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ORIGINAL ARTICLE

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Formulation of a new sustainable feed from food industry discards for rearing the purple sea urchin *Paracentrotus lividus*

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Abstract

The lack of suitable feeds for echinoculture has led to use natural resources already widely exploited by human activities. To move towards a higher sustainability of echinoculture, this study proposes a sustainable feed for *Paracentrotus lividus*. Two experimental formulations were obtained using discarded endive (*Cichorium endivia*) leaves and anchovy (*Engraulis encrasicolus*) industry discards in different proportions, and agar as a binder. The evaluation of the feed stability showed that the feed was stable for 72 hr, allowing a suitable feeding for sea urchins. Both formulations showed a proper nutritional value and fatty acid profile, corresponding to the features of the main ingredients and resulting suitable for echinoculture. A bioenergetic trial was carried out to measure daily ingestion rate, absorption efficiency and gonado-somatic index in sea urchins. They resulted also palatable and well absorbed by sea urchins, especially that one with higher fish content. At the end of the experiment, an increase in gonado-somatic index was also recorded. Despite further analysis is needed to assess the performance of the feed in terms of gonad yield and quality, these encouraging results indicate that food industry discards may be suitable alternative ingredients for the production of sustainable feeds for sea urchin aquaculture.

KEYWORDS
aquaculture, blue economy, echinoculture, fatty acids, feedstuff, sustainability

1 | INTRODUCTION

The edible sea urchin *Paracentrotus lividus* is the most commercially exploited echinoid in Europe (Baião et al., 2019). It is a widespread species along the North Atlantic Ocean and the Mediterranean Sea (Boudouresque & Verlaque, 2007), and the gonads, commonly called roe, are considered a delicacy in many countries worldwide. In the last decades, the increment of its demand has resulted in the overexploitation of natural populations and the consequent collapse of stocks (Gianguzza et al., 2006; Pais, Serra, Meloni, Saba, & Ceccherelli, 2011). Aquaculture is recognized as a possible solution to mitigate harvesting pressure on wild sea urchins. Therefore, many studies have dealt with feeding strategies and diet formulation for optimizing gonad yield and quality (e.g. Cook & Kelly, 2007b; Gibbs, Watts, Lawrence, & Lawrence, 2009; Pearce, Daggett, & Robinson, 2002) and to fill the gap between the growing market request and the natural supply (Carboni, Hughes, Atack, Tocher, & Migaud, 2015). However, so far, one of the main bottlenecks of echinoculture is the lack of an effective and sustainable diet, able to increase gonad production while keeping good nutritional and organoleptic features.

The use of macroalgae in the diet of reared sea urchins has been widely explored (Carrier, Eddy, & Redmond, 2017), as sea urchins are predominantly herbivores and grazers on macroalgae (Boudouresque & Verlaque, 2007). Nevertheless, the use of macroalgae is unlikely to be commercially viable for large-scale culture, since their availability varies throughout the year and transport and storage costs are very high. In addition, their nutritional value and edibility are strongly influenced by the season and sampling site (Cook & Kelly, 2007a; Vadas, Beal, Dowling, & Fegley, 2000). Other ingredients, such as wheat, soybean meals or microalgae, have been added as a partial replacement of macroalgae, and the effect on somatic and gonadic growth (Pearce, Daggett, & Robinson, 2002a, 2004; Pearce et al., 2002b; Woods, James, Moss, Wright, & Siikavuopio, 2008), organoleptic characteristics (Robinson, Castell, & Kennedy, 2002; Suckling, Symonds, Kelly, & Young, 2011) and biochemical composition (Carboni, Hughes, Atack, Tocher, & Migaud, 2013; Liyana-Pathirana, Shahidi, & Whittick, 2002) of the roe was evaluated. Thanks to the promising results of some of these studies, the exploitation of constantly available land-based vegetables is nowadays a better option for formulating aquaculture feeds, due to the reduction in the use of natural marine resources generally included in sea urchin diets. Nevertheless, if the use of proteins and lipids derived from terrestrial plants is widespread in fish aquaculture (Gatlin et al., 2007; Torstensen et al., 2008), that is not the case in sea urchin aquaculture. Sartori and Gaion (2015) evaluated the effect of a diet composed of a mixture of *Maize kernel* and *Spinacia oleracea* on reared *P. lividus*, highlighting good feed ingestion rates and significant increases in the gonado-somatic index. Other studies evaluated the exploitation of fresh agricultural discards as a diet for *P. lividus*, alone [*Beta vulgaris*, *Brassica oleracea*, and *Lactuca sativa* in Vizzini, Miccichè, Vaccaro, and Mazzola (2015) and Vizzini, Visconti, Vaccaro, and Mazzola (2017)] or mixed with egg white and a little amount of commercial fish feed (Vizzini, Visconti, Signa, Romano, & Mazzola, 2019), and reported encouraging results in terms of gonad yield and organoleptic and nutritional features of the roe. More recently, also Raposo et al. (2019), by studying both gonad growth and fatty acid profile of sea urchins fed with terrestrial vegetables, encouraged the use of vegetables instead of cropped macroalgae or commercial feeds.

In this context, this study proposes a sustainable feed for sea urchins, mainly based on discards from the food industry. These discards, which are commonly treated as waste to be disposed, with management costs and environmental impact, could have instead the potential to be recycled as raw materials for the production of formulated feeds, in accordance with the principles of the circular economy. Two feed formulations with different percentages of vegetable and animal discards were tested to assess their feasibility for feeding *P. lividus* in rearing conditions. Feed stability in seawater and both palatability and assimilability of the new sustainable feed for *P. lividus* were tested. A preliminary assessment of the effect of the new feed on gonad growth was also carried out by estimating the gonado-somatic index. The nutritional composition and quality of both ingredients and feed were also assessed through the study of the proximate composition and fatty acid profiles. Indeed, a proper provision of dietary proteins, lipids and fatty acids, such as essential and polyunsaturated fatty acids, especially the *omega*-3 class, is crucial to improve the growth of reared organisms, obtaining also roe of good quality (Carboni et al., 2015; Castell et al., 2004; González-Durán, Castell, Robinson, & Blair, 2008).

