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Effects of Dietary Lipid Sources on the Growth, Gonad Development, Fatty Acid Composition and Spawning Performance of Broodstock, and Early Larvae Quality of Sea Urchin (*Strongylocentrotus intermedius*)

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This study was carried out to investigate the effects of five formulated feeds with different lipid sources (colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO)) and kelp (*Laminaria japonica*) on the growth, reproductive performance of broodstock, and early larvae quality of sea urchin (*Strongylocentrotus intermedius*). The experimental diets were randomly allocated to a total of 48 (eight urchins per experimental group) individually cultured *S. intermedius* (initial weight 90.51 ± 0.82g) and the feeding period lasted for 12 weeks. The results showed that the weight gain rate of female sea urchins fed kelp was significantly higher than those fed formulated feeds while the gonadosomatic index of spawned female sea urchins fed kelp was significantly lower than those fed formulated feeds. *S. intermedius* broodstock fed FO showed the largest egg diameter and fecundity, which could be due to the abundant n-3 LC-PUFA deposited in the gonads of this group. Sea urchins fed SO showed the lowest fecundity and hatching rate, and the highest inflammation level. Sea urchins fed CO showed the highest content of oleic acid in the gonads and eggs, but the lowest fertilization rate. The highest hatching rate was observed in the kelp treatment, which was comparable to that in the LO and PO but was significantly higher than that in CO, FO, and SO. Before mouth opening, all prismatic larvae showed no significant differences in survival during the first 2 days post hatchery (DPH). At the 3 DPH, the survival of *S. intermedius* larvae was highest in the FO group, followed by those in the PO group, with the lowest survival observed in the kelp group. Thus, FO was accepted as the most ideal lipid source based on growth, reproductive performance, and early larval quality. These results could contribute to adopting an efficient feeding strategy to promote the reproductive performance and offspring quality by choosing the optimal lipid source for *S. intermedius* broodstock.

Keywords: lipid sources, *Strongylocentrotus intermedius*, growth, reproductive performance, offspring quality

INTRODUCTION

The gonads of sea urchins are highly accepted as luxury seafood products due to their delicious taste and high nutritional value (Xin et al., 2018; Loureno et al., 2019; Wang et al., 2019). In the last decades, the demand for sea urchins has seen a sharp increase worldwide, which directly causes overfishing and destruction of their wild stocks (Prato et al., 2018; Li et al., 2021; Nhan et al., 2020). To mitigate this issue, aquaculture of sea urchins has been attempted globally and has proven to be an effective alternative strategyforf producing gonads with high yield and market acceptance (Phillips et al., 2010; Onomu et al., 2020). *Strongylocentrotus intermedius*, which is naturally distributed in the coastal areas of North Japan and Far Eastern Russia, was first introduced to China from Japan by Dalian Ocean University in 1989 (Chang et al., 2012). Up to now, it has become one of the most important sea urchin species for aquaculture in the coastal areas of China (Zuo et al., 2018; Wang et al., 2019). At present, fresh kelp *Laminaria japonica* are used as the main food category for *S. intermedius* (Zuo et al., 2018; Li et al., 2020). However, the gonad development of *S. intermedius* was shown to be retarded when they were fed solely macroalgae and could be markedly accelerated after the addition of some mussel *Mytilus edulis* (Zhou et al., 2013). Thus, it is imperative to quantify the nutritional requirement and formulate specialized formulated feeds for the broodstock of this species.

It has been shown that the quality of broodstock diets and their nutrient levels have profound influences on the gonad maturation, reproductive performance, and subsequent offspring quality of numerous aquatic animals (Izquierdo et al., 2001; Mazorra et al., 2003; Sui et al., 2009; Tercero et al., 2015; Yldz et al., 2020). Lipids are not only one of the main energy sources, but also act as the providers of essential fatty acids, carriers of lipid-soluble vitamins, and the major structural components of cellular membranes (Zhou et al., 2007; Turchini et al., 2010; Wang et al., 2012; Gibbs et al., 2015). The level and fatty acid composition of dietary lipids are considered one of the key factors that affect the spawning performance and larvae quality of aquatic animals (Izquierdo et al., 2001). In particular, the absolute level and specific composition of long-chain polyunsaturated fatty acids (LC-PUFAs) and the ratio of members, such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), have been proven to be critical factors in regulating the reproductive performance, egg, and larval viability of rainbow trout *Oncorhynchus mykiss* (Agh et al., 2019; Yldz et al., 2020; Yldz et al., 2021), Nile tilapia *Oreochromis niloticus* (Ng and Wang, 2011), Yucatan octopus *Octopus Maya* (Tercero et al., 2015), Chinese mitten crab *Eriocheir sinensis* (Xiao et al., 2002; Sui et al., 2009), tongue sole *Cynoglossus semilaevis* (Liang et al., 2014), Pearl gourami *Trichogarster leeri* (Mobaraki et al., 2020), European eel *Anguilla anguilla* (Kottmann et al., 2020), and Oriental river prawn *Macrobrachium nipponense* (Li et al., 2020). Fish oil (FO), as the major source of LC-PUFAs, will not be sufficient and continuous for the expanding aquaculture industry (Turchini and Francis, 2009; Ng and Wang, 2011; Tocher, 2015; Yuan et al., 2019). Vegetable oils (VOs), such as colza oil (CO), soybean oil (SO), linseed oil (LO), and palm oil (PO), are viable alternatives for FO due to their relatively stable supply, abundant PUFA, and low cost (Turchini and Francis, 2009; Gibbs et al., 2015; Cuesta-Gomez et al., 2020). However, little information is available about the effects of VO on the reproductive performance and larvae viability of sea urchins, especially when the requirement for LC-PUFA is guaranteed.

Gonads are the reproductive organ and sole edible part of sea urchins. Thus, different feed formulation should be designed for adult sea urchins to acquire market acceptance or reproductive success (Heflin et al., 2012; Brink-Hull et al., 2022). In a recent study, it was found that PO and CO showed comparable effects to FO for *S. intermedius* from the perspective of growth performance and gonad market acceptance (Ning et al., 2022). However, to the best of our knowledge, no information is available about the optimal lipid sources for achieving the best reproductive performance of *S. intermedius*. Thus, this study was conducted to investigate the effects of dietary lipid sources on the gonad development, fatty acid composition, spawning performance of broodstock, and early larvae quality of *S. intermedius*.

MATERIALS AND METHODS

Experimental Broodstock Diets

Five isoproteic (25%) and isolipidic (6.0%) formulated feeds and kelp (*L. japonica*) were used as the experimental diets for sea urchin broodstock in this study. All feed ingredients were ground and passed through a 150 μm mesh. After all the solid ingredients of each formulated feed were thoroughly mixed, one of the following lipid sources, colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO), was added and blended well. At last, about 30% water was mixed with the ingredients above before they were pressed through a die with 2 mm pores by a pellet-making machine (DES-TS1280, Jinan, China). The pellets were then dried at 50°C, tightly packed in sealed bags, and stored at -20° until use. The formulation and nutritional profile of formulated feeds can be found in **Tables 1**, **2**.

Feeding Experiment

Adult *S. intermedius* with the age of 2.5 years were purchased from a local farm (Changhai County, Dalian, China). Then, sea urchins were reared in a rectangular tank (length: width: depth=180cm: 99cm: 70cm), where they were fed fresh kelp to acclimate the experimental conditions for 10 days. After that, sea urchins of similar size (initial weight 90.51 ± 0.82g) were individually assigned to 48 floating cages (22 cm × 28 cm × 48 cm) with each cage holding one individual. All cages were placed in fiber-reinforced rectangular plastic tanks (length: width: depth=95cm: 65cm: 55cm), with each tank holding eight cages. Each experimental diet was randomly allocated to eight urchins. To avoid feed waste, a Petri dish was placed at the bottom of each cage to prevent the feeds from falling through the gaps.

The experimental animals were handfed every day to a state of apparent satiation. To avoid the detrimental effects on

TABLE 1 | Formulation and proximate composition of the experimental diets (% dry weight).

