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Dietary effect of different fermented feed sources on growth performance of early juvenile sea cucumber *Holothuria scabra*

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ABSTRACT

The development of effective, economically viable and locally sourced diets for high-value early juvenile sea cucumber, *Holothuria scabra*, is important to ensure efficient juvenile production for sustainable farming and ranching. The current study applies seven fermented algal/plant diet formulations (C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea,* DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss) in controlled feeding experiments for post-metamorphic juvenile *H. scabra*. The highest overall mean ($\pm$ SE) individual growth rate of 0.09 ± 0.023 g d^{-1} was recorded for the diet green pond algae (DA), however, this diet also exhibited the lowest individual survival rate across all treatments. Combined acceptable growth rates of 0.03 $\pm$ 0.001 and 0.03 ± 0.002 g d^{-1}, and much higher survival rates of 80.63 ± 4.38 and 80.63 ± 2.77%, respectively were recorded for diets *E. acoroides* (C), and *Padina* sp. (DP). The reasons for significant variations and survival rates are not known, however, these results indicate that the diets with acceptable survival rates may be better suited for very early juvenile sea cucumbers. Those diets exhibiting highest overall individual growth rate in the long-term may be better applied to juveniles of larger sizes later in the nursery production process. Overall results indicate good acceptance of most macroalgae fermented diets by juvenile *H. scabra*. Results also indicate that piloting diets at larger scales and at a variety of sizes and ages will allow determination of optimal feeding throughout the nursery production process.

1. Introduction

The tropical holothuriid Sandfish*, Holothuria scabra* is one of the most important sea cucumbers commercially produced in hatcheries, however, information concerning the optimal nutritional and dietary requirements of early juveniles, particularly the newly settled and post-settlement phase remains scarce. In the hatchery, planktonic post-larvae transform into early settled epibenthic juveniles. This is a crucial point in the hatchery process, at the commencement of the post-metamorphic period, when high mortality rates are observed (Lavitra et al., 2009; Laguerre et al., 2020). The metamorphosis rate, as well as settlement rate, determines the overall juvenile production rate (Li et al., 2010). Newly metamorphosed juveniles need the proper diet to increase their growth and reduce mortality (Lavitra et al., 2009). Survival and growth in this phase are affected by the type of diet, the quantity, and the quality of the feeding regime (Lavitra et al., 2009).

The capability of adult and late juvenile *H. scabra* to digest plant-based dietary sources like seagrass and seaweed has been investigated. Different diets from various plant sources have been used for culturing *H. scabra* including *Thalassia hemprichii*, *Thalassodendron ciliatum, Syringodium isoetifolium*, *Sargassum latifolium*, *Gracilaria heteroclada* (Lavitra et al., 2009; Orozco et al., 2014). Previous studies have used many plant-based sources as feed for various species of sea cucumber (Table 1). The most common plant-based source is *Sargassum thunbergii*, which has been used for feeding *Apostichopus japonicus*, *Holothuria spinifera*, and *Isostichopus badionotus*. Some plant-based sources are specific to certain species of sea cucumber, such as *Ascophyllum nodosum* and *Saccharina latissima* for *Cucumaria frondosa*, and *Solieria filiformis* and *Macrocytis piryfera* for *Isostichopus badionotus*.

Algae powder is also common as a food ingredient for a variety of sea

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Table 1
Plant based diet sources of sea cucumber.

Species of sea cucumber	Plant based sources	Ref
Apostichopus japonicus	*Sargassum thunbergii, Sargassum polycystum, Zostera marina, Ulva lactuca,* and *Laminaria japonica*	Xia et al., 2012a
Apostichopus japonicus	*Sargassum thunbergii, Ulva lactuca, Undaria pinnatifida, Laminaria japonica,* and *Schizochytrium* sp.	Anisuzzaman et al. (2017)
Apostichopus japonicus	*Sargassum thunbergii, Undaria* sp., *Laminaria* sp., *Brassica oleracea*, soybean, grain and rice straw powder	Seo et al., 2011a
Apostichopus japonicus	*Sargassum thunbergia, Sargassum horneri, Undaria pinnatiida, Saccharina japonica, Ulva australis*	Kim and Cho (2022)
Apostichopus japonicus	Corn meal, soybean meal, and *Sargassum thunbergii*	Yu et al. (2015)
Apostichopus japonicus	*Laminaria japonica* (both fresh and boiled), *Sargassum thunbergii, Sargassum polycystum, Zostera marina,* and *Ulva lactuca*	Xia et al. (2012a)
Apostichopus japonicus	Corn leaves (Zea mays), *Sargassum thunbergii*	Wu et al. (2015)
Apostichopus japonicus	*Sargassum thunbergii*	Shi et al. (2013)
Apostichopus japonicus	*Sargassum thunbergii, Gracilaria lemaneiformis*	Gao et al. (2011)
Apostichopus japonicus	*Sargassum muticum, Gracilaria lemaneiformis,* and *Ulva lactuca*	Wen et al. (2016)
Apostichopus japonicus	*Sargassum polycystum, Sargassum thunbergii, Sargassum horneri, Enteromorpha prolifera*, and *Macrocystis pyrifera*	Li et al. (2022)
Apostichopus japonicus	Soybean meal	Liao et al. (2015)
Apostichopus japonicus	*Laminaria japonica*	Wang et al. (2015)
Apostichopus japonicus	*Chaetomorpha linum* and *Zostera marina*	Song et al. (2017)
Cucumaria frondosa	*Ascophyllum nodosum* and *Saccharina latissima*	Yu et al. (2020)
Holothuria spinifera	*Sargassum* spp.	Asha and Muthiah (2007)
Holothuria arguinensis	*Saccharina polyschides*	Sousa et al. (2023)
Isostichopus badionotus	*Solieria filiformis* and *Macrocytis piryfera*	Martínez-Milián and Olvera-Novoa, 2016
Isostichopus badionotus	*Macrocystis* sp. and *Sargassum* sp	Zacarías-Soto et al. (2018)
Stichopus mollis	*Macrocystis pyrifera* and *Undaria pinnatifida*	Maxwell et al. (2009)

cucumber species (Seo et al., 2011b). Orozco et al. (2014) mixed sand with another powdered single diet. Feed formulations are frequently not only a single ingredient of macroalgal or marine plant extracts, but also include extracts of algae or algae fermented in controlled processes mixed with other sources (Maxwell et al., 2009; Orozco et al., 2014). Fermenting algae for diet preparation can improve digestibility and reduce refractive diet content (Seo et al., 2011a). The value of such plant-based dietary preparations, including fermentation, remains untested in the diet of early juvenile *H. scabra*.

There is a wide range of research available on the feeding behavior of the temperate aspidochirotid sea cucumber *Apostichopus japonicus*. However, very few studies are found for the newly settled and the post-settlement stages of sea cucumber *H. scabra*, especially as related to diet preference and nutritional requirements. Macroalgae mixed with sea mud is an easy and cost-effective feeding option used in commercial *A. japonicus* hatcheries. This mixture could replace costly periphytic diatoms in other sea cucumber hatcheries. Macroalgae – mud mixes also have the potential to act as a post-settlement diet, especially in the large-scale rearing of juveniles (Li et al., 2010). Such simple mixes remain untested for early juvenile *H. scabra*.

In the current study, a variety of fermented marine plant-based feeds were fed to juvenile *H. scabra* under controlled experimental conditions to determine their suitability as diets based on juvenile survival and growth performance indicators. The selection of plant-based diet sources to be tested is based on natural conditions and resource availability.

Seagrass *Enhalus acoroides,* for example*,* is selected as one of the diet sources, as it also serves as a substrate during the settlement period for *H. scabra* in the natural environment. The leaves are currently pulverized to feed the epibenthic larger juvenile phases of *H. scabra* as industry standard for hatchery production in east Lombok, Indonesia. In addition, *Halimeda discoidea*, *Ulva lactuca*, *Sargassum polycystum*, *Padina australis*, pond moss, and green pond algae are easy to find and available in the wild and were also selected as potential diet ingredients to test. Green algae are photosynthetic microorganisms that can grow naturally in ponds and their abundance often fluctuates based on environmental factors. In shrimp farming ponds, a high biomass of microalgae, including blue-green and green algae, can increase chlorophyll a concentration in the water (Shaari et al., 2011).