2 | MATERIALS AND METHODS

2.1 | Feed formulation

Outermost leaves of *Cichorium endivia* (endive), obtained from unprocessed agricultural discards, and industry discards of *Engraulis encrasicolus* (European anchovy), composed mainly by viscera, head, skin and bones, were used as the main ingredients for producing a new sustainable feed for echinoculture. Endive and anchovy discards were freeze-dried and then ground to fine powder. Two formulations were prepared differing for the percentages of the main ingredients: endive leaves and anchovy discards contributed about 60% and 40% (60/40 formulation) and 80% and 20% (80/20 formulation) to the [6] two feed formulations (Table 1). Agar (Agar-Agar fine powder 100% Food Grade, Intra Laboratories, UK), a non-branched polysaccharide extracted from red algae, was dissolved in boiling Milli-Q distilled water (385 g/L) and mixed until a homogeneous jelly-like solution was obtained. Then, it was allowed to cool to about 60°C and added in different percentage (2.5% and 5%) to both feed formulations, and mixtures were stirred and manually converted into bar-shaped feeds (0.5 cm diameter, 2 cm length, ~1 g wet weight) using a 35-ml syringe. The feed bars were air-dried for 24 hr at room temperature (24°C) and then stored at −20°C until further use and analysis.

2.2 | Stability trial

All the formulations (two feed formulations at two different agar percentages) were tested for stability in seawater, hypothesizing a different stability according to the agar amount. Before the stability trial, six feed bars of each formulation were weighed (WW), oven-dried at 60°C for 48 hr to constant weight and weighed again (DW) to assess the standard dry weight (DW_S % = DW/WW × 100) of each feed formulation.

Afterwards, other six feed bars of each formulation were weighed (WW_I) and put individually inside PVC cylindrical cages (20 cm height and 12 cm diameter) closed on both sides with a nylon net (mesh size 500 µm) and fixed in pairs under the water surface in 80-L tanks (Figure 1a). Environmental conditions were kept stable throughout the stability trial, in terms of seawater temperature: 20.0 ± 1.0°C, salinity: 38.0 ± 0.5 g/kg, photoperiod: 8-hr

TABLE 1 Percentage composition (%) of the two feed formulations, 60/40 and 80/20, with two different agar content (A2.5 = 2.5%, A5 = 5%)

	Feed formulation			
	60/40		80/20	
Ingredient	A2.5 (%)	A5 (%)	A2.5 (%)	A5 (%)
Cichorium endivia	58.8	57.5	78.8	77.5
Engraulis encrasiculos	38.8	37.5	18.8	17.5
Agar	2.5	5.0	2.5	5.0

light and 16-hr dark, and continuous water flow in/out: 5 L/min. At three different times: T1 (24 hr), T2 (48 hr) and T3 (72 hr), two bars of each formulation were randomly collected, oven-dried at 60°C for 48 hr and weighed to assess the final dry weight (DW_F). Feed stability of each formulation was expressed based on the dry weight loss (DW_L) of the feeds at the end of the stability trial, as follows:

$$DW_L\,(\%) = \left[\left(DW_I - DW_F\right)/DW_I\right] \times 100.$$

where DW_I is the dry weight of each feed bar provided, calculated based on the standard dry weight, as follows: DW_I (mg) = ($WW_I \times DW_S$%)/100).

The results of the stability test showed that the agar amount did not affect significantly the feed stability over time (see Section 3), and hence, considering the economic advantages and sustainability of using a lower binder quantity, the feed formulations with the lower amount of agar (2.5%) were selected for the further steps.

2.3 | Proximate composition and fatty acids analysis

The main ingredients, that is discarded outermost leaves of *C. endivia* (endive) and industry discards of *E. encrasicolus* (European anchovy), and the two selected feed formulations with 2.5% agar, 60/40 and 80/20, were freeze-dried, ground and analysed in triplicate. Ash content was determined by combustion in a muffle furnace at 550°C for 4 hr according to Nielsen (2010), and crude protein content was estimated by the Kjeldahl method, with nitrogen to protein conversion factor of 6.25 (Horowitz & Latimer, 2006). Carbohydrate content was also estimated, according to Baião et al. (2019) as follows:

$$\text{Carbohydrates} = 100 - (\text{lipid} + \text{protein} + \text{ash}).$$

A modified version of the Bligh and Dyer (1959) method was applied to measure lipids and fatty acids (FA). Lipids were extracted using a Milli-Q distilled water: methanol: chloroform mixture (1:2:1 v:v:v) with 0.01% BHT (butylated hydroxyl toluene) to avoid lipid oxidation. Samples were then sonicated to improve lipid extraction and centrifuged twice to separate the lipid phase from the aqueous phase. The lipid extracts were evaporated to dryness under gentle nitrogen stream and weighed, and the lipid content was expressed as mg/g dw of dry sample and as percentage. Therefore, lipids were resuspended in n-hexane and subjected to acid-catalysed 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 acid methyl ester (ME C25:0) was used as internal standard for quantification. FA data were expressed as mg/g of dry sample.

2.4 | Bioenergetic trial

Twenty-four *P. lividus* specimens (Test Diameter: 3.7 ± 0.2 cm, Total Wet Weight 23.4 ± 4.1 g) were collected from natural environment and randomly divided into two 80 L tanks. After a starvation period of two weeks, during which sea urchins were kept fasting, six specimens from each tank were randomly collected, sacrificed and wet-weighed, and their gonads were removed and wet-weighed.