Ingredients (%)	Formulated feeds with different lipid sources				
	CO	**FO**	**LO**	**SO**	**PO**
Fish meal[1]	4	4	4	4	4
Kelp meal[2]	10	10	10	10	10
Seaweed meal[3]	29.7	29.7	29.7	29.7	29.7
Soybean meal[4]	13	13	13	13	13
Wheat meal[5]	24	24	24	24	24
Wheat gluten[6]	10	10	10	10	10
Vitamin premix[7]	2	2	2	2	2
Mineral premix[8]	2	2	2	2	2
Calcium propionate	0.18	0.18	0.18	0.18	0.18
Choline chloride	0.1	0.1	0.1	0.1	0.1
Ethoxyquin	0.02	0.02	0.02	0.02	0.02
Soybean lecithin	1	1	1	1	1
Colza oil	4	–	–	–	–
Fish oil	–	4	–	–	–
Linseed oil	–	–	4	–	–
Soybean oil	–	–	–	4	–
Palm oil	–	–	–	–	4
Proximate composition					
Crude protein	25.12	25.20	25.05	25.23	25.01
Crude lipid	6.03	6.10	6.12	6.01	6.05

[1]Fish meal, crude protein 68.7% dry matter, crude lipid 9.7% dry matter.
[2]kelp meal, crude protein 19.31% dry matter.
[3]Seaweed meal, crude protein 8.15% dry matter.
[4]Soybean meal, crude protein 51.56% dry matter, crude lipid 0.9% dry matter.
[5]Wheat meal, crude protein 13.88% dry matter, crude lipid 1.0% dry matter.
[6]Wheat gluten, crude protein 80% dry matter, crude lipid 2.8% dry matter.
[7]Vitamin premix (mg or g kg^{-1} diet), vitamin D, 5 mg; vitamin K, 10 mg; vitamin B_{12}, 10 mg; vitamin B_6, 20 mg; folic acid, 20 mg; vitamin B_1, 25 mg; vitamin A, 32 mg; vitamin B_2, 45 mg; pantothenic acid, 60 mg; biotin, 60 mg; niacin acid, 200 mg; α-tocopherol, 240 mg; inositol, 800 mg; ascorbic acid, 2000 mg; microcrystalline cellulose, 16.47 g.
[8]Mineral premix (mg or g kg^{-1} diet), $CuSO_4 \cdot 5H_2O$, 10 mg; Na_2SeO_3 (1%), 25 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $FeSO_4 \cdot H_2O$, 80 mg; $Ca(IO_3)_2$, 180 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; zeolite, 18.35 g.

water quality, residual feeds and feces were timely removed out of the tanks by siphoning about 6 hours after each feeding operation. Seawater was sand filtered, aerated, and preheated before they were filled into experimental tanks. Water in each tank was exchanged completely every 3 days. During the whole feeding experiment, the water quality parameters were monitored and recorded daily, with temperature maintained at 15 ± 1°C, salinity 33 ± 1‰, pH 8.0 ± 0.1, and dissolved oxygen >8.0 mg L^{-1}. The feeding period lasted for 12 weeks.

Spawning, Egg, and Gonad Collection

At the end of the feeding experiment, the sea urchins were individually weighed after fasting for 24 h, and then artificially induced for spawning as described by Liu et al. (2005). First, 1 ml KCl (0.5 M) was injected into the coelomic cavity *via* the peristomial membrane of each sea urchin. Eggs or sperms began to flood out of the genital pores in about 30 min. Then, three female sea urchins and three male sea urchins were chosen out from each dietary group. The eggs of each sea urchin fed the same diet were collected, counted, and divided into three parts. The first part (about 100 eggs) was put into a 1.5 mL tube with 10% formalin for measuring the egg diameter. The second part was placed in 1.5 ml sterile tubes and then stored at -80°C for later analysis of fatty acids. The third part was placed in a sterile beaker with filtered seawater for the larval culture experiment. The collected sperm of broodstock fed the same diet were mixed and diluted with sterile seawater. Then, the diluted sperm were mixed with the eggs of the same dietary group (sperms/eggs ≈ 1000:1).

Fertilized eggs were hatched and incubated according to the method reported by Chang et al. (2012). Briefly, fertilized eggs collected from each female sea urchin were incubated in a 5L plastic tank (15 eggs/mL), where the water temperature was kept at 15°C ± 0.5°C. After hatching, healthy and strong prismatic larvae were carefully gathered by dragging a net across the one-third upper layer of each tank. Among them, some were transferred to a new 5L container for subsequent larval culture with a density of 1.0 individual/mL. The remaining healthy prismatic larvae in each tank were collected, pooled into a 1.5 tube, and stored at -80°C for later analysis of their fatty acids in response to different maternal nutrition. During the larval culture, the water temperature was maintained at 15 ± 1°C, salinity was 33 ± 1‰, pH was 8.0 ± 0.1, and oxygen was above 7 mg/L. The larval container was illuminated by less than 300 lx of incandescent light, with a natural photoperiod. During the larval culture, no feeding was carried out, and the quality of the prismatic larvae was evaluated by a starvation tolerance test (Quintana et al., 2015). Larvae in each tank were daily sampled to monitor the quantity, developmental stages, body length, and width. If four-armed larvae occurred in any tank, the starvation tolerance test was terminated. At this time, larvae within three tanks of each dietary treatment were

TABLE 2 | Fatty acid composition (g/kg dry matter) of formulated feeds[1].

Fatty acids	Formulated feeds with different lipid sources				
	CO	FO	LO	SO	PO
C14:0	0.57	2.35	0.56	0.78	0.90
C15:0	0.07	0.30	0.06	0.09	0.07
C16:0	7.92	12.09	8.68	10.9	17.6
C17:0	0.08	0.18	0.08	0.12	0.09
C18:0	1.79	2.15	2.53	2.94	2.30
C20:0	0.21	0.18	0.18	0.24	0.20
C21:0	–	–	–	0.16	–
C22:0	0.11	0.09	0.08	0.24	0.09
C23:0	0.04	–	–	0.06	0.04
C24:0	0.17	0.12	0.12	0.13	0.13
ΣSFA[2]	10.94	17.46	12.29	15.66	21.41
C14:1	–	0.06	–	–	–
C16:1	0.41	1.84	0.35	0.53	0.43
C17:1	0.05	0.19	0.03	0.06	0.05
C18:1	23.08	7.4	10.14	11.52	22.26
C20:1	0.60	1.74	0.13	0.36	0.20
C22:1	0.41	0.98	0.13	0.30	0.13
C24:1	0.11	0.24	0.04	0.06	–
ΣMUFA[3]	24.65	12.45	10.82	12.82	23.07
C18:3n-3	–	–	8.03	2.20	0.78
C20:5n-3	0.26	1.82	0.17	0.44	0.25
C22:6n-3	0.37	3.23	0.25	0.68	0.36
∑n-3PUFA[4]	0.63	5.05	8.44	3.32	1.39
C18:2n-6	15.24	8.39	10.63	23.76	13.17
C18:3n-6	–	0.08	–	–	–
C20:3n-6	–	0.05	–	–	0.04
C20:4n-6	0.22	3.06	0.24	0.63	0.27
C22:2n-6	0.04	0.08	–	0.04	–
∑n-6PUFA	15.49	11.65	10.86	24.43	13.48
n-3/n-6PUFA	0.04	0.43	0.78	0.14	0.10
ARA/EPA	0.83	1.68	1.44	1.42	1.08
DHA/EPA	1.39	1.77	1.48	1.53	1.45

[1]Some fatty acids, of which the contents are minor, trace amount or not detected, such as C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:1, were not listed in the table.
[2]SFA saturated fatty acids.
[3]MUFA mono-unsaturated fatty acids.
[4]PUFA poly-unsaturated fatty acids.

pooled into one tube, flash frozen, and stored at -80 °C for later fatty acid analysis.

After spawning, three female sea urchins were weighed and then dissected for gonads and digestive tract. First, the digestive tract of each sea urchin was weighed to calculate the digestive tract index and was placed in a 1.5 ml tube and stored at -80°C until they were used for the analysis of related gene transcription. Then, gonads from three urchins were individually weighed to calculate the gonadosomatic index. After that, a small part of the gonad was carefully put in a 5 ml tube with Bouin's reagent for subsequent histological analysis; the second part was placed in a 1.5 ml tube and stored at -80°C for analysis of gene transcription; the remaining gonads were pooled and then stored at -80°C for later determining the nutritional composition.

Gonadal Histology

The gonad slices were made by following the method of Ning et al. (2022). Briefly, the Bouin's reagent fixed gonad was dehydrated by sinking them into a graded ethanol series (75%–100%) before they were embedded in paraffin. Tissue sections (4 μm in thickness) were prepared by using a microtome (Leica, RM2016). After that, slices were stained with hematoxylin and eosin, which were observed under an optical microscope (40 ×, Leica, Germany).

Nutritional Composition Analysis

The proximate composition of experimental feeds and gonads was analyzed according to AOAC (1995). Briefly, the contents of crude protein and crude lipids were detected by following the methods of Soxhlet and Kjeldahl, respectively. The content of moisture was determined by calculating the mass loss of the samples which were dried at 105°C.