1. This study aims to evaluate the effect of fermented diet on the growth and survival of early juvenile *H. scabra.* Also, this study focuses on the early juvenile stage of *H. scabra*, which is a critical period where individuals begin to digest a new feeding following a larval phase where they consume phytoplankton. At this point, the mortality rate is relatively high, thus necessitating the formulation of feeds that can improve growth performance and survival rates to support mass production and industrial scale. There is limited research concerning fermented diet, especially for species *H. scabra* in the early juvenile stage with an initial average weight of 0.1-0.2 g. To close this gap, our study investigates how different fermented feed sources influence the growth performance and survival of *H. scabra* during this critical developmental period.**Materials and Methods**

1.1. Diet preparation

Macrolagae *U. lactuca*, *H. discoidea*, *S. polycystum*, *P. australis*, seagrass *E. acoroides*, ‘pond moss, and ‘green pond algae’ were collected from the wild (−8.7879715°S, 116.5040435°E) (Fig. 1). Macroalgae and seagrass were collected from the east Lombok beach by snorkeling, while moss and green algae were gathered from unutilized sandfish ponds (1–1.5 m depth), an area of 1000 m^2 which was as previously used as salt evaporation ponds (30 cm depth). Fresh raw material for the diet was taken from the wild every week, aligning with the fermentation schedule. Materials were washed at the beach and rinsed in the laboratory with fresh seawater to remove dirt and were cut into 2 cm by 3 cm pieces and finely ground in a blender. Then, 1000 g of each material was mixed with 1000 mL of 5 μm filtered sea water, 10 mL commercial probiotic decomposer (EM4 for fisheries and pond, manufactured by PT Songgolangit Persada containing *Lactobacillus casei* and *Saccharomyces cerevisiae*), and 10 mL molasses, were placed into a closed tank with a small vent hole and allowed to ferment for approximately 14 days (Table 2). The fermentation process was carried out at a regulated room temperature of 25 ± 2 °C on average. A digital thermometer was used to monitor temperature changes to maintain constant conditions during the fermentation process. Each feed material was fermented in a 10 L plastic container. Each of these containers included a small vent hole to allow for gas exchange and to prevent pressure buildup, which could inhibit the fermentation process. The small vent hole provided minimal aeration, which was shown to be beneficial for the particular probiotic decomposer used, even though the tanks were supposed to be mostly anaerobic. Manual stirring was conducted using gloved hands to ensure the homogeneity of the fermenting material and to distribute microbial activity evenly. To ensure hygiene and minimize cross-contamination, gloves were changed after stirring each fermented diet. Stirring was

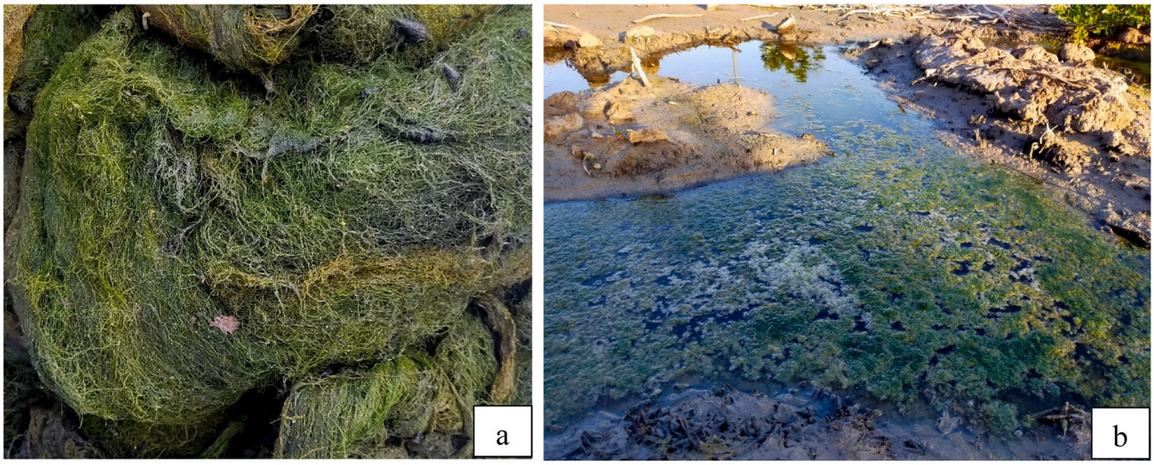

Fig. 1. Pond moss (a); green pond algae (b).

Table 2
Ingredients of experimental fermented diets.

Diet	Ingredients			
	Raw materials (g)	EM4 probiotic (mL)	Molasses (mL)	Filtered seawater (mL)
Enhalus acoroides (C)	1000	10	10	1000
Halimeda discoidea (DH)	1000	10	10	1000
Ulva lactuca (DU)	1000	10	10	1000
Sargassum polycystum (DS)	1000	10	10	1000
Padina australis (DP)	1000	10	10	1000
Green pond algae (DA)	1000	10	10	1000
Pond moss (DM)	1000	10	10	1000

performed once every 24 h. The duration of fermentation was consistently maintained at 14 days, a period determined to be optimal based on preliminary trials that evaluated the activity of the probiotics and the degradation of the raw material. To avoid contamination, all fermentation diet preparation equipment, including blender blades and plastic containers, was sterilized before use. The fresh seawater used for rinsing and mixing was filtered through a 5 μm filter to remove particulate matter and potential pollutants. Fermented materials were sieved using a 1 mm mesh size. 1 mL L^{-1} of the liquid diet was provided every day to early juveniles in each experiment tank. End products were kept fermenting until use within one week and if there were leftovers they were thrown away. New fermented feed products were given the following week. Water flow and aeration were interrupted at feeding for 15 min to allow it to settle.

1.2. Feeding experiment

Early juveniles of *H. scabra* were obtained approximately 80 days post fertilization or 1 day post settlement from the hatchery in east Lombok, Indonesia. Seven fermented diet treatments were prepared as above, they are *E. acoroides* (C) as a control, *U. lactuca* (DU), *H. discoidea* (DH), *S. polycystum* (DS), and *P. australis* (DP), 'green pond algae' (DA) and 'pond moss' (DM) with 4 replicates. We selected seagrass *Enhalus acoroides* as the control feed based on its historical use as a standard in previous research with juvenile *H. scabra*, as described in several studies (Indriana et al., 2017; Ridwanudin et al., 2018; Indriana et al., 2018; Wahyudi et al., 2022). This particular seagrass is not only more easily accessible at our research site, but it is also the standard feed used by the industry for post-settlement juvenile *H. scabra* in East Lombok, Indonesia. The availability and established utilization of *E. acoroides* make it a practical and representative control for our experimental comparisons.

A complete randomized block design was used to arrange the 28 tanks to include quadruplicate tanks for 7 treatment groups. The experimental unit consists of square plastic tanks of 16 L (36×30×14cm). Forty individual juvenile *H. scabra* with an initial average wet weight of 0.16–0.18 g per tank were maintained in the experimental tank. All juveniles were starved for 24 h before treatment. During 56 days experimental period, each tank was supplied with 12 L filtered sea water and provided with gentle aeration continuously. Sea water was replaced 50% with 1 μm filtered sea water daily before feeding to ensure water quality. Feed was given daily at 16:00 h, starting at a rate of 1 mL L^{-1} during week 1. In week 2, the feeding volume was increased to 1.5 mL L^{-1}, and then it continued to increase by 0.5 mL L^{-1} each week, reaching 4.5 mL L^{-1} by week 8.

1.3. Sample collection and measurement

The growth of juveniles was monitored every 28 days. The effect of various feeds was assessed in terms of mortality and body weight gain. A total of 40 individuals in each tank were weighed and averaged monthly. Each individual sea cucumber was dried carefully with blotting paper, weighed using an electronic balance (0.001 g precision), and immediately returned to its experimental tank. Temperature, pH, salinity, and DO were recorded daily. Together with juvenile monitoring, monthly water samples were collected for analysis of Ammonia nitrogen, Nitrite nitrogen, Nitrate nitrogen using a HACH portable spectrophotometer DR-1900. At the same time, water samples were also taken to measure Total Organic Matter (TOM) using titrimetric method. Faeces were dried at 60 °C to constant weight.