The remaining twelve specimens were used for a two-week bioenergetic trial in an indoor tank system made of two groups

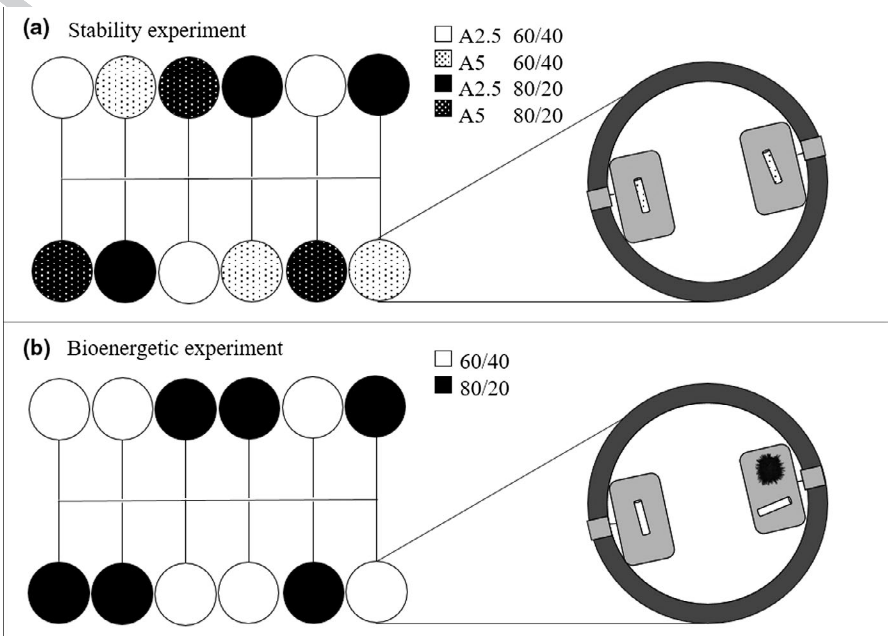


FIGURE 1 Indoor tank system used for the feed stability experiment (a) and the bioenergetic experiment (b). The detail of each tank is showed on the right side of each panel

of six tanks of 80 L, one group per each feed formulation (60/40 and 80/20). In each tank, two PVC cylindrical cages (20 cm height, 12 cm diameter) closed on both sides with a nylon net (mesh size 500 μm) were fixed under the water surface (Figure 1b). The remaining sea urchins were put individually in one of the two PVC cages per tank, while the other cage was left empty as a control treatment, aiming at calculating the feed loss. The same environmental conditions used in the previous stability trial were kept during both the starvation period and the bioenergetic trial. At the beginning of the experiment and every 48 hr (T0-T6), each sea urchin was fed with a known amount of the feed formulations (~1 g WW), and the same amount of feed was put in the correspondent control cage. Before feed provision (T1-T7), all the material contained within both treatment and control cages of each tank was carefully removed, oven-dried to constant weight (48 hr, 60°C) and reweighed. As far as the treatment cages, the collected material was previously separated in feed particles and sea urchin faeces, under a stereomicroscope.

The daily ingestion rate by sea urchins (IR), expressed as dry weight (mg/day), was calculated for each specimen at each sampling time (T1-T7), according to Fernandez and Boudouresque (1998) as follows:

$$\text{IR (mg/day)} = (\text{total provided biomass} - \text{total uneaten biomass})\ /2$$

where the total provided biomass is the dry weight of the feed provided (DW) and calculated from the standard dry weight (DW_S %), likewise the previous stability trial. The total uneaten biomass is given by the dry weight of the feed particles collected in the treatment cages and corrected based on the biomass lost from the control cages, and two are the days between each feed provision.

The absorption efficiency (AE) was calculated for each specimen at each sampling time as follows:

$$\text{AE (\%)} = \left[\frac{(\text{total biomass ingested} - \text{total faeces biomass})}{[\text{total biomass ingested}]}\right] \times 100$$

where total biomass ingested is equal to the following: total provided biomass – total uneaten biomass. At the end of the trial, sea urchins were sacrificed and weighed, and the gonads were extracted and wet-weighed. The gonado-somatic index (GSI) was calculated before the onset (T0), and at the end of the feeding treatment (T7) as follows:

$$\text{GSI (\%)} = [\text{gonad wet weight (g)}\ /\text{total wet weight (g)}] \times 100.$$

2.5 | Data elaboration and statistical analysis

Univariate permutational analysis of variance was used to test the differences in stability among feed formulations at different percentage of agar (factor Agar fixed with two levels: A2.5, A5; factor Feed fixed with two levels: 60/40 and 80/20) across time (factor Time fixed and orthogonal, with three levels: T1, T2, T3). The analysis was run on untransformed data resembled using Euclidean distance.

One-way multivariate permutational analysis of variance (PERMANOVA) was carried out to test the differences in fatty acid (FA) profiles between the two selected feed formulations with 2.5% agar (factor Feed fixed with two levels: 60/40, 80/20). PERMANOVA was carried out on FA data resembled using Euclidean distance after square root transformation. Principal coordinates analysis (PCO) was also run on the FA profiles of the feed formulations, in order to graphically highlight the differences found by PERMANOVA. The nutritional quality of the ingredients and the formulated feed was assessed through a semi-quantitative fatty acid approach: the patterns of the main classes of FA, together with those considered as important biomarkers of nutritional quality in aquaculture [i.e. arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), the sum of ω-3 and ω-6 polyunsaturated fatty acids (Σω-3-PUFA and Σω-6-PUFA), the ratio ω-3/ω-6, and the sum of ω-3 highly unsaturated fatty acids (Σω-3-HUFA), according to Gago, Luis, and Repolho (2009), Sargent, Bell, McEvoy, Tocher, and Estevez (1999) and Vizzini et al. (2019)] were assessed.

Difference in ingestion rate (IR) and absorption efficiency (AE) of the sea urchins fed with the two selected feed formulations across time was also tested using univariate permutational analysis of variance (factor feed fixed with two levels: 60/40, 80/20, factor time fixed and orthogonal, with seven levels: T1–T7). Difference in gonado-somatic index (GSI) between the onset and the end of the trial was also run using univariate permutational analysis of variance with both factors, feed and time, fixed and orthogonal, and both with two levels (Feed: 60/40, 80/20; Time: T0, T7). All the analyses were based on untransformed data resembled using Euclidean distance.