Fatty acid composition of samples was assayed by following the method of Li et al. (2020). Here, the procedures were introduced briefly. First, the fatty acids in the samples were esterified into methyl esters, which were separated with hexane and transferred to a new tube. Before injection, certain amounts of C19:0 were added to each sample obtained above. Finally, the fatty acids were quantified by using gas chromatography (Thermo Fisher Trace 1310 ISQ). The temperature of the injector and detector was

maintained at 290°C. The oven temperature rising procedures were set as follows: 80°C to 200°C at a speed of 10°C/min, 200°C to 250°C at a speed of 5°C/min, and 250°C to 270°C at a speed of 2°C/min. The fatty acid composition (g/kg) was calculated based on their relative peak area.

RNA Extraction and Real-Time Quantitative PCR

The sequences of the specific primers are presented in **Table 3**. The procedures of RNA extraction and real-time quantitative PCR have been previously described (Zuo et al., 2017). Briefly, total RNA was extracted and was reverse transcribed to cDNA by using a commercial kit (TaKaRa, Beijing, China). Then, the reaction regime (20 μL) was prepared as follows: 2 μL of cDNA, 0.8 μL of forward primer and reverse primer (10 mM), 10 μL of FastStart Essential DNA Green Master, and 6.4 μL of sterile distilled water. The quantitative PCR was performed by using the LightCycler® 96 real-time PCR system with the following reaction conditions: 95°C for 10 min; 95°C for 15 s; and 60°C for 60 s (45 cycles); 95°C for 10 s; 65°C for 60 s; and 97°C for 1 s. The formula of $2^{-\Delta\Delta CT}$ was used to calculate the relative gene expression of target genes (Kenneth and Thomas, 2002).

Calculations and Statistical Analysis

Survival rate (SR, %) = $N_f/N_i \times 100$

Weight growth rate (WGR, %) = $(W_b - W_i)/W_i \times 100$

Gonadosomatic index (GSI, %) = $W_g/W_f \times 100$

Digestive tract index (DTI, %) = $W_d/W_f \times 100$

Relative fecundity (RF, 10^3 eggs/g) = E_t/W_b

Fertilization rate (FR, %) = $E_2/E_t \times 100$

Hatching rate (HR, %) = $E_p/E_2 \times 100$

Larvae survival rate (LSR, %) = $N_d/N_t \times 100$

where N_i and N_f were the initial and final number of sea urchins in each dietary group. W_i was the initial body weight of each female sea urchin; W_b was the body weight of each female sea urchin before spawning; W_f, W_g and W_d were the body weight, gonad weight, and digestive tract weight of each female sea urchin after spawning. E_t was the total number of eggs produced by each female sea urchin; E_2 was the number of two-cell stage embryos in the same batch of eggs after fertilization; E_p was the number of healthy prismatic larvae after hatchery. N_t and N_d were the initial number of healthy prismatic larvae and number of live larvae in each tank during the first 3 days post hatchery.

The data were checked for normal distribution and homogeneity of variance before they were statistically analyzed by ANOVA with SPSS 22.0 software. When a significance ($P<0.05$) was detected, Duncan's multiple comparison test was used to compare differences in the means between different treatments. The data were presented as means ± S.E.M (standard error of means).

RESULTS

Survival and Growth Performance

No mortality was observed in any dietary group. Before spawning, the WGR of female sea urchins fed kelp was significantly higher than those fed formulated feeds ($P<0.05$). Among the formulated feed groups, sea urchins fed PO showed a marginally higher WGR than other lipid source groups ($P>0.05$). After spawning, the body weight of female sea urchins fed PO was comparable to those fed LO ($P>0.05$) but was significantly higher than those fed CO, FO, SO, and kelp ($P<0.05$). The gonad weight and GSI of spawned female sea urchins fed kelp were the lowest, which were significantly lower than those fed formulated feeds ($P<0.05$). Among the formulated feed groups, the highest gonad weight and GSI were observed in the PO group, which were significantly higher than those in the other groups ($P<0.05$). The DTI of female sea urchins fed FO was comparable to that of sea urchins fed LO and PO ($P>0.05$) but was significantly higher than those fed CO, SO, and kelp ($P<0.05$) (**Table 4**).

Ovary Histological Observation

A small number of ova were loosely distributed in the space vacated by spawned ova. In the ovary of sea urchins fed kelp, there were still many vitellogenic oocytes that were distributed along the ovarian wall. By contrast, there were only a small number of vitellogenic oocytes observed in the ovaries of sea

TABLE 3 | List of primers used for real time PCR in this study.

Name	Sequence (5'-3')	AT[1] (°C)	Reference
18s rRNA-F	GTTCGAAGGCGATCAGATAC		Li et al. (2020)
18s rRNA-R	CTGTCAATCCTCACTGTGTC	52	
COX-2-F	GAGGTGGATAACCGATTGA		MH516324
COX-2-R	AGCATTGCCCATAGAACAG	60	
AIF-1-F	TCGAACGTGCAAGGTGGCAAG		MH516330
AIF-1-R	CGTCATTGTCATCGAGGTCTCCAC	60	
TNF-α-F	GCTGTAACGGCGTTCGTCTCC		MH516331
TNF-α-R	TGGTGTACTTGTGCTGGTTGTTGG	61.5	
MYP-F	ACCATATGGACTGACGT		LC170478.1
MYP-R	GGGTTCTACCTCGGAGTTGAC	51	

[1]*AT, annealing temperature; COX-2, cyclooxygenase-2; AIF-1, allograft inflammatory factor-1; TNF-α, tumor necrosis factor-α; MYP, major yolk protein.*

TABLE 4 | Effects of different lipid sources on survival and growth performance of female adult sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3)[1].

	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
IBW (g)	89.71 ± 0.70	90.05 ± 0.18	90.21 ± 0.75	90.80 ± 0.83	92.53 ± 1.37	89.77 ± 1.07
BFW (g)	96.53 ± 0.69^{c}	97.47 ± 0.78^{bc}	96.99 ± 1.99^{bc}	97.40 ± 1.02^{bc}	101.68 ± 0.54^{a}	100.74 ± 0.77^{ab}
WGR (%)	7.63 ± 0.46^{b}	8.38 ± 0.05^{b}	8.03 ± 0.69^{b}	7.57 ± 0.70^{b}	10.06 ± 1.02^{b}	12.83 ± 0.79^{a}
AFW (g)	91.62 ± 1.11^{b}	93.06 ± 0.24^{b}	93.56 ± 0.71^{ab}	93.24 ± 0.27^{b}	96.11 ± 0.50^{a}	91.33 ± 0.92^{b}
GW (g)	17.71 ± 0.78^{b}	16.77 ± 0.46^{b}	18.05 ± 1.58^{b}	15.99 ± 0.67^{b}	23.21 ± 1.19^{a}	8.95 ± 0.50^{c}
GSI (%)	18.91 ± 0.50^{b}	17.98 ± 0.94^{b}	18.96 ± 0.86^{b}	19.41 ± 0.53^{b}	24.22 ± 0.71^{a}	9.88 ± 0.94^{c}
DTW (g)	2.87 ± 0.37^{b}	4.38 ± 0.60^{a}	3.42 ± 0.42^{ab}	3.20 ± 0.24^{ab}	3.49 ± 0.05^{ab}	3.12 ± 0.40^{b}
DTI (%)	3.13 ± 0.38^{b}	4.70 ± 0.65^{a}	3.67 ± 0.14^{ab}	3.29 ± 0.34^{b}	3.64 ± 0.06^{ab}	3.42 ± 0.43^{b}

[1]*Mean values with the different superscript letters within the same row are significantly different at P<0.05.*
SR, survival rate; IBW, initial body weight; BFW, before fecundity weight; WGR, weight gain rate; AFW, after fecundity weight; GW, gonad weight; GSI, gonadosomatic index; DTW, digestive tract weight; DTI, digestive tract index.

urchins fed formulated feeds. Generally, sea urchins fed FO showed less vitellogenic oocytes in their ovaries than those fed other lipid sources. The nutritive phagocytes (NP) in the ovary of sea urchins fed the formulated feeds showed a thin and pale meshwork structure, while the nutritive phagocytes in the kelp group showed a dense network structure (**Figure 1**).

Reproductive Performance

The relative fecundity was not significantly affected by diet categories or lipid sources, with a relatively higher value observed in the FO and kelp groups (P>0.05) (**Figure 2A**).