The proximate analysis of the diet followed the standard method of the Association of Official Analytical Chemists International (AOAC). The ash content was determined by incinerating 2–6 g of the sample at a temperature of 550 °C for approximately 4 h. The moisture content was determined by drying the sample using an oven at a temperature of 105 °C for 3 h until it reached a constant weight. The protein content was determined by the Kjeldahl method, where the quantity of protein was calculated by multiplying the nitrogen content by a conversion factor of 6.25. The carbohydrate content and total energy refer to FAO (2003).

Biomass density was calculated in terms of wet weight per unit area using the method following Robinson et al. (2019). In this study, the formula applied was biomass density (g m^{-2}) = Total wet weight of juveniles/area of the tank (0.108 m^2).

1.4. Calculation and statistical analysis

Growth rate (Daily Weight Gain) was calculated as follows:

Growth Rate (g d^{-1}) = (final weight – initial weight)/days

Specific growth rate (SGR) was calculated as follows:

SGR (% d^{-1}) = (ln final weight – ln initial weight)/t x 100

Survival rate (SR) was calculated as follows:

SR (%) = (Number of final/Number of initial) x 100

Feces Production Rate (FPR) was calculated as follows:

FPR (mg g^{-1} h^{-1}) = (dried weight of feces (mg)/ wet weight of the sea cucumber in the tank) (g)/ time (h)

1.5. Data analyses

All data values are presented as mean ± SE, n = no. of replicates. Before data analysis, normality assumptions and variance homogeneity were checked using the Shapiro-Wilk and Levene's tests, respectively. ANOVA was performed to test for significant differences, where the data were parametric, and a Kruskal-Wallis test was used when the data were not parametric. Where significant results were determined, pairwise comparisons were made using a Dunn test, and the p-values corrected for multiple tests using a Bonferroni correction. Generalised linear mixed-effects models (GLMMs) were used to test for effect diet and time on biomass density. The statistical analyses were performed in R Studio 2023.03.0.

2. Results

2.1. Proximate composition and plant-based source diets

The diet green pond algae (DA) showed the highest ash content, diet *U. lactuca* (DU) contained most moisture, while diet *E. acoroides* (C) outperformed the other diets in terms of carbohydrate and total energy content (Table 3). Crude lipid values were < 0.02% for all diets and replicates.

Compared to being used as a single diet source, numerous studies have included macroalgae as component of mixed diet ingredients along with other materials like mud, sand, soil, flour, etc (Table 4). Furthermore, information on the nutritional composition of diet, particularly for sea cucumber *H. scabra*, is scarce.

Table 3
Proximate composition of the experimental diets (mean±SE).

Diet	Ash (%)	Moisture content (%)	Carbohydrate (%)	Crude Protein (%)	Total energy (Kcal 100 g^{-1})
C	7.76 ± 0.07	89.22 ± 0.02	2.46 ± 0.05	0.56 ± 0.01	12.05 ± 0.20
DH	7.92 ± 0.06	89.79 ± 0.04	1.85 ± 0.03	0.45 ± 0.01	9.17 ± 0.14
DU	3.43 ± 0.04	94.06 ± 0.02	1.74 ± 0.02	0.77 ± 0.00	10.04 ± 0.07
DP	6.78 ± 0.04	90.67 ± 0.02	1.93 ± 0.02	0.62 ± 0.01	10.17 ± 0.08
DS	5.21 ± 0.07	92.31 ± 0.02	2.01 ± 0.06	0.46 ± 0.01	9.89 ± 0.21
DA	9.12 ± 0.08	88.81 ± 0.05	1.60 ± 0.03	0.47 ± 0.01	8.28 ± 0.13
DM	7.68 ± 0.09	89.30 ± 0.05	1.91 ± 0.04	1.11 ± 0.02	12.08 ± 0.17

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss.

2.2. Growth performance

Diets *E. acoroides* (C), *P. australis* (DP), and *S. polycystum* (DS) showed strong growth results, with control (C) animals exhibiting the highest survival rate. Diets *H. discoidea* (DH), and pond moss (DM) exhibited comparatively lower growth performance, while diet *U. lactuca* (DU) yielded the lowest levels of growth and survival. Although diet green algae pond (DA) resulted in a lower survival rate, it induced the highest weight increase and the highest growth rate. The growth performance of early juvenile *H. scabra* are influenced by the type of diet provided (Table 5).

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *H. discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values in the same row with different superscripts differ significantly ($p < 0.05$).

2.2.1. Growth rate

There were significant differences in growth rates of juvenile *H. scabra* fed different fermented diets (Fig. 2, $p < 0.05$). The highest and the lowest values of GR±SE (0.09 ± 0.023 and 0.01 ± 0.001 g d^{-1}) were observed in treatment fed with green algae pond (DA), and *U. lactuca* (DU), respectively. Based on the Dunn's post hoc test with Bonferroni correction, the GR of *H. scabra* fed with diet green algae pond (DA) is significantly higher than those fed with diets *Halimeda* sp. (DH), pond moss (DM), and *U. lactuca* (DU), ($p < 0.05$). However, there were no significant differences observed between green algae pond (DA), and *P. australis* (DP), *S. polycystum* (DS). Furthermore, growth when fed diet *U. lactuca* (DU), was significantly lower than pond moss (DM) and *S. polycystum* (DS), ($p < 0.05$). No further significant differences were observed between the diets.

2.2.2. Specific growth rate

The ANOVA test results indicate that there is a significant difference in SGR between the different diets (Fig. 3., $p < 0.001$). Post hoc analysis using Tukey's (HSD) test showed that diet groups of *H. discoidea* (DH), and *E. acoroides* (C) differsignificantly ($p < 0.05$). There is no significant difference between the *P. australis* (DP), and *E. acoroides* (C) diet groups ($p < 0.05$).

2.2.3. Survival rate

The results of the one-way ANOVA indicated a significant effect of different dietary treatments on the survival rate of early juvenile *H. scabra* (Fig. 4., $p < 0.001$). Post hoc analysis using Tukey's (HSD) test showed the mean survival values of green pond algae diet (DA), *H. discoidea* (DH), and pond moss (DM) diets were significantly lower than diet *E. acoroides* (C). Similarly, survival rates for diets green pond algae diet (DA) and *U. lactuca* (DU) were also found to be significantly lower compared to diet *E. acoroides* (C).

2.3. Faeces production rate (FPR)

Different diets significantly affect the Faeces Production Rate (FPR) in early juvenile *H. scabra* at various times, as shown by the significant Kruskal-Wallis test result (Fig. 5., $p < 0.001$). According to the Dunn's post-hoc test, with Bonferroni correction applied, at day 1, there are significant differences between diet *U. lactuca* (DU), and green pond algae (DA) ($p = 0.0133$), *U. lactuca* (DU), and pond moss (DM) ($p = 0.0098$), and *U. lactuca* (DU), and *P. australis* (DP) ($p = 0.0179$). At day 28, significant differences in FPR are observed between diet groups moss (DM) and *H. discoidea* (DH) ($p = 0.0192$), *Sargassum* sp. (DS), and

Table 4
Nutritional composition of various macroalgae diets for different species of sea cucumber.