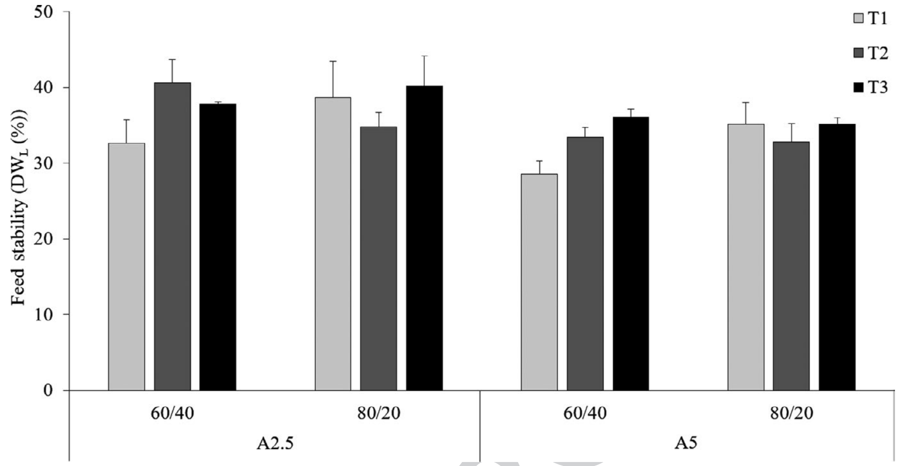
All the statistical analyses were performed using the software PRIMER 6 v6.1.10 & PERMANOVA + β20 (Plymouth, UK). When significant differences were found, pairwise tests were used as a posteriori check of significant effects. The Montecarlo test was also carried out to identify significant patterns when the numbers of permutation were <100.

3 | RESULTS

3.1 | Stability trial

The stability trial carried out on the two new feed formulations (60/40 and 80/20) manufactured with different percentages of agar (2.5% and 5%) revealed that the higher feed loss occurred in the first 24 hr of immersion in seawater and then was overall stable in the following times (48 and 72 hr) (Figure 2). The higher agar amount did not contribute to provide a higher stability to both formulations at all times (MS = 92.23, Pseudo-$F_{(1,12)}$ = 17.27, p = .057); indeed, while the interaction of the factors feed and time was significant (MS = 27.30, Pseudo-$F_{(4,12)}$ = 3.97, p = .036), pairwise tests, carried out to compare the two feed formulations at different agar amount over time, revealed only that the stability of the formulation A5 60/40 was significantly lower at T3 than at T1 (p < .05).

FIGURE 2 Feed stability expressed as dry weight loss (DW_L %, mean ± standard deviation) of the two feed formulations (60/40 and 80/20) prepared with a different agar amount (A2.5:2.5%, A5: 5.0%)



3.2 | Proximate composition and fatty acid analysis

Proximate composition and fatty acid (FA) profiles of the main ingredients, *C. endivia* and *E. encrasicolus* discard, and the two selected feed formulations, 60/40 and 80/20, are shown respectively in Tables 2 and 3. Fish industry discards showed higher lipid, protein and ash content than discarded endive leaves, while endive was richer in carbohydrates than fish discards. These differences were mirrored in the feed formulations: lipids, proteins and ash were more abundant in the formulation with the higher relative content of fish discards (60/40), and carbohydrates were more abundant in the formulation with the higher relative content of endive leaves (80/20) (Table 2).

As regards FAs, the two main ingredients showed very different profiles, being the outermost leaves of endive almost exclusively constituted by 18:3 n3 (α-linolenic acid, ALA), 18:2 n6 (linoleic acid, LA) and 16:0 (palmitic acid), and anchovy discards by a high abundance of essential fatty acids (EFA), namely arachidonic (ARA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (Table 3). As regards the feed formulations, a higher amount of all the three FA classes, saturated, mono- and polyunsaturated FA, characterized the formulation with a higher amount of animal ingredients (∑ SFA, ∑ MUFA, ∑ PUFA: 60/40 > 80/20). Looking through the biomarkers of nutritional quality, the sum of EFA and of ω-3 highly unsaturated FAs (∑ω-3-HUFA) were about twice in the 60/40 formulation, compared with the 80/20. Individual EFAs (i.e. ARA, EPA and DHA) were also higher in the 60/40 formulation, while ALA (18:3n3) and LA (18:2n6), both precursors of EFA (Baião et al., 2019; Castell et al., 2004), showed an opposite trend with a higher amount in the 80/20 formulation than in the 60/40. As a result, the sum of ω-3 and ω-6 PUFA were, respectively, higher in the 60/40 and the 80/20 feed formulation, and their ratio ω-3/ω-6 was also higher in the former, compared with the latter.

PERMANOVA revealed that the FA profiles of the two feed formulations were significantly different (MS = 4.62; Pseudo-$F_{(1,5)}$ = 291.15; $p \le .001$). Principal coordinates analysis (PCO) of the FA profiles of 60/40 and 80/20 formulations confirmed this result, showing a clear separation along the horizontal axis based on the feed formulations with almost the totality of the explained variance (Figure 3). The formulation 60/40 was grouped on the right side of the graph, characterized by a higher abundance of all the FA classes (the sum of SFA, MUFA and PUFA), total and individual EFA, the sum of ω-3 PUFA and HUFA, and the ratio ω-3/ω-6. In contrast, the formulation 80/20 was distributed in the left area of the graph, because of the higher abundance of the sum of ω-6 PUFA and the two dominant fatty acids in the PUFA class, ALA and LA, suggesting that their abundances were an important driver for the distinction between the two formulations.

3.3 | Bioenergetic trial

The daily ingestion rate (IR) recorded in *P. lividus* fed with the two different feed formulations across the seven sampling periods, showed a fluctuating pattern. The mean value of the daily IR calculated for the entire duration of the trial was rather similar for the two feed

TABLE 2 Proximate composition (% dry matter, mean ± standard deviation) of main ingredients and feed formulations

	Ingredient		Feed formulation	
	Cichorium endivia	*Engraulis encrasiculos*	60/40	80/20
Lipid %	3.80 ± 0.17	14.01 ± 1.90	7.08 ± 0.74	5.55 ± 0.20
Protein %	19.14 ± 0.67	40.58 ± 0.38	29.36 ± 0.28	23.86 ± 0.29
Carbohydrate %	64.42 ± 0.96	4.34 ± 2.27	38.89 ± 0.79	50.69 ± 0.84
Ash %	12.63 ± 0.15	41.07 ± 0.01	24.67 ± 0.39	19.89 ± 0.39

TABLE 3 Fatty acid profiles and lipid content (mg/g dw, mean ± standard deviation) of the two main ingredients (*Cichorium endivia* and *Engraulis encrasicolus* discards) and the two selected feed formulations (60/40 and 80/20)