Before fertilization, the egg diameter in the FO group was significantly larger than that in the PO group (P<0.05). After fertilization, the egg diameter in the SO group was significantly larger than that in the other dietary groups (P<0.05). The lowest egg diameter was observed in the PO group, which was comparable to that in FO and CO groups

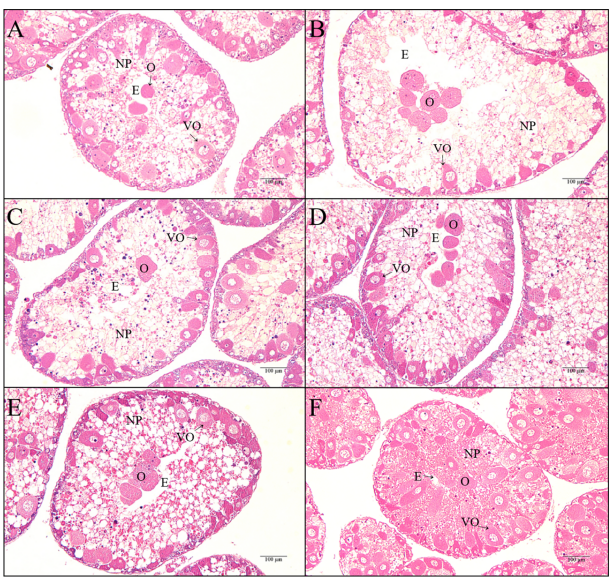

FIGURE 1 | Histology observations in the ovaries of female adult sea urchin (*Strongylocentrotus intermedius*) fed diets with different lipid sources (colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO)). **(A)** Colza oil, stage V; **(B)** Fish oil, stage V; **(C)** Linseed oil, stage V; **(D)** Soybean oil, stage V; **(E)** Palm oil, stage V; **(F)** Feed kelp, stage V. NP, nutritive phagocyte. VO, vitellogenic oocyte. O, ova. E, empty spaces.

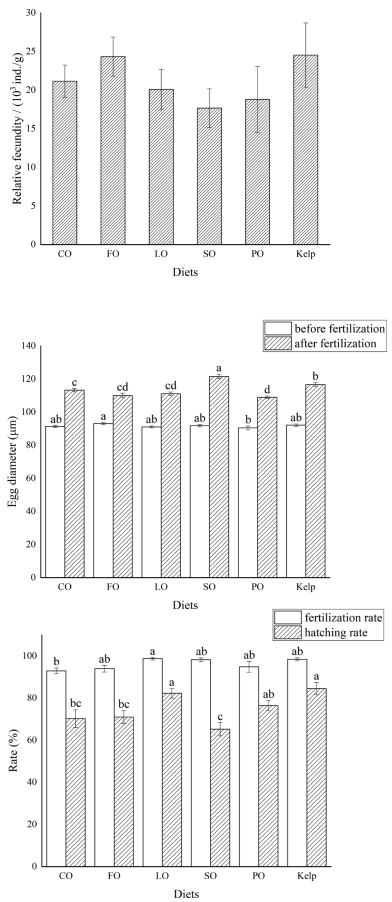

FIGURE 2 | Effects of different lipid sources (colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO)) on the relative fecundity, egg diameter, as well as fertilization rate and hatching rate of sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3). Mean value bars within the same parameter of each chart bearing with different lowercase letters are significantly different at P < 0.05.

(P>0.05) but was significantly lower than that in the other groups (P<0.05) (**Figure 2**).

The fertilization rate was highest in the LO group, which was only significantly higher than that in the CO group (P<0.05). The hatching rate was highest in the kelp group, which was comparable to that in LO and PO (P>0.05) but was significantly higher than that in CO, FO, and SO groups (P<0.05) (**Figure 2**).

Prismatic Larvae Quality

The body length of prismatic larvae was highest in the treatment of LO, which was only significantly higher than that in CO and SO (P<0.05). The body width of prismatic larvae was highest in the kelp group, which was comparable to that in CO (P>0.05) but was significantly higher than that in FO, LO, SO, and PO groups (P<0.05) (**Table 5**).

During the first 2 days post hatching (DPH), no significant difference was observed in the prismatic larvae survival rate (LSR) among dietary groups (P>0.05). On the 3rd DPH, the LSR was significantly decreased in all dietary groups (P<0.05). The LSR in the kelp group was significantly lower than that in the FO group (P<0.05). Among formulated feed groups, LSR was highest in the FO group, followed by PO, CO, SO, and LO groups. However, no significant difference was observed in SR among different formulated feed groups (P>0.05) (**Table 6**).

Transcription of Proinflammatory Genes

The transcription of *COX-2* was highest in the spawned ovary of sea urchins fed SO, which was significantly higher than that in the other groups (P<0.05). The transcription of *AIF-1* was highest in the ovary of sea urchins fed SO, but there was no significant difference among all dietary treatments (P>0.05). The transcription of *TNF-α* was highest in the ovary of sea urchins fed SO, which was comparable to that in CO (P>0.05) but was significantly higher than that in kelp, FO, LO, or PO groups (P<0.05) (**Figure 3**).

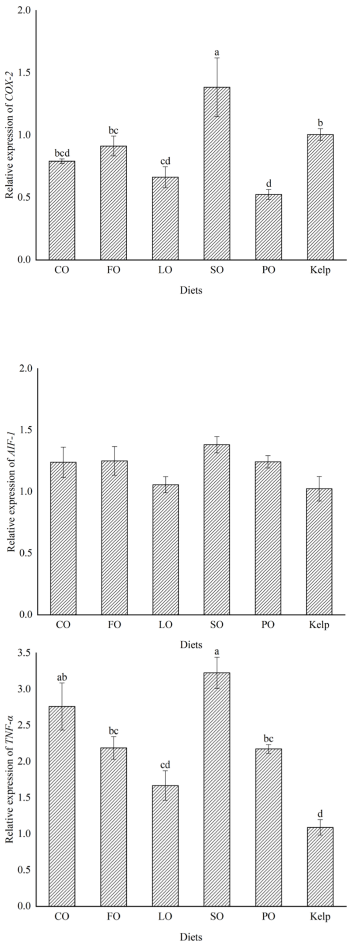

FIGURE 3 | Effects of dietary lipid sources (colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO)) on the inflammatory related gene expression in the ovary of sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3). COX-2, cyclooxygenase-2. AIF-1, allograft inflammatory factor-1. TNF-α, tumor necrosis factor-α. Mean value bars bearing with different lowercase letters are significantly different at P < 0.05.

Transcription of Major Yolk Protein (MYP)

The transcription of *MYP* was highest in the digestive tract of female adult sea urchins fed FO, which was comparable to that in LO (P>0.05) but was significantly higher than that in kelp, CO, SO, or PO groups (P<0.05). The mRNA level of *MYP* was the highest in the ovary of the FO group, which was approximately 2-fold higher than that in the other dietary groups (P<0.05) (**Figure 4**).

Proximate Composition of Ovary

The ovary of sea urchins fed kelp showed significantly lower moisture, but higher protein, than that of sea urchins fed the formulated feeds (P<0.05). Crude lipid in the ovary of sea urchins

TABLE 5 | Effects of different lipid sources on prismatic larvae size of sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3)[1].

	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
Length (μm)	221.1±9.7^{c}	257.7±10.9^{ab}	269.1±9.1^{a}	232.0±6.3^{bc}	239.7±9.9^{abc}	246.5±3.4^{abc}
Width (μm)	146.5±3.8^{ab}	134.5±3.8^{c}	143.4±2.8^{bc}	135.7±1.9^{bc}	141.64±1.9^{bc}	156.2±7.3^{a}

[1]*Mean values with the different superscript letters within the same row are significantly different at P<0.05.*

TABLE 6 | Effects of different lipid sources on the prismatic larvae survival of sea urchin (*Strongylocentrotus intermedius)* at different day post hatching (DPH) (mean ± SEM, n=3)[1].

DPH	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
1	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}
2	96.0 ± 4.0^{A}	100 ± 0.0^{A}	100 ± 0.0^{A}	93.3 ± 6.7^{A}	96. 7 ± 3.3^{A}	100 ± 0.0^{A}
3	56.0 ± 11.6Bab	72.6 ± 11.3Ba	47.0 ± 6.0Bab	55.0 ± 5.0Bab	65.4 ± 7.0Bab	40.4 ± 1.4Bb

[1]Mean values with the different superscript capital letters within the same column are significantly different at P<0.05. Mean values with the different superscript lowercase letters within the same row are significantly different at P<0.05.