Sea cucumber	Diet macroalgae	Type	Ash (%)	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Dry matter (%)	Ref
Apostichopus japonicus	*Sargassum thunbergii*	powder*	76.78	-	3.93	0.18	-	Li et al. (2022)
	Sargassum polycystum	powder*	76.38	-	3.10	0.13	-	Li et al. (2022)
	Sargassum horneri	powder*	75.30	-	5.25	0.13	-	Li et al. (2022)
	Enteromorpha prolifera	powder*	75.30	-	3.05	0.25	-	Li et al. (2022)
	Macrocystis pyrifera	powder*	73.60	-	2.59	0.13	-	Li et al. (2022)
	Ulva fasciata	powder	22.4	7.18	15.70	2.26	-	Liao et al. (2021)
	Gracilaria tenuistipitata	powder	15.40	6.44	17.10	1.40	-	Liao et al. (2021)
	Laminaria japonica	powder	28.98	8.02	10.23	1.25	-	Liao et al. (2021)
	Chaetomorpha linum	powder	48.20	-	16.49	0.53	-	Song et al. (2017)
	Sargassum thunbergii	powder	33.97	-	18.90	0.76	-	Song et al. (2017)
	Laminaria japonica	feedstuff*	37.1	-	14.6	4.60	96.80	Wang et al. (2015)
	Sargassum thunbergii	powder*	75.84	-	5.02	0.75	96.80	Wu et al. (2015)
	Sargassum thunbergii	powder*	30.61	-	16.00	4.42	-	Shi et al. (2013)
	Sargassum thunbergii	powder*	74.93	-	4.65	0.77	96.86	Xia et al. (2012a), (2012b)
	Sargassum polycystum	powder*	73.37	-	5.97	0.85	96.25	Xia et al. (2012a), (2012b)
	Zostera marina	powder*	74.01	-	5.34	0.80	96.48	Xia et al. (2012a), (2012b)
	Ulva lactuca	powder*	76.30	-	3.42	0.50	97.05	Xia et al. (2012a), (2012b)
	Laminaria japonica	fresh*	77.11	-	3.50	0.65	96.98	Xia et al. (2012a), (2012b)
	Laminaria japonica	boiled*	80.86	-	3.23	0.47	97.82	Xia et al. (2012a), (2012b)
Isostichopus badionotus	*Macrocystis* sp	powder*	33.08	10.06	8.54	1.14	-	Zacarías-Soto et al. (2018)
	Sargassum sp	powder*	30.31	8.91	8.37	0.85	-	Zacarías-Soto et al. (2018)
	Macrocystis pyrifera	powder*	33.08	10.06	8.54	-	-	Martínez-Milián and Olvera-Novoa, 2016
	Solieria filiformis	powder*	8.18	6.44	18.8	-	-	Martínez-Milián and Olvera-Novoa, 2016
Stichopus cf. *horrens*	*Sargassum* sp	detritus*	3.34	94.47	1.23	0.04	-	Palomar-Abesamis et al. (2018)

*one of the ingredients in the mixed diet

Table 5
Mean growth performance (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks).

Diet	C	DH	DU	DP	DS	DA	DM
Average initial weight (g)	0.16 ± 0.005	0.17 ± 0.005	0.17 ± 0.003	0.17 ± 0.004	0.17 ± 0.005	0.17 ± 0.005	0.17 ± 0.004
Average final weight (g)	1.70 ± 0.047	1.04 ± 0.102	0.48 ± 0.057	2.00 ± 0.097	2.14 ± 0.079	5.17 ± 1.305	1.30 ± 0.146
Growth rate (g d^{-1})	0.03 ± 0.001^{a}	0.02 ± 0.002^{b}	0.01 ± 0.001^{c}	0.03 ± 0.002^{a}	0.04 ± 0.001^{a}	0.09 ± 0.023^{a}	0.02 ± 0.003^{b}
Specific Growth Rate (% d^{-1})	4.17 ± 0.058^{a}	3.17 ± 0.183^{b}	1.78 ± 0.187^{c}	4.37 ± 0.124^{a}	4.49 ± 0.100^{a}	5.90 ± 0.390^{d}	3.58 ± 0.244^{a}
Survival Rate (%)	80.63 ± 4.375^{a}	69.38 ± 7.526^{ab}	8.75 ± 1.614^{c}	80.63 ± 2.772^{a}	63.75 ± 1.614^{ab}	31.88 ± 8.315^{b}	50.00 ± 4.677^{ab}
Biomass density (g m^{-2})	15.77 ± 0.43^{a}	9.59 ± 0.95^{b}	4.44 ± 0.53^{c}	18.47 ± 0.90^{a}	19.79 ± 0.73^{a}	47.88 ± 12.08^{d}	12.08 ± 1.35^{b}

H. discoidea (DH) ($p = 0.0123$), and *U. lactuca* (DU), and *H. discoidea* ($p = 0.0239$). While at day 56, a significant difference is found between diet *P. australis* (DP), and pond moss (DM) ($p < 0.01$). In this study, early juveniles of *H. scabra* fed with *P. australis* (DP), green pond algae (DA), and pond moss (DM) diets showed the highest FPR on day 1, with the *U. lactuca* (DU) diet recording the lowest rate. However, by day 28, juveniles on the *H. discoidea* (DH), and *P. australis* (DP) diets maintained the highest FPR, while the *S. polycystum.* (DS) and pond moss diets reported the lowest rates. By day 56, only juveniles on the *P. australis* (DP) diet maintained a high FPR, with those on the moss diet showing the lowest rate (Table 6).

2.4. Biomass density

The results of the Generalized Linear Mixed Model (GLMM) analysis indicate that diet and time have a significant effect on biomass density. Regarding the Time variable, the difference between all levels (Day 0, Day 28, and Day 56) is statistically significant ($p < 0.001$). This means that the biomass density significantly differs at each observation time. There are numerous significant Diet and Time interactions for the Diet factor. The difference in biomass density between the green pond algae diet (DA) and other diets at Day 28 and Day 56 is statistically significant ($p < 0.01$). The same applies to diet *U. lactuca* (DU) at Day 28 and Day 56. In addition, there are significant interactions between diet *H. discoidea* (DH) at Day 56, diet green pond algae (DA) at Day 28 and Day 56, and diet *U. lactuca* (DU) at Day 28 and Day 56 ($p < 0.01$).

Diet *S. polycystum* (DS), and green pond algae (DA) diets produced the highest mean biomass densities (± SE) (19.79 ± 0.73 and 47.88 ± 12.08 g m^{-2}, respectively), suggesting their ability to promote sea cucumber growth (Table 4, Fig. 6). The *U. lactuca* (DU) diet, on the other hand, had the lowest result (4.44 ± 0.53 g m^{-2}), implying that it may be less ideal for juvenile *H. scabra*. The other diets, *E. acoroides* (C), *H. discoidea* (DH), *P. australis* (DP), and pond moss (DM), produced outcomes ranging from moderate to low. These data show that types of diet have a major impact on *H. scabra* growth.

2.5. Total organic matter

The statistical analyses using Kruskal-Wallis test and post hoc Mann-Whitney test with multiple comparison adjustment revealed significant differences in Total Organic Matter (TOM) among the diet treatments. The Kruskal-Wallis test indicated a highly significant difference ($p < 0.001$) across the treatments. Further post hoc Mann-Whitney tests, with adjustments for multiple comparisons, revealed a significant difference between the *H. discoidea* (DH), and pond moss (DM) diets. However, no significant differences were found between the remaining pairs of diet treatments. These findings suggest that the impact of diet on TOM varies across different types of diets. Total Organic Matter (TOM)

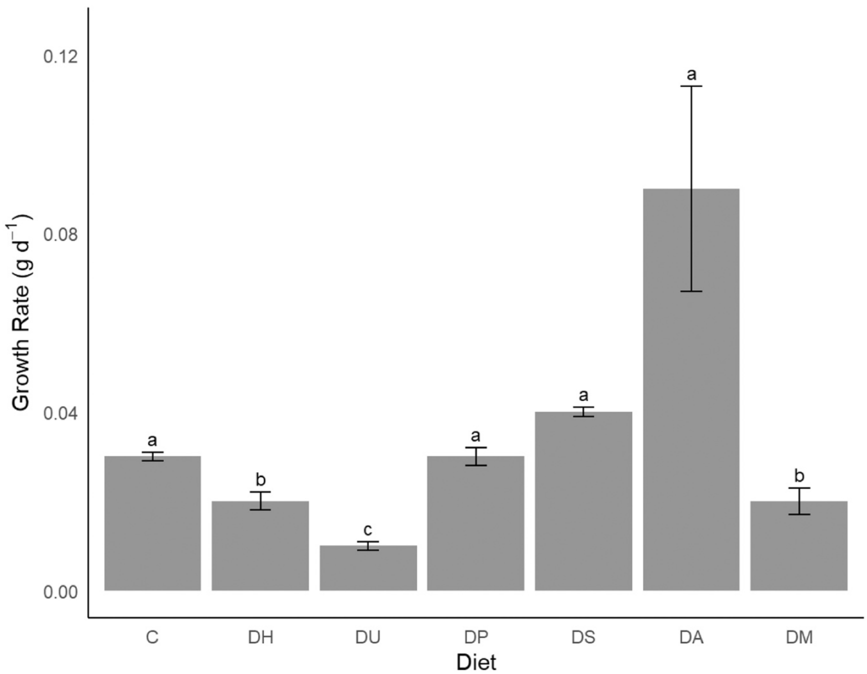

Fig. 2. Mean Growth Rate (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly ($p < 0.05$).