	Main ingredient		Feed formulation	
FAs (mg/g dw)	*C. endivia*	*E. encrasicolus*	60/40	80/20
8:0	0.04 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
10:0	–	0.11 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
11:0	–	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
12:0	0.01 ± 0.00	0.24 ± 0.01	0.02 ± 0.00	0.00 ± 0.00
14:0	0.07 ± 0.01	7.57 ± 0.5	1.86 ± 0.04	0.86 ± 0.06
15:0	0.03 ± 0.00	1.43 ± 0.09	0.37 ± 0.00	0.20 ± 0.01
16:0	2.07 ± 0.01	28.7 ± 1.51	10.49 ± 0.12	6.34 ± 0.21
17:0	0.03 ± 0.00	1.32 ± 0.08	0.61 ± 0.00	0.28 ± 0.02
18:0	0.20 ± 0.01	5.98 ± 0.31	1.82 ± 0.08	1.04 ± 0.02
19:0	–	0.31 ± 0.02	0.10 ± 0.00	0.05 ± 0.01
20:0	0.12 ± 0.00	0.39 ± 0.01	0.12 ± 0.00	0.09 ± 0.00
21:0	0.09 ± 0.01	0.07 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
22:0	0.14 ± 0.01	0.27 ± 0.01	0.13 ± 0.00	0.13 ± 0.00
Σ LCFA (>22:0)	0.26 ± 0.01	0.39 ± 0.08	0.48 ± 0.01	0.47 ± 0.02
Σ SFA	**2.98 ± 0.02**	**47.16 ± 2.42**	**16.06 ± 0.21**	**9.52 ± 0.34**
14:1	–	0.06 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
15:1	0.03 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00
16:1 n7	–	4.15 ± 0.25	1.06 ± 0.01	0.52 ± 0.02
18:1 n7	0.09 ± 0.00	3.01 ± 0.12	0.89 ± 0.01	0.44 ± 0.01
18:1 n9t	–	0.09 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
18:1 n9c	0.19 ± 0.03	14.13 ± 0.68	3.87 ± 0.08	1.92 ± 0.06
20:1 n9	0.03 ± 0.00	0.69 ± 0.03	0.16 ± 0.02	0.08 ± 0.02
20:1 n11	–	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.00
22:1 n9	–	0.19 ± 0.01	0.04 ± 0.00	0.01 ± 0.00
Σ MUFA	**0.35 ± 0.04**	**23.35 ± 1.1**	**6.08 ± 0.12**	**2.99 ± 0.09**
18:2 n6c - LA	3.72 ± 0.31	2.25 ± 0.12	4.12 ± 0.13	5.12 ± 0.14
18:2 n6t	–	0.05 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:3 n3 - ALA	9.31 ± 0.63	1.51 ± 0.09	6.20 ± 0.56	8.26 ± 0.23
18:3 n6	0.09 ± 0.00	0.19 ± 0.03	0.12 ± 0.00	0.16 ± 0.00
18:4 n3	0.03 ± 0.00	2.41 ± 0.16	0.58 ± 0.01	0.32 ± 0.01
20:2 n6	0.07 ± 0.00	0.43 ± 0.02	0.08 ± 0.01	0.04 ± 0.01
20:3 n3	0.03 ± 0.00	0.15 ± 0.01	0.04 ± 0.00	0.02 ± 0.01
20:3 n6	–	0.16 ± 0.06	0.01 ± 0.00	0.02 ± 0.00
20:4 n3	–	0.59 ± 0.04	0.17 ± 0.00	0.10 ± 0.01
20:4 n6 - ARA	–	1.54 ± 0.07	0.28 ± 0.00	0.10 ± 0.01
20:5 n3 - EPA	–	9.74 ± 0.62	2.50 ± 0.02	1.08 ± 0.04
22:2 n6	–	0.14 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:4 n6	–	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
22:5 n3	–	0.92 ± 0.04	0.95 ± 0.01	0.44 ± 0.01
22:6 n3 - DHA	–	24.88 ± 1.51	7.45 ± 0.05	3.28 ± 0.11
Σ PUFA	**13.26 ± 0.50**	**44.96 ± 2.59**	**22.52 ± 0.70**	**18.94 ± 0.55**
Branched	–	1.27 ± 0.11	0.20 ± 0.02	0.08 ± 0.00
-OH	0.30 ± 0.02	0.78 ± 0.03	0.19 ± 0.00	0.17 ± 0.01

(Continues)

TABLE 3 (Continued)

FAs (mg/g dw)	Main ingredient: *C. endivia*	Main ingredient: *E. encrasicolus*	Feed formulation: 60/40	Feed formulation: 80/20
-Δ	0.09 ± 0.03	0.53 ± 0.07	0.13 ± 0.01	0.06 ± 0.01
Σ EFA	–	**36.16 ± 2.20**	**10.23 ± 0.07**	**4.46 ± 0.15**
Σ ω3-PUFA	**9.38 ± 0.63**	**40.20 ± 2.46**	**17.89 ± 0.58**	**13.49 ± 0.39**
Σ ω6-PUFA	**3.88 ± 0.32**	**4.76 ± 0.13**	**4.63 ± 0.12**	**5.45 ± 0.16**
ω3/ω6	**2.44 ± 0.33**	**8.44 ± 0.28**	**3.86 ± 0.00**	**2.47 ± 0.00**
Σ ω3- HUFA	**0.03 ± 0.00**	**38.54 ± 2.36**	**11.65 ± 0.07**	**5.22 ± 0.16**
Σ FA	**16.98 ± 0.61**	**118.06 ± 6.32**	**45.12 ± 0.00**	**31.72 ± 0.00**
Lipid content (mg/g dw)	**38.02 ± 1.66**	**140.10 ± 18.97**	**70.82 ± 7.36**	**55.49 ± 1.97**

Note: Main FA classes (SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA); main biomarkers of nutritional quality are also indicated. LCFA: long-chain FA; LA: linoleic acid, ALA: α-linolenic acid; ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, Branched: branched-chain saturated FA, -OH: hydroxyl FA, -Δ: cyclopropyl FA.

formulations: 104.0 ± 25.5 mg DW per day and 111.9 ± 25.1 mg DW per day, respectively, for 60/40 and 80/20. A mean IR decrease was evident in the early stages of the experiment (T1-T3), followed by a slight increase (T4-T5) and a further reduction (T6-T7) (Figure 4). This ambiguous temporal trend, coupled with a high individual variability, resulted in a lack of significant differences between feed formulations, times and their interaction (Table 4).