TABLE 7 | Effects of different lipid sources on the proximate composition (% wet weight) in the ovary of adult sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3)[1].

	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
Moisture	74.68 ± 0.33^{a}	74.86 ± 0.46^{a}	73.04 ± 0.63^{b}	74.3 ± 0.80^{ab}	73.64 ± 0.34^{ab}	69.19 ± 0.15^{c}
Crude lipid	5.47 ± 0.42^{a}	5.79 ± 0.37^{a}	5.76 ± 0.16^{a}	5.49 ± 0.15^{a}	5.35 ± 0.01^{ab}	4.38 ± 0.05^{b}
Crude protein	12.26 ± 0.27^{b}	11.67 ± 0.04^{b}	12.10 ± 0.27^{b}	12.00 ± 0.25^{b}	11.83 ± 0.10^{b}	13.70 ± 0.58^{a}

[1]Mean values with the different superscript letters within the same row are significantly different at P<0.05.

fed kelp was significantly lower than that of formulated feed groups except for the PO group (P<0.05). Among the formulated feed groups, the moisture was highest in the ovary of sea urchins fed FO and CO, which was only significantly higher than that of sea urchins fed LO (P<0.05). However, there were no significant differences in the protein and lipid among the formulated feed groups (P>0.05) (**Table 7**).

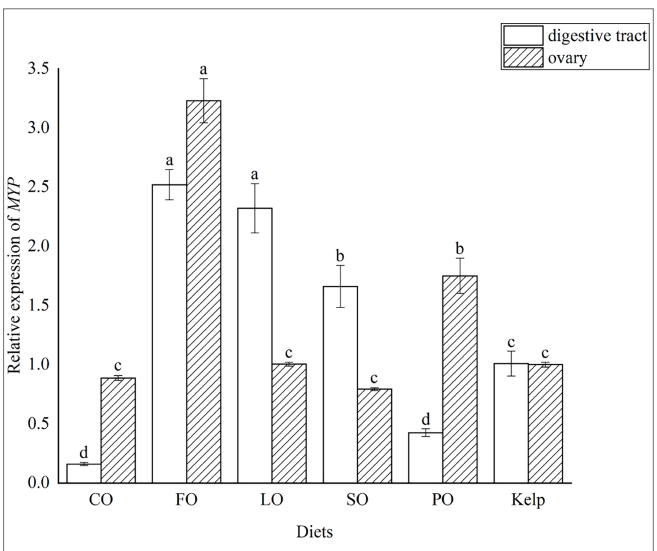

FIGURE 4 | Effects of dietary lipid sources (colza oil (CO), fish oil (FO), linseed oil (LO), soybean oil (SO), or palm oil (PO)) on the major yolk protein (MYP) expression in sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3). Mean value bars bearing with different lowercase letters are significantly different at P < 0.05

Fatty Acid Composition of the Spawned Ovary, Eggs, and Prismatic Larvae

Generally, the spawned ovary of sea urchins showed characteristic fatty acid categories of the ingested lipid sources, with the highest amounts of oleic acid (OA, C18:1n-9), EPA (C20:5n-3) and DHA (C22:6n-3), linolenic acid (LNA, C18:3n-3), linoleic acid (LA, C18:2n-6) and palmitic acid (PA, C16:0) detected in the CO, FO, LO, SO, and PO groups, respectively. Sea urchins fed kelp showed significantly higher EPA and n-3/n-6 PUFA but lower LA in the spawned ovary than those fed formulated feeds (P<0.05). The ARA in the spawned ovary of sea urchins fed CO, FO, and kelp was significantly higher than that in the other groups (P<0.05). ARA/EPA of the spawned ovary was highest in the PO group, which was comparable to that in the CO group (P>0.05) but was significantly higher than the other groups (P<0.05). The lowest ARA/EPA was observed in the spawned ovary of sea urchins fed kelp, which was comparable to that in the FO and LO groups (P>0.05) but was significantly lower than that in the other groups (P<0.05). DHA/EPA was highest in the FO group, followed by the CO group, which were significantly higher than that in the other groups (P<0.05). Sea urchins fed kelp showed the lowest DHA/EPA in the spawned ovary, which was significantly lower than that in the other groups (**Table 8**).

The EPA content in the eggs of sea urchins fed kelp was significantly higher than that in the formulated feed groups (P<0.05). The DHA and ARA contents were highest in the eggs of sea urchins fed FO, which were significantly higher than that in the other dietary groups (P<0.05). However, sea urchins fed LO and CO produced eggs with significantly (P<0.05) higher n-3 PUFA and n-6 PUFA, respectively. The n-3/n-6 PUFA in the eggs of sea urchins fed kelp was significantly higher than that in the formulated feed groups (P<0.05). The ARA/EPA and DHA/EPA showed a similar changing

TABLE 8 | Effects of different lipid sources on the fatty acid composition (g/kg dry matter) in the ovary of adult sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3)[1].

FA	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
C14:0	15.52 ± 0.72b	18.76 ± 0.44a	18.98 ± 0.74a	14.14 ± 0.60b	15.07 ± 0.56b	14.84 ± 0.94b
C15:0	0.76 ± 0.18	0.93 ± 0.10	0.57 ± 0.09	0.76 ± 0.26	0.45 ± 0.03	0.64 ± 0.05
C16:0	39.1 ± 1.31ab	37.9 ± 0.77b	37.48 ± 1.74b	31.17 ± 1.08c	37.11 ± 1.27b	42.07 ± 0.85a
C17:0	0.31 ± 0.04	0.30 ± 0.04	0.24 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	0.24 ± 0.02
C18:0	5.47 ± 0.37b	4.82 ± 0.47b	5.52 ± 0.69b	5.16 ± 0.36b	4.33 ± 0.41b	7.45 ± 0.23a
C20:0	0.63 ± 0.06b	0.56 ± 0.03b	0.65 ± 0.07b	0.64 ± 0.10b	0.42 ± 0.05b	2.92 ± 0.17a
C21:0	35.51 ± 0.21a	21.42 ± 1.24c	19.91 ± 0.80cd	18.34 ± 0.70d	27.04 ± 1.05b	12.49 ± 0.51e
C22:0	0.34 ± 0.03a	0.27 ± 0.04abc	0.22 ± 0.03bc	0.28 ± 0.03ab	0.18 ± 0.01c	0.29 ± 0.02ab
C23:0	0.77 ± 0.13a	0.54 ± 0.03ab	0.43 ± 0.09b	0.43 ± 0.05b	0.58 ± 0.06ab	0.34 ± 0.03b
C24:0	0.19 ± 0.03ab	0.12 ± 0.01b	0.17 ± 0.04ab	0.22 ± 0.00a	0.15 ± 0.01ab	0.14 ± 0.02b
ΣSFA[2]	98.59 ± 2.44a	85.63 ± 0.96b	84.16 ± 2.80b	71.4 ± 2.87c	85.57 ± 1.86b	81.43 ± 0.88b
C14:1	0.91 ± 0.20ab	1.27 ± 0.11a	1.35 ± 0.15a	0.87 ± 0.21ab	1.33 ± 0.19a	0.68 ± 0.16b
C16:1	8.87 ± 0.60ab	11.02 ± 0.61a	7.74 ± 0.96b	6.54 ± 1.16b	8.99 ± 0.55ab	8.28 ± 0.56b
C18:1	29.95 ± 0.82a	8.65 ± 0.81d	11.43 ± 0.65c	12.99 ± 1.03c	26.69 ± 0.67b	5.22 ± 0.43e
C20:1	12.61 ± 0.78a	7.74 ± 0.89b	6.37 ± 0.81b	6.70 ± 0.99b	8.82 ± 0.60b	8.35 ± 0.39b
C22:1	18.66 ± 0.57b	17.97 ± 1.69b	14.28 ± 0.95c	21.37 ± 0.92b	12.98 ± 1.07c	28.89 ± 1.08a
C24:1	0.72 ± 0.12a	0.55 ± 0.05ab	0.32 ± 0.06b	0.38 ± 0.09b	0.47 ± 0.02b	0.40 ± 0.03b
ΣMUFA[3]	71.73 ± 1.43a	47.20 ± 0.66c	41.48 ± 1.55d	48.84 ± 1.19c	59.29 ± 2.8b	51.81 ± 1.41c
C18:3n-3	8.46 ± 0.47b	2.13 ± 0.25d	19.72 ± 0.77a	4.28 ± 0.65c	1.64 ± 0.12d	4.84 ± 0.33c
C20:3n-3	1.47 ± 0.13c	0.93 ± 0.06cd	4.05 ± 0.43b	1.33 ± 0.36c	0.33 ± 0.04d	7.72 ± 0.44a
C20:5n-3	5.75 ± 0.65cd	11.61 ± 1.21b	8.26 ± 0.64c	5.45 ± 1.51cd	2.86 ± 0.37d	26.49 ± 0.39a
C22:6n-3	1.31 ± 0.14b	10.34 ± 1.74a	1.01 ± 0.09b	1.21 ± 0.18b	1.41 ± 0.27b	0.48 ± 0.08b
∑n-3PUFA	16.99 ± 0.48d	25.01 ± 3.24c	33.04 ± 1.86b	12.28 ± 1.91d	6.24 ± 0.77e	39.53 ± 0.32a
C18:2n-6	31.32 ± 0.60b	13.25 ± 1.96e	19.13 ± 0.71d	36.89 ± 1.37a	24.22 ± 0.73c	3.12 ± 0.24f
C18:3n-6	0.13 ± 0.02b	0.22 ± 0.00b	0.11 ± 0.02b	0.20 ± 0.06b	0.11 ± 0.03b	0.63 ± 0.13a
C20:2n-6	6.85 ± 0.58b	5.58 ± 1.02bc	6.23 ± 0.87bc	9.90 ± 0.80a	4.19 ± 0.31c	5.75 ± 0.34bc
C20:3n-6	4.05 ± 0.36a	2.26 ± 0.32c	2.80 ± 0.28bc	3.74 ± 0.70ab	2.75 ± 0.07bc	1.58 ± 0.15c
C20:4n-6	12.2 ± 0.24a	10.27 ± 1.20a	7.20 ± 0.62b	7.79 ± 0.59b	7.71 ± 0.47b	10.96 ± 0.44a
C22:2n-6	0.26 ± 0.04a	0.24 ± 0.05ab	0.27 ± 0.06a	0.33 ± 0.02a	0.12 ± 0.01b	0.26 ± 0.01a
∑n-6PUFA	54.82 ± 0.84^{a}	31.82 ± 1.97^{c}	35.72 ± 0.92^{bc}	58.84 ± 3.03^{a}	39.1 ± 0.62^{b}	22.29 ± 0.84^{d}
ΣPUFA[4]	71.81 ± 1.29a	56.83 ± 2.16c	68.76 ± 2.47ab	71.11 ± 4.78a	45.34 ± 0.64d	61.82 ± 0.53bc
n-3/n-6PUFA	0.31 ± 0.00^{c}	0.80 ± 0.15^{b}	0.92 ± 0.05^{b}	0.21 ± 0.03^{c}	0.16 ± 0.02^{c}	1.78 ± 0.08^{a}
ARA/EPA	2.17 ± 0.20ab	0.88 ± 0.06c	0.87 ± 0.03c	1.63 ± 0.37b	2.78 ± 0.35a	0.41 ± 0.02c
DHA/EPA	0.23 ± 0.00c	0.88 ± 0.07a	0.12 ± 0.01cd	0.26 ± 0.08c	0.49 ± 0.04b	0.02 ± 0.00d