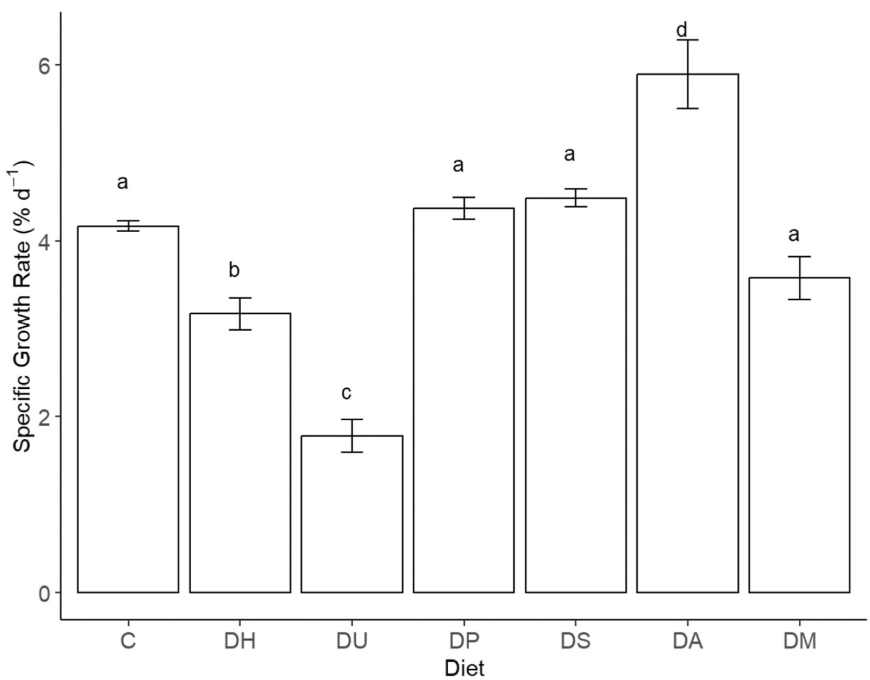

Fig. 3. Specific Growth Rates (SGR) (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly ($p < 0.05$).

observations revealed varying trends between treatments (Fig. 7, Table 7). Throughout the monitoring period, there was a steady increase in TOM concentration in the *H. discoidea* (DH), diet. The mean TOM concentration (± SE) was 39.50 ± 1.83 mg L^{-1} at the start of the experiment (day 0). On day 56, this concentration increased to 58.78 ± 2.00 mg L^{-1}. The moss diet (DM), on the other hand, exhibited a distinct dynamic. The TOM content in this diet decreased on day 28, then increased to 82.16 ± 2.10 mg L^{-1} on day 56.

2.6. Water quality

Overall, the parameters of temperature, pH, salinity, and Dissolved Oxygen (DO) were similar across seven diet treatments. The range of water temperature was between 24.40 - 30.80 °C, salinity between

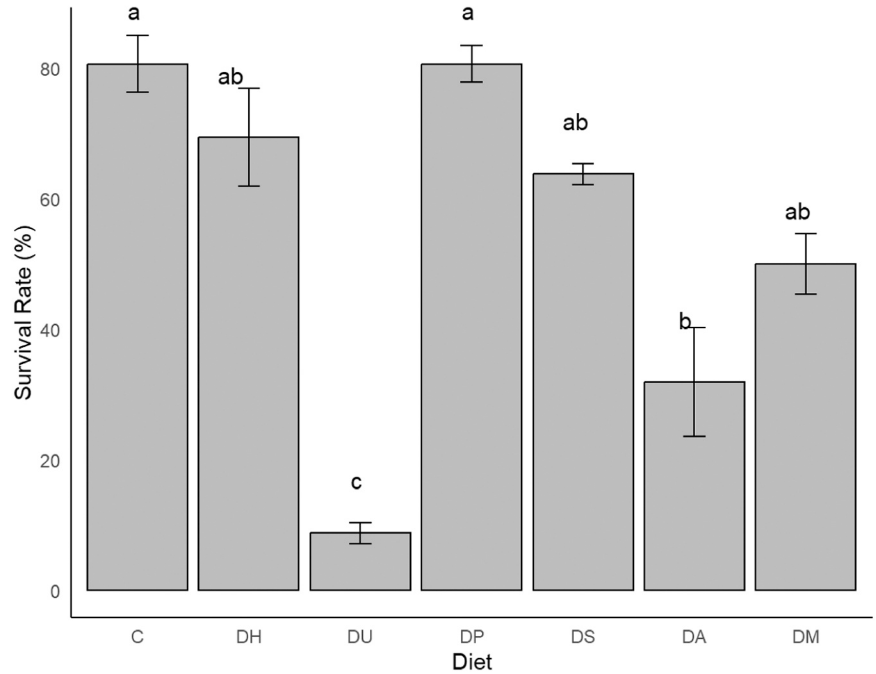

Fig. 4. Survival Rates (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly (p < 0.05).

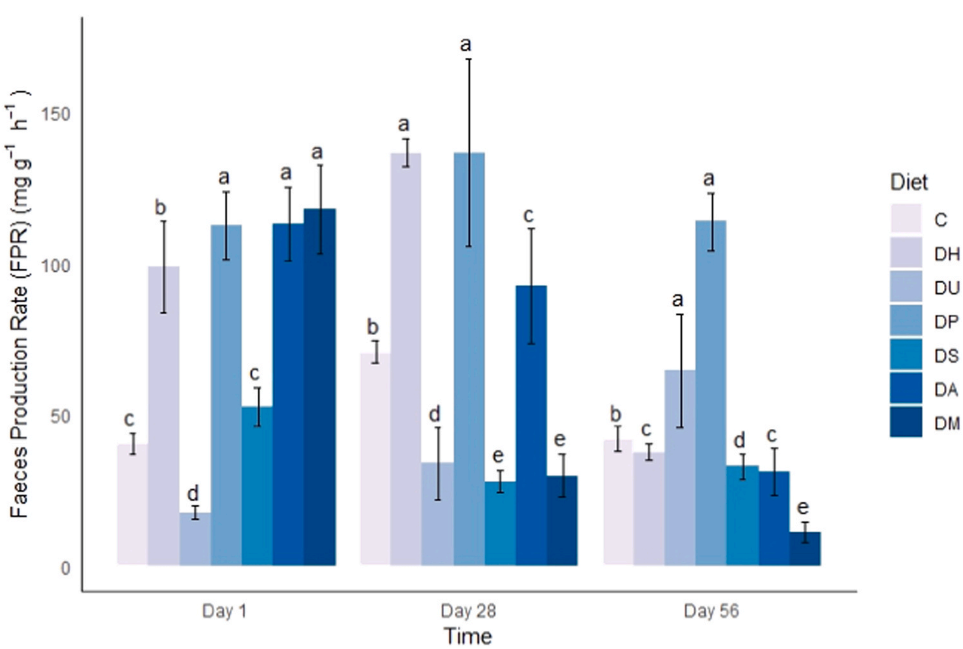

Fig. 5. Faeces Production Rate (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly (p < 0.05).

33–37 ppt, pH between 7–8.20, and DO between 4.40–6.70 mg L^{-1} (Table 8). The mean values of each parameter indicate that the water quality conditions were generally consistent across all diet treatments.

2.7. Nitrite nitrate and ammonia

Diet *E. acoroides* (C) produced the highest concentrations of ammonia and nitrate. Diets *H. discoidea* (DH), and *U. lactuca* (DU) show the lowest concentrations of nitrite, which could mean a minimal negative impact on the nitrogen balance in the system. Diets *P. australis* (DP), and *S. polycystum* (DS) indicated low and stable nitrate concentrations, suggesting good nitrate absorption. Diet green pond algae (DA) produced high nitrite concentrations on the 56th day, which could indicate potential toxicity (Table 9).

3. Discussion

The results of this study showed that fermented plant-based diets had

Table 6
Mean Faeces Production Rate (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks).