The absorption efficiency (AE) recorded in the sea urchins fed with the two feed formulations showed a fluctuating pattern, similarly to that observed for IR. After the early stages of the trial, where the AE values were similar in the sea urchins fed with the 80/20, and tended to decrease in those fed with the 60/40 formulation, higher AE values were recorded in the sea urchins fed with the formulation with the higher fish content (i.e. 60/40) (Figure 5). The average AE calculated for the whole trial was higher, indeed, for the 60/40 formulation than for the 80/20 (63.6 ± 6.4% vs. 55.1 ± 10.3%, respectively) (Table 4), while differences among times and for the interaction of the two factors were not detected.

Despite the short duration of the trial (2 weeks), the gonado-somatic index (GSI) showed a clear increase in sea urchins fed with both formulations (from 0.8 ± 0.7% to 2.8 ± 0.6% and from 0.9 ± 0.7% to 2.7 ± 1.4% in the sea urchins fed with the 60/40 and 80/20 formulations, respectively). Univariate permutational analysis of variance showed significant differences between times (MS = 21.55; Pseudo-$F_{(1,22)}$ = 23.73; $p < .01$), but not between feeds (MS = 0.002 and Pseudo-$F_{(1,22)}$ = 0.002, $p > .05$) or the interaction of the two factors (Pseudo-$F_{(1,22)}$ = 0.13, $p > .05$).

4 | DISCUSSION

To move towards a higher sustainability of echinoculture, this study proposes a new sustainable feed through the reuse of discards from the food industry. Two experimental formulations were prepared using discarded endive (*C. endivia*) leaves and anchovy (*E. encrasicolus*) industry discards in different proportions and were tested for stability in seawater. Nutritional composition and quality of the main ingredients and the formulations were evaluated through the analysis of proximate composition and fatty acid profiles and biomarkers. Finally, both formulations were tested for palatability, absorption efficiency and effect on gonad growth of the purple sea urchin *P. lividus*.

The stability trial showed a comparable pattern between the feed formulations at different agar amount: the greater feed loss occurred in the first 24 hr of immersion in seawater and then remained fairly stable in the subsequent times, ranging overall between 30% and 40%. These patterns clearly indicate that the different amount of agar in the feed formulation affected only marginally the feed

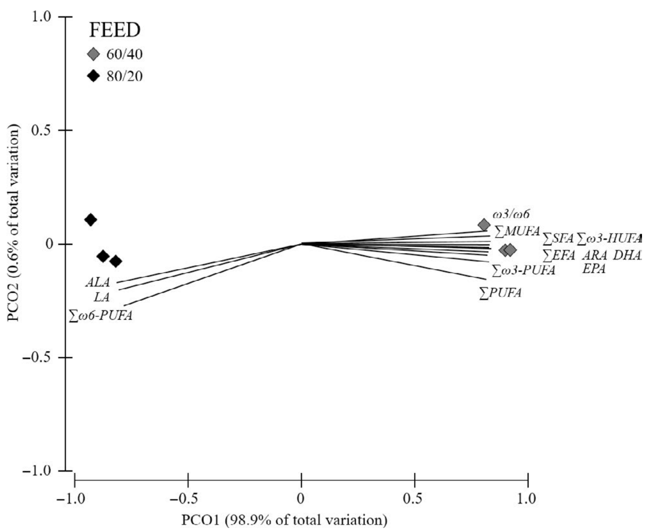


FIGURE 3 Principal coordinates analysis (PCO) of the fatty acid profiles of the two feed formulations (60/40, 80/20). The main fatty acid classes and the main indicators of nutritional quality selected in this study are superimposed to the graph. The meaning of the acronyms is the same as in Table 3

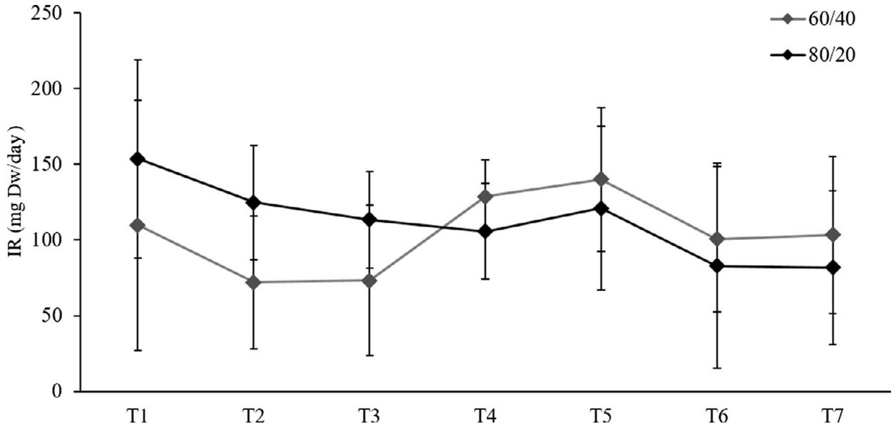


FIGURE 4 Ingestion rate IR (mg dw per day, mean ± standard deviation) of *Paracentrotus lividus* for the two feed formulations across time

