolic pattern (Monroig and Kabeya, 2018). Krill, fish, mussel and fish discards meal (Ciriminna et al., 2020; Ezgeta-Balić et al., 2012; Hagen et al., 2001; Šimat et al., 2020) are all characterized by a high content of EFA, allowing sea urchin to obtain EFA also from feed and limiting energy consumption for their production. Results on feed proximate composition showed that lettuce-based formulations had a protein (25–27%) and lipid (4–6%) content suitable and adequate for sea urchin gonad growth (Baião et al., 2019, Lourenço et al., 2020) in spite of the low proportion (about 8%) of animal meal. Grosso et al. (2022) tested mixed diets on adult *P. lividus* obtaining GI values of about 13 and 9% using 20 and 40% of animal meal. Similarly, Prato et al. (2018) obtained about 10% of GI feeding *P. lividus* with a diet based on krill (30%). The mix of lettuce discards and only 8% animal meal therefore appears to be a satisfactory trade-off between productivity and sustainability, resulting a better alternative also to macroalgae-based feeds. In fact, although some algae can be cultivated by limiting the dependence from natural resources, the use of food processing discards is a preferable ingredient as it allows the re-use of nutrient-rich organic matter and facilitates the management of the organic waste produced by the food industry. Finally, the introduction of *L. calcareum* in the experimental feeds as a source of inorganic carbon and other minerals may have a positive effect in sea urchin oocyte production. Minerals are involved in physiological processes, in particular in test production (Ebert, 2013), and were usually extracted from algae and seawater with energy consumption (Hermans et al., 2010). Despite the effect of dietary mineral content has not been thoroughly investigated, it was observed an increase in somatic growth of juvenile *Stongylocentrotus droebachiensis* when fed with a mineral-enriched diet (Kennedy et al., 2007), while Cirino et al. (2017) found a positive effect on *P. lividus* reproductive cycle management adding calcium carbonate ($CaCO_3$) in the prepared food.

Sea urchin gonads, besides being appreciated for their taste, are considered an excellent nutritional source, being rich in protein and low in fat. Moreover, they are characterized by high amounts of ω3- and essential fatty acids (EFA), molecules that bring several benefits to human health (Glick and Fischer, 2013; Wulandari and Warsito, 2022). All produced sea urchin caviar showed high protein content and a low relative concentration of lipids, a nutritional profile comparable to the gonad composition reported in literature (e.g. Lourenço et al., 2021; Prato et al., 2018; Wulandari and Warsito, 2022). The results were in accordance with expectations, as the nutrients accumulated in the gonads are progressively transferred to the oocytes to support the early stages of larval development, until feeding competence is achieved (Byrne et al., 2008). Analysis of fatty acids showed a high content of α-Linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and EFA, molecules that humans need to assume directly by diets (Kaur et al., 2014). The ω3/ω6 ratio was always higher than 1, which is the recommended value for a healthy diet (Simopoulos, 2002), while PUFA/SFA was always higher than 0.45, as recommended by the Department of Health (1994). Similarly, lipid quality indices, which depend on the relative proportions of specific single saturated and unsaturated fatty acids, confirmed the good quality of sea urchin caviar. The lower the values of these indices, the greater the benefits for human health in terms of cardiovascular disease prevention (Stanek et al., 2011). All obtained caviar was therefore qualitatively suitable for human consumption, although by comparing the nutritional profiles, statistical analysis highlighted some differences, confirming the influence of the diet on the reared sea urchins. In particular, the Caviar-UL was characterized by the lowest amount of proteins, ALA, DHA, ω3-HUFA and ω3-PUFA, resulting the caviar with the poorest nutritional content. Similarly, as regards caviar color, Feed-UL promoted the lowest quality colouration, with the production of pale orange and cream orange caviar, consistent with literature (Pearce et al., 2004). On the contrary, all the four lettuce-based feeds showed a better performance with Feed-S resulting the most efficient. The color in gonads is one of the most important features, as visible at a first sight influencing the visual assessment (Cuesta-Gomez and Sánchez-Saavedra, 2018). In sea urchins, gonad color is related to carotenoid dietary intake and accumulation, in particular the amount of β-carotene plays a fundamental role being an echinenone precursor, the most representative carotenoid in sea urchin gonads (Symonds et al., 2009). As observed by Kim et al. (2016) lettuce leaves have high content of β-carotene, thus the positive performance of the four lettuce-based feeds could be influenced by feed carotenoid content, as already found by Vizzini et al. (2014).

5. Conclusion

Findings of the present study showed that sustainable feeds, composed mainly by *L. sativa* discards (outermost leaves), were efficient as feeds for *P. lividus*. All the four tested feeds resulted characterized by an adequate nutritional composition, with a proper content of proteins, lipids and carbohydrates. Feed assimilation promoted an increase in total weight and caviar production. Finally, sea urchin caviar showed a suitable nutritional content for human consumption and an adequate colouration, important as quality feature. In addition, all the four sustainable feeds resulted more effective than macroalgae feed in caviar production and quality. These results confirm the suitability of food processing discards as ingredients for aquaculture feed production, as an alternative to more expensive and less sustainable sources, in accordance with the principles of circular economy.

Funding

This work was funded by the PON Project (CUP:

E86C18001440008), the INSAIL Project (CUP: B66C18000480005) both funded by the Italian Ministry of Education, University, and Research.

CRediT authorship contribution statement

Arnold Rakaj: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Luca Grosso:** Investigation, Writing – review & editing. **Davide Pensa:** Investigation, Writing – review & editing. **Alessandra Fianchini:** Supervision, Writing – review & editing. **Antonio Mazzola:** Conceptualization, Supervision, Writing – review & editing. **Salvatrice Vizzini:** Conceptualization, Supervision, Validation, Writing – review & editing. **Laura Ciriminna:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, 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

Data will be made available on request.

Acknowledgments

The authors are grateful to Francesca Perinelli and Massimiliano Graziani for their assistance in the laboratory work, and the fish company Balistreri Girolamo & C.snc, Aspra (PA), Italy, for collaborating in the project providing *Engraulis encrasicolus* discards

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102017.

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