Diet	Day 1	Day 28	Day 56
C	40.35 ± 3.57^{c}	70.87 ± 3.85^{b}	42.07 ± 4.05^{b}
DH	99.09 ± 15.19^{b}	136.59 ± 4.70^{a}	37.62 ± 2.69^{c}
DU	17.65 ± 2.13^{d}	33.82 ± 12.17^{d}	64.56 ± 18.83^{a}
DP	112.69 ± 11.29^{a}	136.74 ± 31.12^{a}	113.99 ± 9.54^{a}
DS	52.53 ± 6.28^{c}	27.86 ± 3.81^{e}	32.92 ± 4.29^{d}
DA	113.05 ± 12.13^{a}	92.40 ± 19.04^{c}	30.89 ± 7.79^{c}
DM	117.97 ± 14.48^{a}	29.82 ± 7.22^{e}	11.18 ± 3.56^{e}

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values in the same row with different superscripts differ significantly ($p < 0.05$).

a substantial influence on the growth performance of early juveniles of *H. scabra.* Several of the diets outperformed the control in terms of individual growth and overall biomass production. This study found that the diet consisting of *U. lactuca* (DU) resulted in the lowest growth rate (Table 4). This finding contrasts with the research conducted by Xia et al. (2012a), which suggested that sea cucumber *A. japonicus* exhibit faster growth when fed diets of *Ulva lactuca* or *Laminaria japonica*, compared to a diet of *Sargassum spp*. Further, Xia et al. (2012a) indicated that *U. lactuca* and *L. japonica* are the optimal types of seaweed for cultivating the commercially valuable sea cucumber species, *A. japonicus*.

Numerous studies have demonstrated that the composition of the diet has a substantial impact on the growth rate of sea cucumber. The growth of *A. japonicus* is significantly influenced by the type of seaweed ingested (Xia et al., 2012a). Similar to this, the type of feed generally impacts aspects like ingestion rate and digestibility, which have an impact on growth rates of organisms like sea cucumbers (Xia et al., 2012a). Additionally, diet composition significantly affected the growth rate of *I. badionotus* (Zacarías-Soto et al., 2018).

Specific Growth Rate (SGR) of many sea cucumbers species, including sub-adult *H. scabra*, juvenile *A. japonicus*, and juvenile *I. badionotus*, is significantly influenced by the type and composition of their diet (Wu et al., 2015; Song et al., 2017; Zacarías-Soto et al., 2018; Broom et al., 2021).

The results of the present study show that the highest SGR is obtained when feeding green pond algae (DA) diet ($5.90 \pm 0.390\%\ d^{-1}$), followed by the *S. polycystum* (DS) ($4.49 \pm 0.100\%\ d^{-1}$), *P. australis* (DP) ($4.37 \pm 0.124\%\ d^{-1}$), and the diet *E. acoroides* (C) ($4.17 \pm 0.058\%\ d^{-1}$). Further pilot application and testing as a diet for other aquaculture species is highly recommended. The species composition of the green algae in the current study is unknown, however generally the green-tide algae include *Ulva prolifera* and *Ulva linza*, as mentioned by Luo et al. (2012). The potential variability of species make-up and seasonal changes in the composition of green pond algae should be considered prior to further development of its use as a potential diet source for early juvenile sea cucumbers.

The diet of *U. lactuca* (DU) resulted in the lowest SGR value of $1.78 \pm 0.187\%\ d^{-1}$. According to research by Anisuzzaman et al. (2017), the SGR of juvenile *A. japonicus* fed with a diet containing 15% *Ulva lactuca* powder reached $1.58\%\ d^{-1}$, the highest value recorded when compared to all other diet treatments, including those with 15% *Sargassum thunbergia* powder. In addition, Wu et al. (2015) used *S. thunbergii* as one of the components in the feeding treatment for *A. japonicus*, and their results showed that a diet consisting of 70% marine mud, 15% *S. thunbergii,* and 15% corn leaves yielded the highest SGR ($0.59 \pm 0.04\%\ d^{-1}$) compared to other diets.

The control diet source in this study was the seagrass *E. acoroides* which is commonly used in pulverized form in hatcheries for later juvenile stages of *H. scabra*. Song et al. (2017), showed powdered seagrass *Zostera marina* feed for *A. japonicus*, resulted in an SGR of $0.29 \pm 0.04\%\ d^{-1}$ when mixed in a ratio of 40% *Z. marina* combined with 60% muddy sediment. In comparison, the seagrass diet provided in the current study resulted in an SGR of $4.17 \pm 0.058\%\ d^{-1}$ indicating a substantially higher SGR for juvenile *H. scabra* when fed with *E. acoroides* compared to *A. japonicus* fed with *Z. marina*.

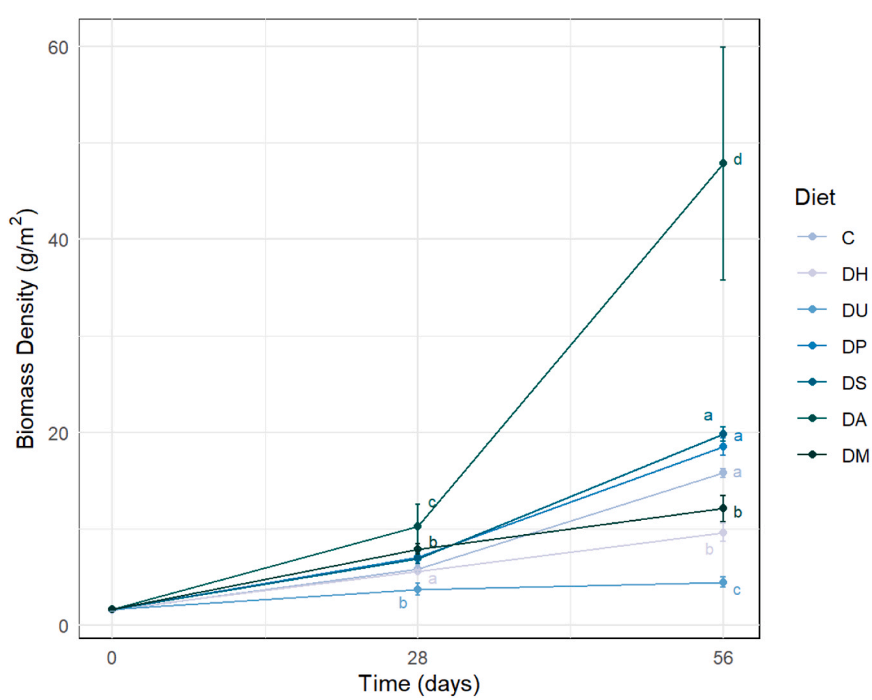

Fig. 6. Biomass density (± SE) of juvenile *Holothuria scabra* fed different fermented diets over a period of 56 days under controlled conditions. N = 4 (Tanks). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly ($p < 0.05$).

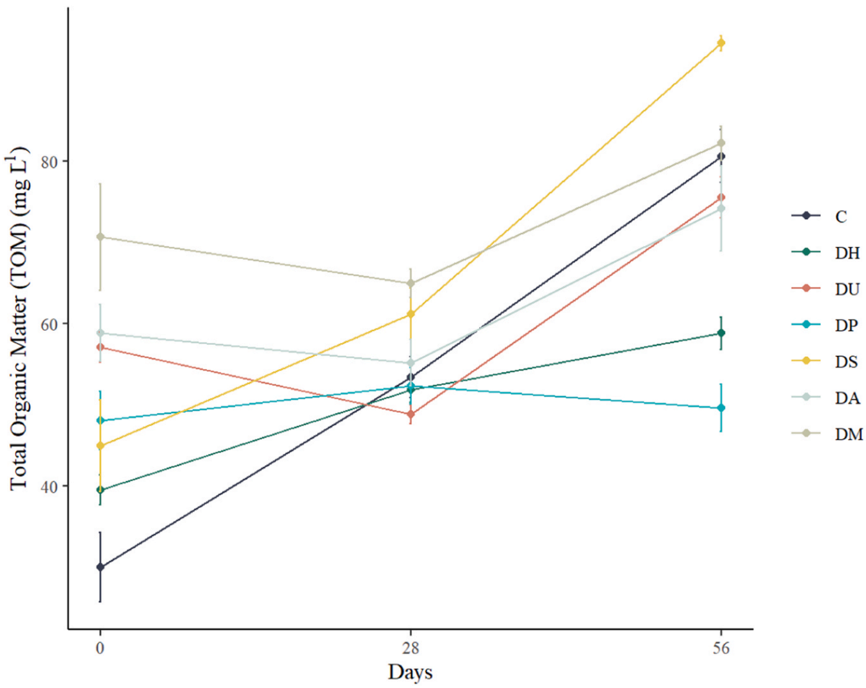

Fig. 7. Total Organic Matter of different diets treatments for juvenile *Holothuria scabra*. Values were given as mean±SE (n = 4). C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss. Values with different superscripts differ significantly ($p < 0.05$).