stability over time, consistent with previous studies (Argüello-Guevara & Molina-Poveda, 2013; Fabbrocini et al., 2012). The macromolecular structure of the gel formed by agar is deemed, indeed, as a strong binder as it confers a high feed stability at ambient temperature by limiting nutrient loss through leaching (Fabbrocini et al., 2012; Leclercq, Graham, & Migaud, 2015) and water absorption (Paolucci, Fasulo, & Volpe, 2015). Moreover, as *P. lividus* takes at least 2–3 days to eat the feed offered in confined conditions (Fabbrocini, Volpe, Coccia, D'Adamo, & Paolucci, 2015), the very limited feed loss observed between 24 and 72 hr makes both feed formulations enough stable over time and then resulting a suitable choice in the production of sustainable feeds for sea urchins. Additionally, the present findings revealed that the use of a commercially affordable product (i.e. agar powder for home baking) rather than a laboratory product, for the production of aquaculture feeds ensured good results coupled with a substantial cost reduction. In contrast, although other binders, such as pork gelatine, may result in a higher feed stability in water (Pearce, Daggett, & Robinson, 2002a), the higher cost and quantity needed to produce gelatine-based pellets make them economically unsustainable. Furthermore, agar-based feeds may have a positive effect on growth rate, as previously observed in reared crustaceans (Palma, Bureau, & Andrade, 2008) and on both gamete production and gonad growth of *P. lividus* (Fabbrocini et al., 2012). For all these reasons, chiefly the comparable stability over time coupled with the greater sustainability of using lesser amount of binders in the context of industrial-scale feed production, the further steps were conducted using only the two formulations with the lower agar amount.

Following the stability trial, the two selected feed formulations and their main ingredients were characterized in terms of nutritional composition and quality. Both formulations appeared nutritionally balanced, with carbohydrates as the most representative macronutrient, followed by proteins and lipids. As expected, the differences found between the formulations were essentially driven by the different nutritional contributions of the main ingredients. Indeed, endive discarded leaves and anchovy industry discards showed major differences in both lipid and fatty acid content, the two ingredients being respectively of plant and animal origin and hence characterized by a different nutritional profile (Rana, Siriwardena, & Hasan, 2009). Being constituted mainly of fish skin, bones, heads and internal organs, the protein and lipid content of anchovy discards was much higher than that of endive leaves (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013). This was mirrored in the two feed formulations, where proteins and lipids decreased proportionally with the ratio of vegetal versus animal ingredients, consistent with the literature (Fernandez & Boudouresque, 2000). On the other hand, discarded endive leaves and the formulation 80/20 were characterized by the highest content of carbohydrates.

A proper nutritional composition of the feeds is crucial in echinoculture. Previous studies showed that carbohydrate and protein levels similar to those found in this study (~40% and 20%) provide the proper amount of energy and essential amino acids needed to foster growth and reproduction (Cuesta-Gomez & Sánchez-Saavedra, 2018; Hammer et al., 2012). Also, the source of proteins is important, as revealed by Fernandez and Boudouresque (2000) who found the highest values of gonado-somatic index in the sea urchins fed with diets with intermediate levels of animal ingredients. Also, dietary lipids have a key role as structural components, source of energy and precursors of bioactive molecules (Carboni et al., 2013), and additionally they influence the FA composition and organoleptic attributes of the roe (Martínez-Pita, García, & Pita, 2010; Siliani et al., 2016; Vizzini et al., 2019). Consequently, a high lipid content of the diet may favour gonad development and contribute to the restoration of energy supplies following the starvation, during which sea urchins tend to consume the nutrients present in their tissues (Guillou & Lumingas, 1998).

Turning to the FA profiles, the high concentration of SFA and MUFA found in the formulation characterized by a higher content of anchovy discards (60/40) is mainly attributable to a higher content of 16:0 and 18:1n9 in fish discards than in endive leaves, consistently with the high typical abundance of SFA and MUFA in the common anchovy (Öksüz & Özyilmaz, 2010; Zlatanos & Laskaridis, 2007). In contrast, the high concentration of PUFA observed in both formulations is mainly due to the high content of linoleic (LA) and α-linolenic (ALA) acids, being both very abundant in the endive leaves, but not in the fish discards. Endive is a 18:3 metabolism plant, and its PUFA profile is composed almost exclusively by 18:3n3 and 18:2n6 (Le Guedard, Schraauwers, Larrieu, & Bessoule, 2008; Vizzini et al.,

TABLE 4 Univariate permutational analysis of variance results testing the effects of the feed formulations across time on the ingestion rate IR (a) and absorption efficiency AE (b) of *Paracentrotus lividus*

Main test		a) IR			b) AE		
Source of variation	df	MS	Pseudo-*F*	*p (perm)*	MS	Pseudo-*F*	*p (perm)*
Feed	1	1,321.7	0.50	.48	1529.3	4.43	**.04**
Time	6	3,914.9	1.48	.18	544.3	1.58	.18
Feed × Time	6	3,766.7	1.43	.24	340.3	0.99	.45
Residual	70	2,636.8			345.4		

Note: Significant *p* values are highlighted in bold.

2019), which, in contrast, are fatty acids generally not abundant in the common anchovy (Öksüz & Özyilmaz, 2010).

The higher abundance of essential FA (EFA) in the 60/40 formulation than in the other (80/20) is also consistent with the high EFA concentration in *E. encrasicolus* discards. In turn, the EFA content in fish discards is consistent with what is reported in the literature for anchovy tissues [about 1%, 10% and 15% of the total FA content for arachidonic (ARA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, respectively, Öksüz and Özyilmaz (2010)], due to the high EFA assimilation and storage ability of fish (Bendiksen, Johnsen, Olsen, & Jobling, 2011). EFA are deemed suitable indicators of high nutritional quality in aquaculture feeds as they play a key role in many physiological functions and then represent an added value in the market of sea urchins. The abundance in the proposed formulation may also boost gamete production and gonad growth (Watts, Lawrence, & Lawrence, 2013). The preliminary assessment of gonado-somatic index carried out in this study confirms this, but longer-time experiments are needed for further consideration.