[1]*Mean values with the different superscript letters within the same row are significantly different at P<0.05. Some fatty acids, of which the contents are minor, trace amount or not detected, such as C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:1, C17:1, were not listed in the table.*

[2]*SFA, saturated fatty acids.*

[3]*MUFA, mono-unsaturated fatty acids.*

[4]*PUFA, poly-unsaturated fatty acids.*

tendency to that of spawned ovary in all dietary groups, with significantly higher ratios observed in the PO and FO, respectively (P<0.05) (**Table 9**).

The EPA content of the prismatic larvae was highest in the kelp group, followed by that of FO, LO, and CO groups, with the lowest EPA observed in SO and PO groups. Although no statistical analysis was made, DHA content in the prismatic larvae of the FO group was extremely higher than that in the other dietary groups. ARA of prismatic larvae showed comparable levels among dietary groups, with marginally higher value observed in PO and kelp groups. The prismatic larvae in the kelp group showed the lowest n-6 PUFA and the highest n-3 PUFA and n-3/n-6 PUFA among all dietary groups. Among the prismatic larvae of formulated feed groups, the lowest n-6 PUFA and the highest n-3 PUFA and n-3/n-6 PUFA were observed in the FO group. The highest ARA/EPA of prismatic larvae was observed in the PO group, followed by the SO and CO groups. While DHA/EPA of prismatic larvae was highest in the FO group, followed by the PO group, with the lowest ratio observed in the kelp group (**Table 10**).

DISCUSSION

In the present study, the WGR of female adult *S. intermedius* fed kelp was significantly higher than those fed formulated feeds. Similar results have been obtained in the juvenile *S. intermedius* (Li et al., 2020). This could be due to the presence of unknown immune and growth stimulant substances in the fresh kelp. A low molecular weight fucoidan, obtained from the kelp *Laminaria japonica*, played an important role in antioxidant and anticoagulant activity (Wang et al., 2010). In the present study, female sea urchins fed SO showed the worst growth performance. Similar results have been reported by Gibbs et al. (2015), who found that the WGR of juvenile *Lytechinus variegatus* fed SO was lower than that of sea urchins fed FO. Li et al. (2020) reported

TABLE 9 | Effects of different lipid sources on the fatty acid composition (g/kg dry matter) of eggs of adult sea urchin (*Strongylocentrotus intermedius*) (mean ± SEM, n=3)[1].