Table 7
Total Organic Matter (± SE) over a period of 56 days under controlled conditions.

Diet	Total Organic Matter (mg L^{-1})		
	Day 0	Day 28	Day 56
C	30.02 ± 4.27	53.40 ± 2.51	80.58 ± 3.29
DH	39.50 ± 1.83	51.82 ± 1.57	58.78 ± 2.00
DU	57.04 ± 1.81	48.82 ± 1.14	75.52 ± 2.51
DP	48.03 ± 3.55	52.30 ± 2.20	49.61 ± 2.92
DS	44.93 ± 5.59	61.15 ± 3.14	94.48 ± 0.91
DA	58.78 ± 3.51	55.14 ± 2.87	74.10 ± 5.25
DM	70.63 ± 6.54	64.94 ± 1.76	82.16 ± 2.10

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss.

The survival rate of *H. scabra* fed over 56 days trial period varied between 8% and 80%. In comparison to diets *E. acoroides* (C), and *P. australis* (DP), which had the same survival rate (80.6%), the diet green pond algae (DA) had a lower survival rate (31.9%), despite having the highest growth rate value. The statistical analysis demonstrated that there was a significant difference between Survival Rate on various different diets. In particular, survival of diets green pond algae (DA), pond moss (DM) and *U. lactuca* (DU), all had significantly lower rates compared to the diet *E. acoroides* (C). These findings suggest that the influence of diet on survival rate is likely to differ from one type of diet to another and may vary depending on the specific composition of the diet. A previous study demonstrated that juvenile *H. scabra*, starting with an initial weight of 14 g and reared for 20 weeks on an ad libitum mixed diet of sea grass *E. acoroides*, Napier grass *Pennisetum purpureum*, and cow manure, had survival rates that were lower than the results of this study, with 28.21 ± 2.29% in the treatment indoor concrete tanks and 56.67 ± 6.67% in indoor fiberglass tanks (Indriana et al., 2017). Diet *U. lactuca* had the lowest rate of survival (8.75 ± 1.61%). Therefore, this diet, which uses a fermentation process using the macroalgae *U. lactuca* as the basic material, is not recommended.

In this study, we continuously collected each species of macroalgae from their respective locations as raw material for fermented diet, ensuring uniformity throughout experimental period. The composition and distribution of macroalgae are heavily influenced by changing environmental and physical factors such as nutrient availability, water quality, light intensity, salinity, temperature, and the characteristics of the water column and sediments, which vary significantly across

Table 8
Mean and range of temperature, salinity, pH and dissolved oxygen over the 56 experimental days, measured at 16:00.

Diet	Water temperature (°C)		Salinity (ppt)		pH		Dissolved Oxygen (mgL^{-1})	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
C	27.49	24.50 - 30.00	34.55	33 - 36	7.91	7.60 - 8.10	5.86	5.00 - 6.70
DH	27.60	24.70 - 30.20	34.57	33 - 36	7.91	7.70 - 8.10	5.83	5.30 - 6.60
DU	27.59	24.40 - 30.10	34.45	33 - 37	7.77	7.40 - 8.20	5.58	4.80 - 6.60
DP	27.56	24.70 - 30.10	34.60	33 - 36	7.90	7.00 - 8.10	5.93	5.00 - 6.70
DS	27.51	24.50 - 30.10	34.50	33 - 36	7.85	7.00 - 8.10	5.87	4.90 - 6.60
DA	27.46	24.60 - 30.00	34.55	33 - 36	7.94	7.70 - 8.10	5.86	5.40 - 6.50
DM	27.59	24.70 - 30.80	34.60	33 - 37	7.85	7.60 - 8.10	5.68	4.40 - 6.60

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australis*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss.

Table 9
Physico-chemical parameters over the 56 experimental days.

Diet	ammonia NH3-N			nitrite NO2-N			nitrate NO3-N		
	0	28	56	0	28	56	0	28	56
C	0.26 ± 0.21	0.46 ± 0.15	0.16 ± 0.03	0.008 ± 0.00	0.118 ± 0.06	1.835 ± 0.08	1.13 ± 0.05	1.75 ± 0.38	0.08 ± 0.00
DH	0.13 ± 0.04	0.24 ± 0.04	0.10 ± 0.03	0.005 ± 0.00	0.003 ± 0.00	0.020 ± 0.01	1.08 ± 0.33	1.00 ± 0.11	0.02 ± 0.00
DU	0.02 ± 0.01	0.14 ± 0.07	0.19 ± 0.13	0.009 ± 0.00	0.006 ± 0.00	0.010 ± 0.00	2.03 ± 0.13	0.85 ± 0.03	0.04 ± 0.00
DP	0.14 ± 0.04	0.17 ± 0.05	0.15 ± 0.04	0.003 ± 0.00	0.002 ± 0.00	0.009 ± 0.00	0.98 ± 0.11	0.93 ± 0.05	0.02 ± 0.00
DS	0.08 ± 0.05	0.12 ± 0.05	0.04 ± 0.02	0.010 ± 0.00	0.001 ± 0.00	0.007 ± 0.00	1.85 ± 0.15	0.78 ± 0.05	0.02 ± 0.00
DA	0.05 ± 0.02	0.09 ± 0.08	0.16 ± 0.08	0.006 ± 0.00	0.016 ± 0.00	1.575 ± 0.72	1.38 ± 0.22	1.68 ± 0.41	0.06 ± 0.00
DM	0.20 ± 0.08	0.50 ± 0.04	0.15 ± 0.03	0.003 ± 0.00	0.002 ± 0.00	0.018 ± 0.00	1.15 ± 0.13	0.93 ± 0.03	0.03 ± 0.00

C (control), diet seagrass *Enhalus acoroides*; DH, diet macroalgae *Halimeda discoidea*, DU, diet macroalgae *Ulva lactuca*, DP, diet macroalgae *Padina australi*, DS, diet macroalgae *Sargassum polycystum*, DA, diet green pond algae; DM, diet pond moss.

different regions and even seasonally within the same region (Fong et al., 2001; Collado-Vides et al., 2011; Costa et al., 2021). The nutritional content of macroalgae is greatly affected by geographic location, resulting in variances in specimens observed in different places (Wan et al., 2019). Due to the variability in macroalgal composition and nutrient content across different regions and environmental conditions, it is likely that the nutritional value of macroalgae used as feed for sea cucumbers will not be the same when sourced from different locations.

In addition, some studies revealed contrasting feeding strategies among sea cucumbers in different climates. According to Slater et al. (2011) the temperate sea cucumber *Australostichopus mollis* demonstrated strong organic selectivity, particularly in areas containing a high proportion of organic content, but this selectivity decreases when Total Organic Matter (TOM) exceeds 3%. On the other hand, Uthicke and Karez (1999) study on tropical species such as *Stichopus chloronotus* and *S. variegatus* showed significant sediment patch selectivity, preferring patches with higher microalgal biomass, while *Holothuria atra, H. edulis,* and *H. nobilis* revealed minimal to no patch selectivity. This difference in feeding behaviors between temperate and tropical sea cucumbers indicates their adaptability to local environments, this aspect is crucial for understanding their ecological functions and informs the development of sustainable aquaculture practices in diverse climatic regions.

In the current study, the stocking density was recorded at 370.37 juveniles m^{-2} with an average initial weight of 0.168 g. This approaching the hatchery industry in east Lombok, Indonesia that uses geomembrane round-pools (diameter 6 m) for the rearing of early juveniles of *H. scabra* (0.1 to 0.2 g) with the stocking density at 353.73 juveniles m^{-2}.

During the first 21 days of the experiment using the green pond algae diet (DA), juveniles exhibited a pronounced decline in the survival rate. This could be a result of their adaptation process to the new diet and potential stress experienced by some individuals. However, following this adaptation period, the survival remained stable from day 21 to day 56, reaching a figure of almost 32%. This suggests that the experimental conditions might have been conducive and supportive of the growth and survival of the juveniles. Nonetheless, a deeper analysis regarding the nutritional composition of the green pond algae diet (DA) is highly recommended for future studies.