The two selected formulations were provided to sea urchins to assess both palatability and absorption efficiency of *P. lividus*. The bioenergetics trial highlighted a similar fluctuating pattern for both ingestion rate (IR) and absorption efficiency (AE), characterized by high initial values followed by an overall decrease during the first phases of the experiment and then increased again. The early pattern may have been influenced by the previous period of starvation. Under food limitation, sea urchins rely on internal stores of nutrients to meet their energy requirements for maintenance (Guillou, Lumingas, & Michel, 2000; Lares & Pomory, 1998), while, once food become available, the level of hunger may lead sea urchins to increase the consumption of food regardless of its nutritional content (Castilla-Gavilán, Cognie, Ragueneau, Turpin, & Decottignies, 2019). After that, the reduction of food ingestion may be an effect of the stomach fullness (Lawrence, Plank, & Lawrence, 2003). Moreover, the fluctuating IR pattern observed in *P. lividus* may be also due to an intrinsic periodicity of food ingestion resulting in high peaks spaced out by a few fasting days (Nédélec, Verlaque, & Dallot, 1983). Comparisons with sea urchins fed with natural food (i.e. macroalgae and seagrasses) revealed contrasting results depending on the species used. Mean IR of the two formulations was higher than that observed for *Corallina elongata*, *Flabellia petiolata*, *Halopteris scoparia* and *Ulva lactuca*, comparable to the IR measured for *Dictyota* sp., *Laurencia* sp., *Padina pavonica*, *U. rigida* and *Posidonia oceanica*, and lower than the IR for *Codium* sp. and *Dictyopteris* sp. (Ruocco et al., 2018; Sartori & Gaion, 2015). Nevertheless, present IR values were overall comparable with those previously measured in *P. lividus* fed with commercial and experimental pellets (Ruocco et al., 2018; Sartori & Gaion, 2015). Although agar was observed to confer a high palatability to manufactured feeds without, however, affecting the digestibility (Barker, Keogh, Lawrence, & Lawrence, 1998; Fabbrocini et al., 2012, 2015; Leclercq et al., 2015), the low concentration (2.5%) used here in the preparation of the sustainable feed may have had a negligible influence on the IR values.

Similarly to the IR, the patterns observed for the absorption efficiency (AE) showed that sea urchins responded to the resumption

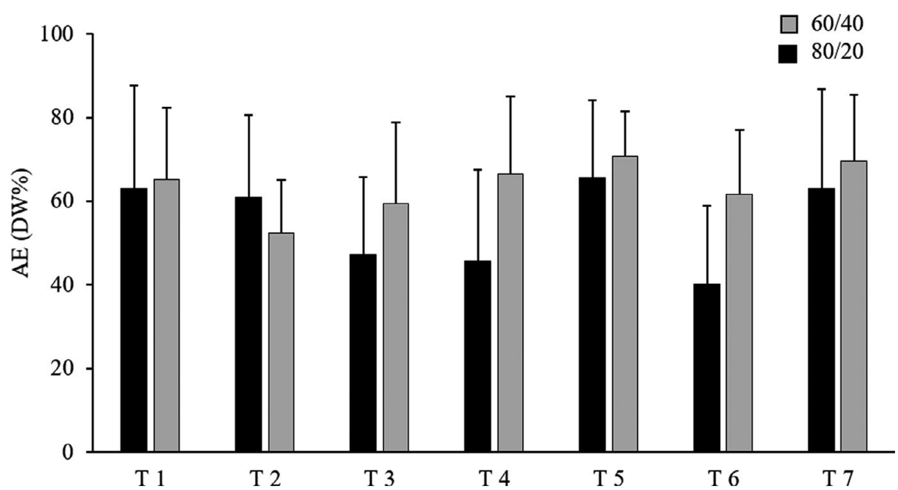


FIGURE 5 Percentage absorption efficiency AE (DW%, mean ± standard deviation) of *Paracentrotus lividus* for the two new feed formulations across time

of feed provision with high feed absorption, for meeting their nutritional requirements, regardless of the type of the food provided. After that, there was an evident difference in AE based on the formulation provided, with a higher assimilation of the formulation with a higher content of fish ingredients, than the other. This is consistent with the literature: also Fernandez and Boudouresque (2000) found a different absorption efficiency in *P. lividus* according to the food provided. In particular, a different AE seems to depend on the assimilation of carbohydrates: vegetables are characterized by a higher amount of insoluble carbohydrates, not digestible by echinoids, that are instead poorly represented in fishmeal (Fernandez & Boudouresque, 2000, present study). This is supported also by the higher biomass of faeces found in the cages where sea urchins were fed with the 80/20 formulation, compared with those where sea urchins were fed with the 60/40 (data not showed), where the ingredients of plant and animal origin are more balanced. Despite the differences found between formulations, the mean absorption efficiency observed for both was comparable with the AE recorded in the Australian sea urchin, *Heliocidaris erythrogramma*, fed with mixed feed (Senaratna, Evans, Southam, & Tsvetnenko, 2005), confirming the suitability of the new sustainable feed.

Finally, as previously mentioned, this preliminary short-time assessment of the effect of the sustainable feed on gonad growth, based on the evaluation of the gonado-somatic index (GSI), revealed a significant increase in GSI for both formulations, regardless of the ratio of vegetal versus animal ingredients. Although GSI is usually estimated over longer-time scales, our findings are overall consistent with the literature (e.g. Vizzini et al., 2019; Zupo et al., 2019) and revealed that the sea urchins fed with the new sustainable feed had good feed intake and nutrient conversion even in a very short time (2 weeks).

5 | CONCLUSION

A new sustainable feed, produced using anchovy and endive food industry discards with the addition of a low amount of agar, resulted suitable for feeding *P. lividus*. Two formulations at a different ratio of vegetal versus animal ingredients were tested. Both showed a good stability in seawater, and a balanced nutritional composition and fatty acid (FA) profiles, which are basic requirements for feeding sea urchins. Main biomarkers of nutritional quality (PUFA, ω-3 HUFA, EFA and the ratio ω-3/ω-6) were higher in the formulation with the higher content of fish discards. This formulation was also absorbed more efficiently by the sea urchin, resulting as attractive as the other formulation, but more digestible for *P. lividus*. Finally, despite the short experimental period, the gonado-somatic index increased in all the reared sea urchins, regardless of the provided formulation. These encouraging results showed that food industry discards are suitable and promising alternative ingredients for the production of sustainable feeds for sea urchins, by meeting also the requirements of bio- and blue economy that promote sustainable development. Moreover, on first analysis, the formulation with a more balanced ratio of vegetal versus animal content (60/40) seemed more suitable in echinoculture, but further studies are needed to assess the effect of this new feed on gonad yield, in order to obtain a marketable while sustainable product.

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DATA AVAILABILITY STATEMENT

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

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