FA	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
C14:0	10.88 ± 0.21^{c}	19.21 ± 0.31^{a}	12.21 ± 0.45^{b}	9.06 ± 0.26^{d}	10.18 ± 0.17^{c}	10.94 ± 0.21^{c}
C15:0	0.63 ± 0.03^{b}	1.01 ± 0.01^{a}	0.53 ± 0.03^{c}	0.38 ± 0.04^{d}	0.36 ± 0.00^{d}	0.41 ± 0.02^{d}
C16:0	32.64 ± 0.62^{b}	42.71 ± 0.71^{a}	30.25 ± 1.12^{cd}	22.8 ± 0.64^{e}	31.33 ± 0.54^{bc}	28.5 ± 0.60^{d}
C17:0	0.28 ± 0.01^{b}	0.39 ± 0.02^{a}	0.26 ± 0.01^{b}	0.20 ± 0.00^{cd}	0.21 ± 0.01^{c}	0.17 ± 0.01^{d}
C18:0	5.65 ± 0.09^{b}	7.45 ± 0.12^{a}	7.03 ± 0.26^{a}	4.85 ± 0.12^{c}	4.56 ± 0.10^{c}	5.98 ± 0.12^{b}
C20:0	0.48 ± 0.01^{c}	0.69 ± 0.01^{b}	0.63 ± 0.02^{b}	0.45 ± 0.01^{cd}	0.39 ± 0.02^{d}	1.95 ± 0.05^{a}
C21:0	33.89 ± 0.60^{a}	27.51 ± 0.57^{b}	19.76 ± 0.72^{d}	14.46 ± 0.42^{e}	24.32 ± 0.47^{c}	8.71 ± 0.20^{f}
C22:0	0.27 ± 0.01^{b}	0.33 ± 0.03^{a}	0.27 ± 0.01^{b}	0.22 ± 0.01^{c}	0.18 ± 0.00^{c}	0.21 ± 0.01^{c}
C23:0	1.06 ± 0.02^{b}	0.97 ± 0.04^{c}	0.67 ± 0.03^{d}	0.37 ± 0.01^{e}	1.46 ± 0.01^{a}	0.22 ± 0.01^{f}
C24:0	0.16 ± 0.00^{b}	0.18 ± 0.00^{b}	0.22 ± 0.00^{a}	0.21 ± 0.00^{a}	0.17 ± 0.00^{b}	0.12 ± 0.02^{c}
ΣSFA[2]	85.94 ± 1.60^{b}	100.45 ± 1.76^{a}	71.83 ± 2.63^{c}	53.00 ± 1.50^{d}	73.15 ± 1.28^{c}	57.21 ± 1.20^{d}
C14:1	0.68 ± 0.05^{c}	1.34 ± 0.04^{a}	0.69 ± 0.07^{c}	0.58 ± 0.06^{c}	0.89 ± 0.03^{b}	0.43 ± 0.02^{d}
C16:1	7.65 ± 0.14^{b}	12.43 ± 0.15^{a}	5.26 ± 0.20^{d}	4.65 ± 0.15^{e}	7.05 ± 0.10^{c}	5.42 ± 0.13^{d}
C18:1	28.02 ± 0.52^{a}	9.94 ± 0.13^{cd}	10.62 ± 0.39^{c}	9.22 ± 0.27^{d}	24.21 ± 0.46^{b}	3.03 ± 0.07^{e}
C20:1	9.47 ± 0.17^{a}	8.55 ± 0.16^{b}	5.13 ± 0.18^{d}	3.86 ± 0.11^{e}	6.84 ± 0.13^{c}	4.89 ± 0.10^{d}
C22:1	18.76 ± 0.30^{c}	25.83 ± 0.63^{a}	19.59 ± 0.70^{c}	19.68 ± 0.54^{c}	14.75 ± 0.31^{d}	22.59 ± 0.52^{b}
C24:1	0.93 ± 0.02^{b}	1.01 ± 0.03^{a}	0.54 ± 0.02^{d}	0.39 ± 0.01^{e}	0.85 ± 0.02^{c}	0.44 ± 0.02^{e}
ΣMUFA[3]	65.51 ± 1.19^{a}	59.10 ± 1.05^{b}	41.83 ± 1.54^{d}	38.38 ± 1.13^{de}	54.6 ± 0.97^{c}	36.79 ± 0.81^{e}
C18:3n-3	7.94 ± 0.15^{b}	1.97 ± 0.05^{e}	19.79 ± 0.76^{a}	6.44 ± 0.18^{c}	1.53 ± 0.02^{e}	3.92 ± 0.09^{d}
C20:3n-3	1.46 ± 0.02^{c}	0.97 ± 0.04^{d}	5.19 ± 0.19^{b}	1.44 ± 0.04^{c}	0.26 ± 0.00^{e}	6.01 ± 0.14^{a}
C20:5n-3	5.41 ± 0.09^{d}	15.94 ± 0.27^{b}	10.15 ± 0.35^{c}	4.47 ± 0.13^{e}	2.57 ± 0.05^{f}	22.35 ± 0.49^{a}
C22:6n-3	1.44 ± 0.02^{b}	12.69 ± 0.22^{a}	1.18 ± 0.03^{bc}	0.93 ± 0.03^{c}	1.14 ± 0.03^{c}	0.28 ± 0.01^{d}
∑n-3 PUFA	16.26 ± 0.28^{c}	31.57 ± 0.49^{b}	36.32 ± 1.33^{a}	13.28 ± 0.37^{d}	5.49 ± 0.11^{e}	32.55 ± 0.72^{b}
C18:2n-6	29.8 ± 0.55^{a}	15.74 ± 0.22^{e}	19.16 ± 0.70^{d}	26.00 ± 0.75^{b}	22.52 ± 0.42^{c}	1.92 ± 0.05^{f}
C18:3n-6	0.12 ± 0.00^{c}	0.22 ± 0.01^{b}	0.09 ± 0.01^{d}	0.10 ± 0.01^{cd}	0.09 ± 0.01^{d}	0.37 ± 0.01^{a}
C20:2n-6	7.72 ± 0.13^{ab}	8.24 ± 0.13^{a}	7.97 ± 0.28^{a}	7.37 ± 0.24^{b}	4.67 ± 0.10^{c}	3.83 ± 0.09^{d}
C20:3n-6	2.61 ± 0.05^{a}	2.32 ± 0.09^{b}	1.91 ± 0.07^{d}	2.15 ± 0.06^{bc}	1.99 ± 0.04^{cd}	0.94 ± 0.03^{e}
C20:4n-6	10.63 ± 0.21^{b}	11.51 ± 0.21^{a}	7.23 ± 0.26^{c}	4.99 ± 0.14^{d}	7.11 ± 0.13^{c}	7.00 ± 0.12^{c}
C22:2n-6	0.26 ± 0.01^{b}	0.27 ± 0.01^{b}	0.44 ± 0.01^{a}	0.27 ± 0.00^{b}	0.16 ± 0.01^{d}	0.21 ± 0.01^{c}
∑n-6 PUFA	51.13 ± 0.95^{a}	38.30 ± 0.65^{bc}	36.80 ± 1.31^{c}	40.88 ± 1.18^{b}	36.53 ± 0.70^{c}	14.27 ± 0.30^{d}
ΣPUFA[4]	67.39 ± 1.23^{b}	69.86 ± 1.14^{ab}	73.12 ± 2.64^{a}	54.16 ± 1.55^{c}	42.01 ± 0.80^{e}	46.83 ± 1.02^{d}
n-3/n-6 PUFA	0.32 ± 0.00^{e}	0.82 ± 0.00^{c}	0.99 ± 0.00^{b}	0.32 ± 0.00^{d}	0.15 ± 0.00^{f}	2.28 ± 0.00^{a}
ARA/EPA	1.96 ± 0.01^{b}	0.72 ± 0.00^{d}	0.71 ± 0.00^{d}	1.12 ± 0.00^{c}	2.77 ± 0.01^{a}	0.31 ± 0.00^{e}
DHA/EPA	0.27 ± 0.00^{c}	0.80 ± 0.00^{a}	0.12 ± 0.00^{e}	0.21 ± 0.00^{d}	0.44 ± 0.00^{b}	0.01 ± 0.00^{f}

[1]Mean values with the different superscript letters within the same row are significantly different at P<0.05. Some fatty acids, of which the contents are minor, trace amount or not detected, such as C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:1, C17:1, were not listed in the table.

[2] SFA, saturated fatty acids.

[3] MUFA, mono-unsaturated fatty acids.

[4] PUFA, poly-unsaturated fatty acids.

that the WGR of juvenile *S. intermedius* fed SO was lower than those fed LO and FO. In a recent study, we also found that the WGR of adult *S. intermedius* fed SO was lower than those fed PO, CO, and FO (Ning et al., 2022). This indicated that a high amount of linoleic acid (LA, 18:2n-6) had detrimental effects on the growth performance of sea urchins, just as observed in most marine carnivorous fish species (Zuo et al., 2015). The GSI of female *S. intermedius* fed kelp was significantly lower than those fed formulated feeds, which was consistent with the findings of a variety of previous studies (Ning et al., 2022). Although n-3 LC-PUFA is essential for normal gonad development, results of this study and some previous studies showed that sea urchins seem to have a low n-3 LC-PUFA requirement for sustaining normal gonad development (Lv et al., 2020). In this study, sea urchins fed PO showed higher GSI than those fed other lipid sources. This could be due to the abundant PA in PO, which has also been found in high quantities in the gonads of wild sea urchins (Wang et al., 2019).

Although sea urchins belong to inveterate, they are similar to fish in some physiological processes, including fatty acid metabolism, gonad development, and reproduction (Roöttinger et al., 2008; Silvia et al., 2015; Wang et al., 2021). Fecundity generally depends on the species, age, size, and nutritional conditions of spawners (Izquierdo et al., 2001; Kamler, 2005; Wanke et al., 2017). In the present study, the relative fecundity of sea urchins was not significantly affected by the experimental diets. This was consistent with the findings on rainbow trout *Oncorhynchus mykiss*, which showed that the relative fecundity of female individuals was not significantly affected by dietary lipid sources (Yldz et al., 2020). However, some studies have shown that dietary lipids can significantly improve the fecundity of broodstock, such as zebrafish *Danio rerio* (Jaya-Ram et al., 2008), yellowfin sea bream *Acanthopagrus latus* (Zakeri et al., 2009), Atlantic cod *Gadus morhua* (Rjbek et al., 2014), and three-spot gourami *Trichopodus trichopterus* (Berenjestanaki et al., 2014). Egg size (may be expressed as egg diameter) has been

TABLE 10 | Effects of different lipid sources on the fatty acid composition (g/kg dry matter) in the prismatic larvae of sea urchin (*Strongylocentrotus intermedius*) (n=1)[1].