Different fermented diet can affect the Faeces Production Rate (FPR) of early juveniles *H. scabra*, which correlates with growth and survival. This is in line with the research by Yuan et al. (2006) on *A. japonicus*. In this study, significant variations were found in FPR based on diet and throughout experimental period. Diet *E. acoroides* (C), *H. discoidea* (DH), *P. australis* (DP), and *S. polycystum* (DS), appear to demonstrate good digestive adaptation to fermented diet, which was reflected in the high SGR and survival. Although FPR for some of these diets was high at certain points compared to other diets, this balance and variability seemed to be acceptable in the context of good growth and survival. The optimal FPR value may indicate that early juveniles are adapting their digestion to diet, thereby increasing feed efficiency and their growth. Conversely, diet green pond algae (DA) and pond moss (DM) showed a significant decrease in FPR, which may indicate increased digestive efficiency or reduced food consumption.

Liu et al. (2009) indicated that the FPR of *A. japonicus* decreases as the percentage of dietary sea mud or yellow soil increases, but there is no significant difference in FPR among *A. japonicus* fed diets containing the same proportion of sea mud and yellow soil. The average FPR values for three size groups of *A. japonicus* were reported as 4.0 ± 0.8 (small), 4.9 ± 0.4 (medium), and 4.8 ± 0.4 mg g^{-1} h^{-1} (large) according to Sun et al. (2015). Zamora and Jeff (2011) determined diet treatments with 1%, 4%, 12%, and 20% TOM (Total Organic Matter) for *Australostichopus mollis*, resulting in mean FPR of 2.5 ± 0.8, 3.8 ± 1.6, 3.4 ± 0.8, and 3.0 ± 0.4 mg g $ww^{-1}h^{-1}$, respectively.

In terms of biomass density, the results of the study show that the diet green pond algae (DA) have the highest biomass density (47.88 ± 12.08 g m^{-2}), making it the most effective diet to support the growth rate of early juveniles *H. scabra*, followed by diet *S. polycystum* (DS), and *Padina* sp. (DP). Meanwhile, diets *E. acoroides* (C), pond moss (DM), and *H. discoidea* (DH), are less effective, and diet *U. lactuca* (DU), is the least effective among all the diets tested (4.44 ± 0.53 g m^{-2}).

Senff et al. (2022) indicated that the biomass density of *H. scabra* with the initial wet weight of 16.8 ± 0.6 g treated with bagasse significantly outperformed that without supplementation, with respective values of 205.71 ± 11.08 g m^{-2} and 134.76 ± 14.09 g m^{-2}. A study by Robinson et al. (2015) indicated that the biomass density of *H. scabra* with initial weight of 7.16 ± 0.22 g from oxic redox regime was 461.68 ± 18.01 g m^{-1}, whereas in oxic-anoxic conditions there was a higher final biomass density of 715.76 ± 75.97 g $m^{-1}$with the initial weight 7.16 ± 0.33 g. Further research conducted by Robinson et al. (2019) found that during the bioremediation of aquaculture waste, *H. scabra* with initial weight 4.08 ± 0.58 g in the starch treatment reached a final biomass density of 1.011.46 ± 75.58 g m^{-2} by the fourth month, while the control tanks only reached 702.12 ± 35.93 g m^{-2}. Robinson et al. (2015) argued the importance of C:N ratio and the important role of bacterial vectors in improving food digestibility and nutrient availability, as achieved in the current study via fermentation.

The correlation between the growth of sea cucumbers and the fermentation process of their feed is indeed a pivotal aspect of this research. Fermentation is known to increase the bioavailability of nutrients in the feed, enabling the juveniles to assimilate these nutrients more efficiently, thereby improving growth performance. Generally, sea cucumber feed uses macroalgae powder as one of the component ingredients mixed with other substances. However, this still has some limitations; therefore, a new type of alternative feed needs to be developed for sea cucumbers, particularly in *H. scabra* species. Fermented feed could be a considerable alternative for providing mass-scale sea cucumber feed, especially during the juvenile phase. The results of this study indicate that fermented feed affects the growth and survival of early juvenile *H. scabra*. Seo et al. (2011a) suggest that microbial activities reduce antinutritional factors and enhance lipid absorption, thereby increasing the nutrient value of the diet during fermentation. In addition, Sun et al. (2016) determined that fermenting juvenile sea cucumber diet with *Bacillus* and *Lactobacillus casei* not only improved the feed quality but also led to higher growth rates without compromising effects on water quality, suggesting a sustainable method to boost aquaculture growth performance. The general effects of macroalgal fermentation in terms of macronutrient composition and nutrient avai-lablility are increased protein and decreased carbohydrates however this varies markedly across algae and fermentation techniques. It can be expected in the current study that fermented diets will contains more protein and more available bioactives and bacterial biomass and exudates.

Pérez-Alva et al. (2022); Norakma et al. (2022) indicate that macroalgae fermentation has the potential to improve nutritional value and bioactive properties. The fermentation process is increasing bioavailability of trace elements and digestibility, as well as changing the balance between nutritive and anti-nutritive components in macroalgae. During the fermentation process, significant changes occur in the amino acid composition, phenolic profiles, and volatile chemical profiles, all of which lead to an enhancement in biological functioning and nutrient bioavailability. Furthermore, fermentation is a promising alternative to traditional extraction methods and provides advantages such as increased yields and enhanced features of valuable compounds like enzymes and organic acids. In addition, Ang et al. (2021) investigated the specific impacts of macroalgae fermentation on its nutritional content. Protein content consistently increases after fermentation, while the effect on carbohydrates and lipids varies according to the type of microbial fermentation used. Bacterial fermentation may decrease carbohydrate content, whereas fungal fermentation tends to increase it. Lipid content generally declines in fermented macroalgae, despite there are slight increases in fat and fatty acid content. Ash content also shows improvement after fermentation. In general, the fermentation process of macroalgae significantly improves nutritional components including ash, proteins, carbohydrates, and lipids, it represents a valuable approach to enhancing the nutritional composition of algae.

1. The fermented feed had a greater growth rate and better growth performance than the unfermented feed, making it a useful practice for enhancing growth rates while minimizing environmental impact. Furthermore, to optimize the fermentation process and enhance the nutritional value of the feed intended for *Apostichopus japonicus*, Jiang et al. (2015) selected various microbes intending to improve the health and growth of sea cucumbers.**Conclusions**

Results suggest that the green pond algae diet can trigger rapid growth in early juvenile *H. scabra*. However, the survival rate of sea cucumbers on diet green pond algae (DA) is low, indicating that this diet also potentially causes stress or other negative effects on very early juvenile *H. scabra*, impacting their mortality rate. These adverse effects could be caused by various factors, such as an unbalanced nutrient composition of the diet. Further analysis is required to identify these causative factors. The FPR results show early juveniles fed diet green pond algae experienced a significant decrease in feces production with increasing experimental period. This reduction could indicate that early juveniles are experiencing stress affecting metabolism and nutrient assimilation, which may also contribute to the low survival rate. While diet green pond algae (DA) appear promising in terms of growth and shows the highest biomass value among other diet treatments, these results suggest that DA may not be optimal for early juveniles *H. scabra* cultivation in the long run. Therefore, further adjustments and optimization of this diet are needed to achieve a balance between growth and survival of early juveniles *H. scabra*.

Diet green pond algae (DA) might be more suitable for larger and stress-tolerant juvenile *H. scabra*, while diets *E. acoroides* (C), *P. australis* (DP), *S. polycystum* (DS), and pond moss (DM) might be more appropriate for smaller and sensitive juveniles. Diet *U. lactuca* (DU) is unsuitable as a diet source for early juveniles due to its low growth and survival rates. Diet *E. acoroides* (C), and *P. australis* (DP) have competitive growth rates, high survival (>80%), and reasonably constant values for all parameters. These two diets, which produce a moderate FPR that suggests efficient digestion, could be considered as potential candidates for early juvenile *H. scabra* diet.

CRediT authorship contribution statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm.

that the order of authors listed in the manuscript has been approved by all of us.We understand that the Corresponding Author is the sole contact for the Editorial process.He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lisa Fajar Indriana reports financial support was provided by German Academic Exchange Service.

Data availability

I have shared the link to my data/code at the attach file step.

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