FA	Formulated feeds with different lipid sources					Kelp
	CO	FO	LO	SO	PO	
C14:0	5.60	7.96	5.36	3.79	5.25	9.53
C15:0	0.31	0.45	0.30	0.34	0.27	0.48
C16:0	16.44	17.38	14.12	9.95	17.89	29.95
C17:0	0.13	0.18	0.16	0.10	0.12	0.21
C18:0	3.35	3.65	4.09	3.00	3.80	5.73
C20:0	0.35	0.37	0.35	0.22	0.22	1.63
C21:0	15.94	10.87	8.96	5.86	12.33	7.27
C22:0	0.20	0.19	0.20	–	0.28	0.17
C23:0	0.51	0.39	0.33	0.27	0.41	0.20
C24:0	0.18	0.14	0.17	0.40	0.47	0.25
ΣSFA[2]	43.02	41.58	34.02	23.91	41.02	55.41
C14:1	0.31	0.51	0.28	0.17	0.34	0.22
C16:1	3.57	5.05	2.43	1.67	3.31	4.73
C18:1	13.32	4.40	5.53	4.26	14.97	2.94
C20:1	4.73	3.42	2.35	1.81	3.51	4.98
C22:1	11.96	13.29	13.89	10.02	9.33	19.30
C24:1	0.62	0.52	0.35	0.32	0.61	0.41
ΣMUFA[3]	34.51	27.20	24.82	18.23	32.05	32.58
C18:3n-3	3.54	0.90	5.74	1.06	0.76	3.15
C20:3n-3	0.72	0.43	1.69	0.57	0.21	5.53
C20:5n-3	3.22	7.82	4.47	2.20	1.72	19.25
C22:6n-3	0.71	5.38	0.58	0.37	0.71	0.20
∑n-3PUFA	8.18	14.54	12.47	4.20	3.40	28.13
C18:2n-6	13.73	6.49	12.97	10.40	12.36	1.63
C20:2n-6	3.81	3.25	4.17	3.72	2.37	3.49
C20:3n-6	1.42	0.92	1.16	0.67	0.93	0.77
C20:4n-6	6.90	6.08	4.54	5.63	7.96	7.31
C22:2n-6	0.15	0.15	0.24	0.28	0.19	0.23
∑n-6PUFA	26.01	16.88	23.07	20.70	23.81	13.43
ΣPUFA[4]	34.19	31.42	35.54	24.89	27.21	41.56
n-3/n-6PUFA	0.31	0.86	0.54	0.20	0.14	2.10
ARA/EPA	2.14	0.78	1.02	2.56	4.62	0.38
DHA/EPA	0.22	0.69	0.13	0.17	0.41	0.01

[1]*Some fatty acids, of which the contents are minor, trace amount or not detected, such as C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:1, C17:1, were not listed in the table.*

[2]*SFA, saturated fatty acids.*

[3]*MUFA, mono-unsaturated fatty acids.* [4]*PUFA, poly-unsaturated fatty acids.*

shown to be an indicator of evaluating reproductive performance in most aquatic animals (Lund et al., 2008; Wanke et al., 2017; Stuart et al., 2020), with the assumption that larger eggs represent a higher reserve of metabolic energy substrates (Brooks et al., 1997; Kamler, 2005; Nazari et al., 2009; Stuart et al., 2020). In the present study, sea urchins fed FO produced eggs with the largest size. This was consistent with the findings of Xu et al. (2017) who found that the egg diameter of tongue sole *Cynoglossus semilaevis* was significantly increased by feeding diets enriched with n-3 LC-PUFA. On one hand, the n-3 LC-PUFA could be used for the formation of cell membranes and substances reserved inside the eggs. On the other hand, n-3 LC-PUFA could promote the synthesis, transportation, and deposition of yolk protein in the eggs. Indeed, results of this study showed that the mRNA level of *MYP* in the ovaries of sea urchins fed FO was approximately 2-fold higher than that in the other groups. However, the egg size of Nile tilapia (Ng and Wang, 2011), flame angelfish *Centropyge loriculus* (Callan et al., 2014), and three-spot gourami (Berenjestanaki et al., 2014) was not significantly affected by dietary LC-PUFA levels. The discrepancies could be due to differences in oil droplet presence and egg size between species.

In the present study, the fertilization rate and hatching rate of sea urchins fed LO were relatively high, which could be related to the relatively higher ratio of n-3/n-6 PUFA in the eggs of sea urchins. Jaya-Ram et al. (2008) reported that egg quality and hatching rate of zebrafish were positively correlated with dietary n-3/n-6 PUFA. On the other hand, this could be due to the relatively lower inflammation in the ovary of sea urchins fed LO. Crespo et al. (2010) reported that the pro-inflammatory cytokines (e.g., *TNF-α*) in the ovaries of brown trout *Salmo trutta* broodstock negatively affected the ovarian function and egg quality. In this study, sea urchin broodstock fed SO had a poor reproductive performance. This could be due to the increased linolic acid derived inflammation, which has been found to be responsible for the infertility. It was previously found that auraptene, as a coumarin derivative with anti-inflammatory properties, was effective in improvement of oocyte maturation and fertilization rate in polycystic ovary syndrome patients

(Abizadeh et al., 2020). Furthermore, the sperm quality is important for the success of fertilization. Vassallo-Agius et al. (2001) found that semen obtained from rainbow trout fish fed n-3 PUFA-sufficient showed higher sperm motility rates. Butts et al. (2015) reported that European eel fed diets with deficient n-3 fatty acids showed lower sperm motility. Therefore, the higher fertilization in the LO group of this study could be due to the better sperm quality of sea urchins. However, sperm quality parameters were not analyzed in this study and following studies are needed to clarify this point. It is well known that the steps of fertilization include the acrosome reaction, cell membrane fusion of both gametes, and genetic materials interchange (Pomin, 2015). Previous studies have shown that membrane fluidity plays a critical role in the fusion process of gametes, which is positively correlated with the content of n-3 PUFAs (Asturiano et al., 2001). Despite high n-3 LC-PUFA promoting fertilization, fertilization was lower in the FO group, which was possibly due to oxidation of seminal plasma (Jedrzejczak et al., 2005). This indicated that oxidation levels should be assayed when sea urchin broodstock are conditioned with fish oil to acquire satisfactory fertilization rates.

Larvae survival during a short period of starvation has been widely used as an index of evaluating their viability (Quintana et al., 2015). In the present study, at the 3rd day post hatching (DPH), the larvae survival was the highest in the FO treatment, which paralleled with egg size, n-3 LC-PUFA content, and MYP expression level. Tamada and Iwata (2005) reported that the survival rate of *Rhinogobius* larvae was positively correlated with the egg size during a period of 72 h starvation. Stuart et al. (2020) reported that the starvation-resistant capacity of California yellowtail *Seriola dorsalis* larvae was positively correlated with their egg size. It is commonly accepted that larger larvae can live longer under the starvation test (Miller et al., 1998; Kamler, 2005). However, body length and body width of prismatic larvae were almost the highest in the kelp group, which was inconsistent with the poorest survival performance of this group. Thus, in addition to body size, body nutritional composition was also important for the survival of larvae before mouth opening. Carboni et al. (2012) showed that the increasing levels of DHA of sea urchin *Paracentrotus lividus* larvae fed with *Pleurochrysis carterae* and *Cricosphaera elongata* promoted better larval performance. It was previously found that *P. lividus* larvae has specific dietary requirements for high level of n-3 LC-PUFA, including DHA and EPA, low DHA/EPA and high EPA/ARA (Liu et al., 2007; Carboni et al., 2012). In this study, prismatic larvae with high DHA/EPA showed a higher survival rate. Thus, DHA/EPA could be used as a sensitive indicator of evaluating the survival of early larvae which still relies on endogenous nutrition. Furthermore, survival of larvae in the CO group was the lowest among all dietary groups. This could be due to the high amount of oleic acid (OA, 18:1n-9) in the CO. Samaee (2010) reported that the increasing level of OA in eggs reduced the survival rate of early larvae of common dentex *Dentex dentex*. Callan et al. (2012) reported that the increasing level of oleic acid in eggs has a negative impact on the egg vitality of flame angelfish *Centropyge loriculus*.

In conclusion, sea urchins fed PO showed higher GSI than those fed other lipid sources. Egg diameter and fecundity of *S. intermedius* were largest in the FO group, which could be due to the abundant n-3 LC-PUFA deposited in their gonads. The higher inflammation level could account for lower fecundity and hatching rate of sea urchins fed SO. Sea urchins fed CO showed the highest content of oleic acid in the gonads and eggs, and the lowest fertilization rate. The highest hatching rate was observed in the kelp treatment, which was comparable to that in the LO and PO but was significantly higher than that in CO, FO, and SO. At the 3rd DPH, the survival of *S. intermedius* larvae was highest in the FO treatment, which was only significantly higher than those in Kelp. Thus, FO was accepted as the most ideal lipid source based on growth, reproductive performance, and early larval quality. These results could contribute to adopting an efficient feeding strategy to promoting the reproductive performance and offspring quality by choosing the optimal lipid source for *S. intermedius* broodstock.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

RZ and YN designed the experiment under the help of JD. YN and RZ performed the feeding experiment. YN, WD, and YH analyzed the data. YN drafted the manuscript. RZ, JD, JS, and YC revised the manuscript. All authors contributed to the article and approved the submitted version.

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